2024 GLOBAL NF CONFERENCE

The EGG Brussels
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Organized by:

CHILDREN'S TUMOR FOUNDATION
ENDING NF THROUGH RESEARCH

EUROPEAN NEURO FIBROMATOSIS GROUP

Hosted by:

CHILDREN'S TUMOR FOUNDATION EUROPE

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ABSTRACTS

Speaker Abstracts

CLINICAL CARE SYMPOSIUM

Session Co-Chairs: Scott Plotkin, MD, PhD, *Massachussets General Hospital, US;* Amedeo Azizi, MD, *Medizinische Universitaet Wien, Austria*

Topical Therapies for Cutaneous Neurofibromas

Friday, 21 June, 8:45 – 9:15

Carlos Romo, MD, *Johns Hopkins, US*

We will review exciting results and ongoing clinical trials evaluating topical, injectable, and non-invasive devices for the treatment of cutaneous neurofibromas (cNFs). In addition, we will discuss two clinical trials evaluating systemic MEKi for cNFs.

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Breast Cancer Risk in NF Patients

Friday, 21 June, 10:00 – 10:30

Juha Peltonen, MD, PhD, *University of Turku, Finland*

Dr. Juha Peltonen received his MD in 1981 and PhD in 1985 from the University of Turku, Finland, with his doctoral research focusing on "Connective Tissue in von Recklinghausen's Neurofibromatosis." In 1987, he moved to the United States, joining the Jefferson Institute of Molecular Medicine at Thomas Jefferson University in Philadelphia, PA, where he served as a postdoctoral researcher and later as an associate professor. Returning to Finland, Dr. Peltonen has held professorships at the Universities of Oulu and Turku. His research primarily explores the cellular composition and development of dermal neurofibromas and the skeletal manifestations in Neurofibromatosis type 1 (NF1). In recent years, Dr. Peltonen's research has expanded to include comprehensive population analyses of cancer in NF1 patients. His team established the Finnish Neurofibromatosis Registry, the first comprehensive population-based registry of its kind. This registry has facilitated significant studies on cancer associations in NF1 patients and has helped elucidate the natural history of the disorder.

GENE THERAPY

Session Co-Chairs: Elisabeth Castellanos, PhD, ErCLG, *Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Spain;* Kathrin Meyer, PhD, *Alcyone Therapeutics, US;* Peggy Wallace, PhD, *University of Florida, US*

RNA Therapeutic Approaches for Neurofibromatosis Type 1

Friday, 21 June, 13:10 – 13:35

Santiago Vernia, PhD, *Imperial College London, UK*

Neurofibromatosis type 1 (NF1) is a relatively common autosomal dominant disorder caused by mutations in *NF1* gene which encodes neurofibromin, a tumour suppressor with RAS-GTPase activity.

Affected individuals can have multiple clinical manifestations including benign tumours in the skin (neurofibromas) or in the nerves (plexiform neurofibromas), which in a 10% of patients progress to malignant peripheral nerve sheath tumours (MPNSTs). When surgery for cutaneous neurofibromas and large plexiform neurofibromas is not possible, treatment with MEK inhibitors, such as selumetinib has shown beneficial effects. However, not all patients respond to this treatment and adverse effects have been described. Consequently, there is an urgent need for novel therapeutic options.

RNA therapeutics is an emerging area of research. The specificity of hybridization by Watson-Crick base pairing allows a highly targeted approach to be carried out that results in minimal off-target affects. Recent advances in the design and improved pharmacokinetics of RNA-based oligonucleotides would enable the development of modulators for previously undruggable targets. However, this avenue is largely unexplored for multiple pathologies. Our work is focused on the development of RNA-based therapeutic approaches for NF1 patients. We are developing short oligonucleotides and exosome-mediated mRNA delivery aimed to restore neurofibromin tumour-suppressor activity.

Platform: Gene Editing Corrects an NF1 Causal Mutation and Restores Neurofibromin Expression *In Vitro*

Friday, 21 June, 13:35 – 13:50

Madeleine J. Sitton, *Duke University, Durham, North Carolina, US*

The **purpose** of this study is to determine if base editing, a cutting-edge CRISPR technology, can correct a causal *NF1* mutation resulting in functional neurofibromin *in vitro*.

Methods: *In silico* tools were used to design guide RNAs (gRNAs) targeting the $NF1^{Arg681*}$ mutation. Mouse embryonic fibroblasts (MEFs) were then generated from $DhhCre+;NF1^{Arg681*/4Fl};Rosa26-lsl-nls-tdTomato/+$ mice and immortalized by knocking-out *p53.* Lentiviral vectors were designed to express a gRNA, either targeting the Arg681* mutation or a non-targeting negative control sequence, and the adenine base editor (ABE8e) linked to the puromycin resistance gene via a P2A cleavage site. MEFs were transduced and treated with puromycin. DNA, RNA, and protein were collected at various timepoints to 1) quantify editing rates using next-generation sequencing, 2) quantify the number of corrected transcripts using digital droplet PCR (ddPCR), and to 3) evaluate neurofibromin protein restoration by Western blot.

Results: We identified a S. *pyogenes* Cas9 (SpCas9) and a *Staphylococcus aureus* Cas9 (SaCas9) gRNA predicted to revert the premature stop codon back to an arginine. We transduced MEFs with lentivirus expressing the base editing components and tracked gene editing over time. By day 9, MEFs treated with the SaCas9 gRNA showed a 23% correction rate compared to controls. Cells treated with the SaCas9 ABE8e base editing technology exhibited a 15% increase in wild-type NF1 transcripts and restored neurofibromin protein expression.

We observed that gene editing activity increased over the first 9 days. However, editing activity began to decline at day 16, such that over time we found that the percent edited cells decreased to undetectable levels by day 42. We hypothesize that restoration of functional neurofibromin in edited cells results in a growth disadvantage whereby unedited cells out-compete edited cells.

Conclusions: We developed a base editing strategy to correct a nonsense mutation found in individuals with NF1 and demonstrated that this technology restores the wild-type NF1 sequence, resulting in the production of wild-type transcripts and neurofibromin protein. Our data showing that edited cells diminish in cultures over time likely suggests corrected cells harbor functional NF1 and are outcompeted by non-edited, NF1 mutant cells. Ongoing experiments aim to functionally characterize the signaling and proliferation in edited cells and test combination treatment with MEK inhibitor therapy. To our knowledge, this work represents the first example of gene editing applied to mutations causing NF1 and has important implications for the use of gene editing to treat existing tumors.

Full List of Authors: Madeleine J. Sitton, Joel D. Bohning, Lixia Luo, Andrea R. Daniel, David S. Van Mater, David G. Kirsch, Charles A. Gersbach

Platform: Preclinical Development and *In Vivo* Delivery of Antisense Oligonucleotides for Targeted *NF1* Exon 17 Skipping

Friday, 21 June, 13:50 – 14:05

Deeann Wallis, PhD, *University of Alabama at Birmingham, US*

Neurofibromatosis type I arises from germline mutations across the *NF1* gene. Our research has published *in vitro* evidence highlighting the therapeutic potential of antisense oligonucleotides (ASOs) for numerous NF1 pathogenic variants through targeted exon skipping, including exon 17. To provide pre-clinical validation of ASOs *in vivo* and to facilitate translational development, various mouse models have been developed. The first is a conditional tamoxifen-inducible systemic knockout *Nf1* mouse model with floxed NF1 alleles. Following tamoxifen inactivation of floxed *Nf1* alleles, adult CAGGCre-ER^{TM};*Nf$^{Flox/Flox}$* mice lose expression/function of *Nf1* systemically; we refer to this as the "acute" model as mice die 12 days post tamoxifen induction. We have bred the acute model to the humanized exon 17 allele with patient-specific pathogenic variant G629R to obtain CAGGCre; NF$^{Flox/hG629R}$ mice. We have used these mice to evaluate two ASO delivery platforms: conjugation of ASOs with morpholino chemistry (PMO) to cell penetrating peptides (PPMO) and recombinant Adeno-Associated Viral (AAV) vectors with AAV9 serotype carrying U7-SnRNA expression cassettes to give proof-of-concept of *in vivo* exon skipping efficacy of our optimized ASOs. We treated five CAGGCre; NF$^{Flox/hG629R}$ mice with a single ICV dose of 1.34 mg/kg exon 17 PMO conjugated to a CPP (PPMO) designed for cell membrane penetration and endosomal escape (5 mice with PBS injection were used as control). One week post PPMO/Ctrl injection tamoxifen induction was performed. We see PPMO-driven skipping of the humanized allele in RNA from brain and optic nerve (both NF1 relevant tissues). We also see restoration of neurofibromin protein expression and partial functionality (as measured by reduced pERK/ERK) in brain. PPMO has been limiting to date. We are working on improving PPMO solubility, purity, and production scale to allow multiple dosing schedules. We were also able to treat a very small cohort of P3 neonatal CAGGs-Cre$^{Flox/hG629R}$ mice with 2 x 10^{11} vp/mouse AAV9-U7-SnRNA vectors (3 mice), or PBS (2 mice) delivered by ICV. At six weeks, we induced *Nf1* loss with tamoxifen. We then used body condition score mandated euthanasia for tissue collection. We have been able to show evidence of *in vivo* exon skipping of the humanized allele in the brains of AAV9-U7-SnRNA treated mice using q-RT-PCR. While this small number of mice does not yet give statistical significance, it demonstrates suggestive evidence of *in vivo* efficacy and gives rationale that this dose and route may be effective in future studies with improved vectors. Viral delivery of AAV9-U7-SnRNA-2A-GFP-luciferase will be tested in neonatal mice to evaluate biodistribution, RNA skipping, protein restoration, and Ras activity.

Conclusions: This preliminary data is the first to show *in vivo* splice modulation and restoration of neurofibromin protein expression in NF1-relevant tissues including the brain and optic nerve.

Full List of Authors: Marc Moore, Hui Liu, Xiaoxia Zhang, Gretchen Long, Erik Westin, Robert Kesterson, Jiangbing Zhou, Linda Popplewell and Deeann Wallis

Schwannoma Gene Therapy: AAV- Mediated Delivery of the Inflammasome Adaptor, ASC

Friday, 21 June, 14:30 – 14:55

Gary Brenner, MD, PhD, *Massachusetts General Hospital, US*

Gary J. Brenner is a clinician-investigator in with medical expertise in Pain Medicine and over 30 years of experience investigating the interaction between the immune and nervous systems with an emphasis on the role of these interactions in health and disease. After a doctoral dissertation focused on neuro-immune interactions responsible for stress-induced alterations in immune function, he performed post-doctoral research on the neurobiology of pain focused in part on the role of immune responses in the development of pathological pain. More recently, Dr. Brenner's primary research focus has been on the development of novel gene-and cell-based treatment, including immunotherapeutic, for schwannoma. This work has been NIH-funded and resulted in multiple peer-reviewed publications and patent applications.

In addition to leading a research laboratory, Dr. Brenner has substantial mentorship and administrative experience through over 20 years as Program Director of the MGH Pain Medicine Fellowship. He has developed a national presence and reputation in the field of pain medicine through leadership positions in several key organizations including The Association of Pain Program Directors (past president), The Society of Academic Associations of Anesthesia and Perioperative Medicine (past president, subspecialty component), the American Society of Regional Anesthesia and Pain Medicine (research committee, past vice-chair), and the American Board of Anesthesiologists (Pain Medicine Maintenance of Certification Committee, current member; Pain Medicine Exam Committee, past member). Dr. Brenner has also served CTF in a variety of roles including grant review panels (ongoing), Clinical Care Advisory Board member (current), and Research Advisory Board member (past).

Platform: Promising Adeno-Associated Viral (AAV) Gene Replacement Therapy for NF2 Effectively Reduces Tumor Growth in *In Vitro* and in *In Vivo* Schwannoma Models

Friday, 21 June, 14:55 – 15:10

Krizelle Alcantara, MS, *Center for Gene Therapy, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, US*

In recent years, gene therapy has emerged as a promising treatment option for rare monogenic disorders with limited treatment options. Particularly, adeno-associated viral vectors (AAVs) have been demonstrated to effectively target the nervous system with a remarkable safety profile. Due to its monogenic nature, NF2-related schwannomatosis (*NF2*-SWN) is a promising candidate for AAV gene therapy. This study aims to verify the targeting and efficacy of our lead candidate vector developed for AAV-mediated gene replacement therapy for *NF2*-SWN loss-of-function pathology.

We generated multiple AAV vectors comparing several truncated NF2 promoters expressing either reporter green fluorescent protein (AAV.NF2.GFP) or wild-type NF2 (AAV.NF2.NF2). We tested our vectors *in vivo* by intracerebroventricular (ICV) injection in wild-type mice followed by immunofluorescence (IF) staining and Western blot (WB) analysis to determine targeting and expression in relevant tissues and cell types (Figure 1). We further tested the efficacy of the top candidate AAV.NF2.NF2 vector in reducing tumor growth *in vivo* via intratumoral injection in two intrasciatic xenograft schwannoma murine models of NF2 (Figure 1), and *in vitro* through transduction in NF2 patient-derived induced Schwann cells (iSCs) with three distinct *NF2* gene mutations, primary human vestibular schwannoma (VS) tumor cells, and U87 glioblastoma cancer cells. Expression of NF2 and relevant biomarkers were assessed through immunofluorescence (IF) imaging, RT-qPCR, and WB analysis (Figure 2). The effect of vector treatment on tumor growth was measured *in vivo* through bioluminescence/IF imaging and caliper measurements (Figure 1), and *in vitro* through cell proliferation assays (Figure 2).

Confocal Z-stack imaging of brain regions with reported high native NF2 expression were examined for GFP expression in wild-type mice injected with our AAV.NF2.GFP vectors by IF imaging. Results show overall highest GFP expression and colocalization with the Schwann cell/astrocyte marker S100 from NF2 Promoter A. Injection of the corresponding AAV.NF2.NF2 vector demonstrated the same results, as confirmed by WB analysis of NF2 expression in these brain regions. Intratumoral injection in the schwannoma xenograft mouse models targeted tumor cells and significantly reduced tumor growth compared to PBS injected controls. *In vitro* transduction of the top candidate AAV.NF2.NF2 vector in iSCs, primary VS cells, and glioblastoma cells increased NF2 expression, reduced cell proliferation, and rescued the tumorigenic phenotypes of these cells as assessed through expression of biomarkers for mature Schwann cells, stem cells, and the cancer marker nuclear beta-actin.

Our robust data indicates our gene therapy approach is highly promising and warrants further development towards IND-enabling studies.

Figure 1. *In Vivo*

Figure 2. *In Vitro*
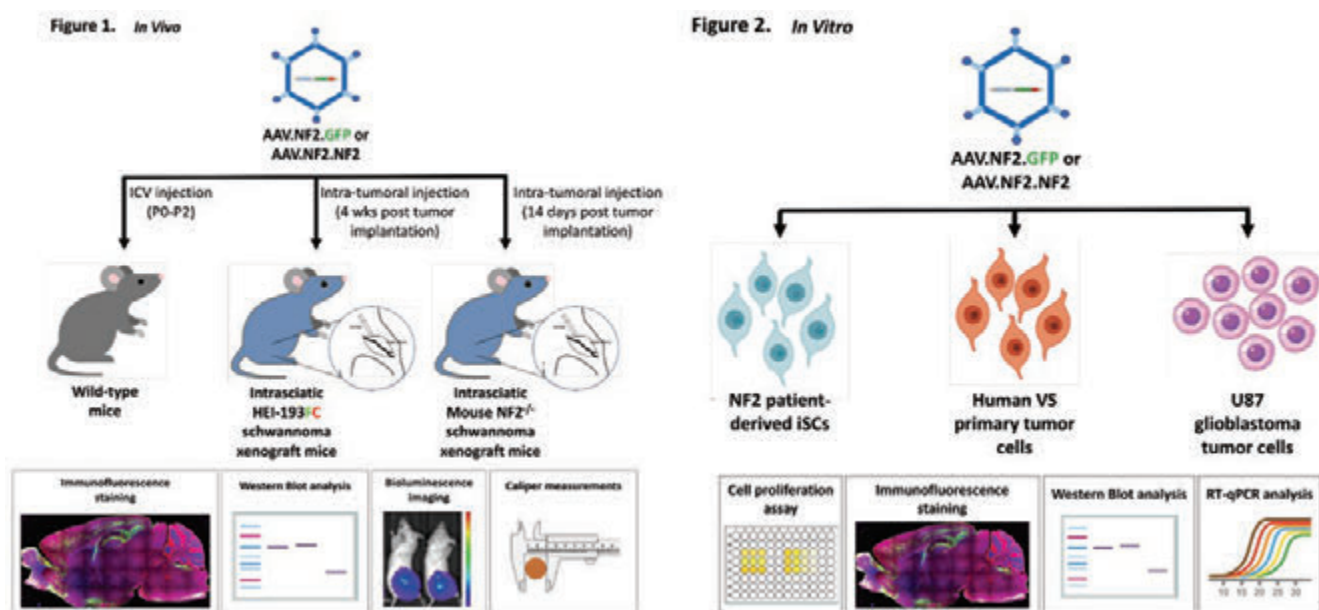
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<u>Platform</u>: Testing the Potential of Antisense Gene Therapy for *NF2*-Related Schwannomatosis in an iPSC-Based Model

Friday, 21 June, 15:10 – 15:25

Gemma Casals Sendra, MSc, *Clinical Genomics Research Group, Germans Trias i Pujol Research Institute, Spain*

NF2-related Schwannomatosis (*NF2*-related SWN) is an inherited autosomal dominant disorder caused by loss of function (LOF) variants in the *NF2* gene and, currently, with no effective treatment. Previously, we have shown that a Phosphorodiamidate Morpholino Oligomers (PMOs) antisense can induce the skipping of exon 11 of the *NF2* gene harbouring a nonsense variant, and partially rescue the *NF2* phenotype in fibroblasts[1]. Here, we aim to test the potential of this therapy approach in a more appropriate cellular model for *NF2*-related SWN and evaluate its efficacy to recover merlin function.

With this objective, we edited induced pluripotent stem cells (iPSCs) with CRISPR/Cas9 to obtain *NF2*(+/-) and *NF2*(-/-) lines with truncating variants on exon 11 of *NF2* gene to achieve an inexhaustible cell source that can be differentiated toward the Neural Crest - Schwann Cell axis (NC-SC), called iPSCmut11.

The use of PMOs was tested in the iPSCmut11 lines to force the in-frame skipping of the exon 11 harbouring the truncating variants, and to generate less deleterious merlin forms. Moreover, we designed an expression panel to discriminate the RNA signature based on the *NF2* genotype and the cellular stage of differentiation, considering expression profile of 50 transcripts identified on previous data[2]. We have seen that the iPSCmut11 are able to reproduce expression panel profile observed in other *NF2* deficient iPSC cells differentiated towards NC-SC axis[2]. Furthermore, after analysing the expression profile of these 50 transcripts before and after exon 11 PMO treatment, we observed that the vehicle used to deliver the PMOs has a dominant effect on the overall expression, but it also unveils a subtle recovery of the expression levels of some markers in the treated cells, as ANXA2, COL6A3, CYBRD1 or MEST, indicating that PMO targeting exon 11 could have being inducing some recovery in merlin function.

Currently, we are differentiating these iPSC-e11 towards the NC-SC axis and testing a new *NF2* exon 11 PMO approach that does not require any delivery method in SC-like spheroids.

Full List of Authors: Gemma Casals-Sendra[a], Miguel Cidràs[a], Inma Rosas[a,b], Sandra Bonache[a,b], Ester Romera[a,b], Aida Silverio[a,b], Emilio Amilibia[c], Angela Callejo[c], Eduard Serra[d], Ignacio Blanco[a,b], Núria Catasús[a,b], and Elisabeth Castellanos[a,b]

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References:
1. Catasús N, Rosas I, Bonache S, Negro A, Torres-Martin M, Plana-Pla A, et al. Antisense oligonucleotides targeting exon 11 are able to partially rescue the NF2-related schwannomatosis phenotype in vitro. Mol Ther Nucleic Acids. 2022 Dec 13;30:493–505.
2. Núria Catasús, Miguel Torres-Martin, Alex Negro, Bernd Kuebler. Merlin-Deficient iPSCs Show Altered Pluripotency and Constitute a Potential in Vitro Model for NF2-Related Schwannomas. Preprint. 2023

KEYNOTE #1: GENE THERAPY OF METACHROMATIC LEUKODYSTROPHY FROM BENCH TO BEDSIDE AND THE MARKET: A ROADMAP TO THE DEVELOPMENT OF NEW ADVANCED GENE AND CELL THERAPIES

Friday, 21 June, 15:25 – 16:30

Luigi Naldini, MD, PhD, *San Raffaele University, Italy*

Luigi Naldini, M.D., Ph.D., is Professor of Cell and Tissue Biology and of Gene and Cell Therapy at the San Raffaele University School of Medicine and Scientific Director of the San Raffaele Telethon Institute for Gene Therapy (Milan, Italy). He has received his medical degree from the University of Turin (Italy) and his PhD from the University "La Sapienza" of Rome (Italy). For the past 30 years he has pioneered the development and applications of lentiviral vectors for gene therapy, which have become one of the most widely used tools in biomedical research and are providing a long-sought hope of cures for several otherwise deadly human diseases. Throughout this time, he has continued to investigate strategies to overcome the major hurdles to safe and effective gene therapy, bringing about innovative solutions that are not only being translated into new therapeutic strategies for genetic diseases and cancer, but have also allowed novel insights into hematopoietic stem cell function and tumor angiogenesis. He also contributed to pioneer and advance the use of artificial nucleases for targeted genome editing in cell and gene therapy. He has published >300 scientific papers. SCOPUS Author h-index: 108.

Member of the European Molecular Biology Organization (EMBO), has been President of the European Society of Gene and Cell Therapy (ESGCT), appointed as expert on the "Human Gene Editing Study" of the US National Academies of Sciences and of Medicine, and on the Italian National Committee for Biosafety, Biotechnology and Life Sciences. He was awarded the Outstanding Achievement Award from the American Society of Gene and Cell Therapy (ASGCT) in 2014 and from ESGCT in 2015, an Honorary doctorate from the Vrije University, Brussel, in 2015, the Jimenez Diaz Prize in 2016, the Beutler Prize from the American Society of Hematology (ASH) in 2017 and the 2019 Jeantet-Collen Prize for Translational Medicine. He was nominated "Grande Ufficiale dell'Ordine Al Merito della Repubblica Italiana", one of the highest-ranking honor in Italy, and elected member of "Accademia dei Lincei", the oldest and most prestigious national academic society. He is also co-founder of three innovative biotech start-up companies: Genenta (recently listed on Nasdaq), Epsilen Bio (now acquired by Chroma Medicine) and Genespire.

KEYNOTE #2: THE SCIENCE AND THE ART OF RESILIENCE: 5 LESSONS LEARNED FROM PATIENTS, COMMUNITIES, AND SOCIETY

Saturday, 22 June, 9:00 – 10:00

Abby Rosenberg, MD, *Boston Children's Hospital / Harvard University, US*

Resilience is a concept that is both common and commonly misunderstood. It is also important; resilience helps people navigate myriad stressful experiences ranging from chronic illness to natural disaster. In this session, Dr. Rosenberg shares what she and her team have learned from studying resilience for over a decade. Beginning with lessons-learned from children with serious or chronic illnesses and their parents, she expands to lessons-learned during the COVID-19 pandemic and other transformative societal events. She ends with thought-provoking questions and advice for how all of us can help each-other — and ourselves — to be more resilient with whatever comes next.

COMPREHENSIVE CARE

Session Co-Chairs: Ignicio Blanco, MD, PhD, *Hospital Universitari Germans Trias i Pujol, Spain;* Hilde Brems, PhD, *KU Leuven, Belgium;* Justin Jordan, MD, MPH, *Massachusetts General Hospital, US;* Laura Iesse, MD, PhD, *University of Texas Southwestern Medical Center, US*

## Platform: Exploring the Interplay Between Lipid Metabolism and LZTR1 in Peripheral Nerve Pathologies

Saturday, 22 June, 11:00 – 11:15

Georgia Daraki, MSc, *Fritz Lipmann Institute-Leibniz Institute on Aging, Jena, Germany*

Schwannomatosis is characterized by a prominent feature — neuropathic pain. Patients who carry an *LZTR1* deletion (*LZTR1*-related schwannomatosis) experience heightened pain compared to those with other disease-associated mutations. Despite this association, the cause of neuropathic pain and its link to *LZTR1* deletion remains elusive. To address this gap and understand the underlying molecular mechanism, we utilize a mouse model where *Lztr1* is knocked out in Schwann cells (*Lztr1*-KO P0) and carry out molecular analysis and sensory tests. Already at a young age, this model exhibited increased sensitivity to mechanical stimuli (Figure 1), partially recapitulating the clinical phenotype. Analysis of sciatic nerves from these mice revealed disrupted stoichiometry of structural myelin proteins, altered myelin sheath structure, and an inflammatory response that is often observed in demyelinating diseases (Figure 2). Proteomic and lipidomic data from *Lztr1*-deficient mice (*Lztr1*-KO P0 mice and global heterozygous *Lztr1* mice) revealed a novel role for LZTR1 in the regulation of lipid metabolism, with the deficiency leading to problems with fatty acid metabolism and composition. Using *in vitro* models (HEK293T and SHSY-5Y cells), we were able to identify novel interactors of LZTR1 associated with translational control and nutrient sensing, and demonstrate lipid dysregulation upon LZTR1 depletion. Upon nutrient stress, HEK393T LZTR1 knockout cells (LZTR1 KO) exhibited hyperactivation of the ERK component of the MAPK signaling cascade (Figure 3) and an upward tendency of autophagy (Figure 4). Considering the crucial role of lipid metabolism in peripheral nerve maintenance and function of the myelin sheath, we hypothesize that LZTR1 deficiency contributes to the disruption of lipid homeostasis, influencing peripheral nerve myelination, and ultimately contributing to neuropathic pain development.
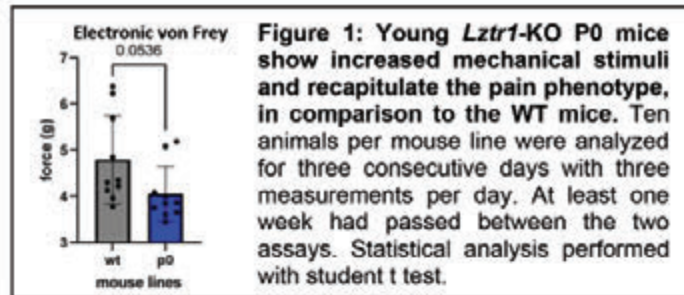

Figure 1: Young *Lztr1*-KO P0 mice show increased mechanical stimuli and recapitulate the pain phenotype, in comparison to the WT mice. Ten animals per mouse line were analyzed for three consecutive days with three measurements per day. At least one week had passed between the two assays. Statistical analysis performed with student t test.
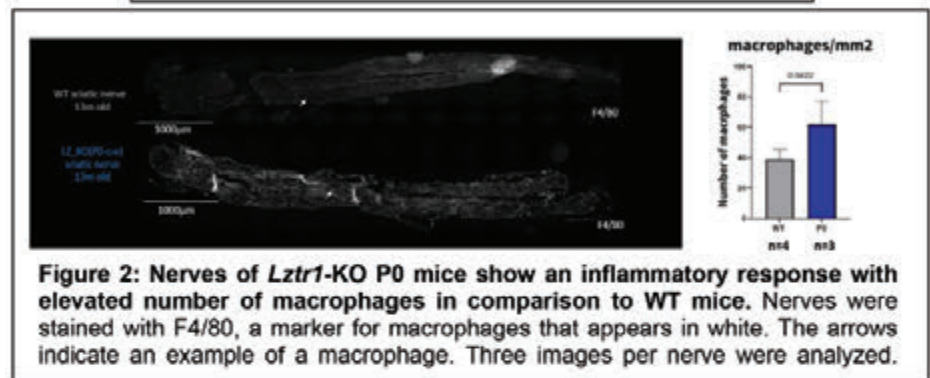
Figure 2: Nerves of *Lztr1*-KO P0 mice show an inflammatory response with elevated number of macrophages in comparison to WT mice. Nerves were stained with F4/80, a marker for macrophages that appears in white. The arrows indicate an example of a macrophage. Three images per nerve were analyzed.
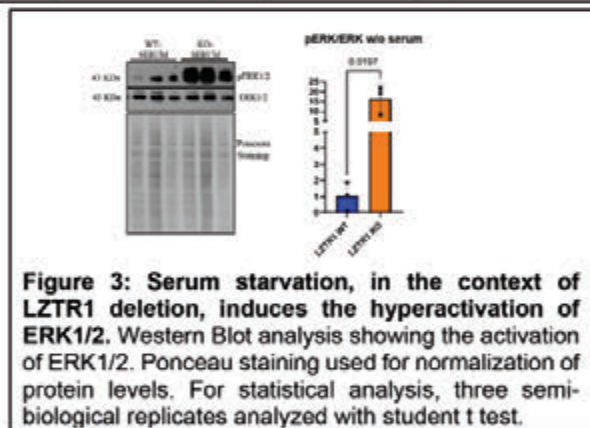
Figure 3: Serum starvation, in the context of LZTR1 deletion, induces the hyperactivation of ERK1/2. Western Blot analysis showing the activation of ERK1/2. Ponceau staining used for normalization of protein levels. For statistical analysis, three semi-biological replicates analyzed with student t test.
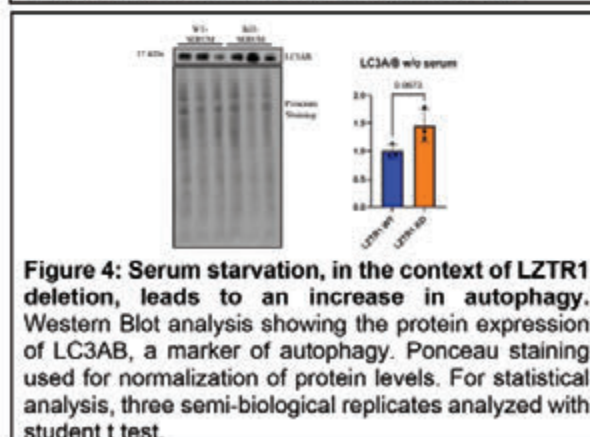
Figure 4: Serum starvation, in the context of LZTR1 deletion, leads to an increase in autophagy. Western Blot analysis showing the protein expression of LC3AB, a marker of autophagy. Ponceau staining used for normalization of protein levels. For statistical analysis, three semi-biological replicates analyzed with student t test.

Full List of Authors: Daraki Georgia, Ricciardi Luisa, Sabah Majed, Uta Papke, Riecken Lars Bjorn, Reuter Michael, Morrison Helen

Platform: Disruption of Core Clock Gene Expression in NF1-Associated Schwann Cells

Saturday, 22 June, 11:15 – 11.30

Anja Harder, MD, *University Medical Center, Johannes Gutenberg University Mainz; CURE-NF Research Group, Martin Luther University Halle-Wittenberg, Germany*

Purpose: Patients with neurofibromatosis type 1 (NF1) develop sleep disorders. Circadian clock studies in NF1 have revealed a role for MAPK signaling, cAMP-dependent PKA, calcium levels and ALK signaling. Mammalian astrocytes show a disturbed rhythm when deprived of *NF1.* Whether gene expression in the peripheral counterpart of the central glia, the human Schwann cell, is rhythmic, e.g. in normal (*NF1+/+*) or NF1-associated Schwann cells (*NF1+/-*), is unknown.

Methods: We analyzed both normal human Schwann cells and NF1-derived MPNST for rhythmic gene expression. The transcript levels of *JUN, TGFA, PER2, PROK2, CLOCK, VEGFA, MYC, MAP3K8, CREM, ARNTL/BMAL1, CRY2, CCND1, TIMESLESS, CRY1, PER1, NR1D1, DBP, CSNK1, HPRT1* were analyzed by next generation sequencing. Three types of gene expression were defined in the data analysis, comparing expression under serum shock and starvation and categorized into rhythmic, inducible and spontaneous gene expression.

Results: We were able to demonstrate rhythmic expression of several central clock genes in normal human Schwann cells *(ARNTL, CLOCK, CRY1, CRY2)* as well as *CSNK1E* and *NRD1D1.* Thus, normal human peripheral glia exhibit rhythmic clock gene expression. In addition, we demonstrated that the normal rhythmic nuclear clock gene expression of Schwann cells is abolished in MPNST: there was not only a loss of rhythmic nuclear clock expression, but also an increase in rhythmic expression of oncogenes and growth factors *(MYC, VEGFA).*

Conclusions: Disturbed circadian rhythms can promote tumor development. In NF1, the common neurofibroma originates from Schwann cells. Disruption of rhythmic gene expression of clock genes and enhancement of rhythmic expression of oncogenes in NF1-associated MPNST illuminates our understanding of physiological processes in peripheral glia as well as tumorigenesis of Schwann cell-derived tumors and stimulates chronotherapeutic approaches for MPNST in NF1.

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Platform: Abnormal Circadian Excretion of 6-Sulfatoxymelatonin in Women with NF1

Saturday, 22 June, 11:30 – 11:45

Isis Atallah, MD, PhD, *Division of Genetic Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland*

Purpose: As sleep troubles are frequently described in NF1 individuals and melatonin is the main circadian pacemaker in humans, we aimed to study the circadian pattern of melatonin excretion in individuals with NF1.

Material and Methods: We measured the excretion of diurnal (10:00-22:00) and nocturnal (22:00-10:00) 6-sulfatoxymelatonin (6-SM) in the urine of 20 individuals with NF1 using an ELISA kit from Biosource. The mean age of the participants was 34.0 years, IQR 23.0 – 53.75 years. 65% (N=13) were women. Seven patients complained of sleep troubles.

Results: The median diurnal and nocturnal excretion of 6-SM was 216.5 ng/h (13.4 – 1225.3) and 125.8 ng/h (25.4 - 381.5), respectively. The night-day ratio was of 0.9016 (0.1094 – 2.939). NF1-women excreted 5 folds more 24h 6-SM than men. Their increase in diurnal and nocturnal 6-SM excretion levels was 11fold and 2fold compared with men. An inverse circadian pattern of 6-SM excretion was observed only in women 0.34 (0.089 -1.09). We did not observe statistical differences among 24h, diurnal and nocturnal 6-SM excretion, age, BMI, tumor burden, or sleep troubles in NF1 individuals.

Graph 1: Gender-stratified levels of 24h, diurnal and nocturnal 6-SM (ng) in NF1 individuals and their night/day ratios.
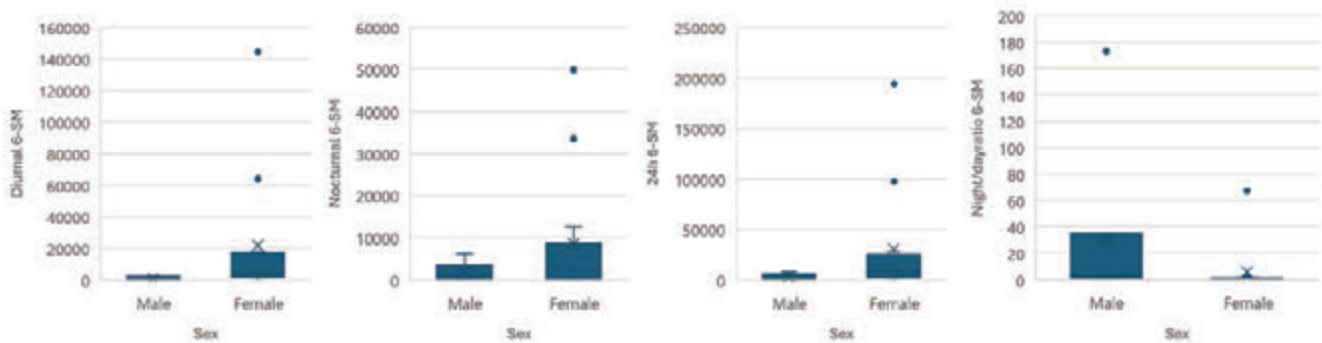
Conclusion: Loss of neurofibromin has been reported to disrupt circadian rhythms of locomotor activity in Drosophila[1] and sleep rhythms in mice[2]. As sleep troubles such as parasomnias, difficulties initiation sleep, early morning awakenings and excessive sleep/wake transition are frequently described in NF1 patients[3], we studied the circadian pattern of excretion of 6-SM, the major melatonin metabolite in the urine of 20 individuals with NF1. Our results showed higher levels and an inverted circadian pattern of 6-SM excretion in women. Based on this result, a hormonal regulation of melatonin secretion is highly suspected. A feedback mechanism between melatonin and estradiol was priorly observed in primary human trophoblast. Estradiol actively enhances melatonin production through the GPER1-PKA-CREB signaling pathway while melatonin negatively regulates estradiol production by suppressing the level of estrogen synthetase aromatase[4]. Interestingly, GPER1 receptors are constitutively expressed in cutaneous neurofibromas[5] and neurofibromin has already been implicated in the E2 signaling pathway by repressing ERα signaling in breast cancer cells[6]. Studying the underlying mechanisms responsible for excessive melatonin synthesis in women with NF1 is crucial to understanding the complex pathogenesis associated with NF1.

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4. Shao X, Yang Y, Liu Y, Wang Y, Zhao Y, Yu X, et al. Orchestrated feedback regulation between melatonin and sex hormones involving GPER1-PKA-CREB signaling in the placenta. J Pineal Res. 2023;75(4):e12913.
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6. Zheng ZY, Anurag M, Lei JT, Cao J, Singh P, Peng J, et al. Neurofibromin Is an Estrogen Receptor-alpha Transcriptional Co-repressor in Breast Cancer. Cancer Cell. 2020;37(3):387-402 e7.

Platform: Age Related Change in the Magnetic Resonance Imaging Characteristics of Plexiform Neurofibromas in Patients with Neurofibromatosis Type 1

Saturday, 22 June, 11:45 – 12:00

Eva Dombi, MD, *National Cancer Institute, Bethesda, Maryland, US*

Purpose: Plexiform neurofibromas (PN) show characteristic bright signal on fluid sensitive magnetic resonance imaging (MRI) sequences, such as short T1 inversion recovery (STIR). In participants on our long-term observational study for pediatric and adult patients with neurofibromatosis type 1 (NF1) we noted that STIR signal intensity of PN decreased with age in some patients. The purpose of this systematic review is to assess the change in imaging characteristics of PN in a cohort of NF1 patients ≥30 years of age.

Methods: Participants of the National Cancer Institute's NF1 Natural History study (NCT00924196) were included in this retrospective analysis if they were ≥30 years of age at their last imaging evaluation and had at least one PN with ≥10 years of MRI follow up using STIR technique. Patients may have received PN directed therapy other than MEK inhibitors that are known to alter the natural history of the disease. PN type was characterized as diffuse or fascicular/multi-nodular. PN burden was categorized as localized, whole-body, or combined. The absolute value of MRI signal intensity varies between scans and there is no established method for normalization, therefore the subjective brightness of PN signal was visually compared between time points.

Results: Seventeen participants were identified for this analysis. The median age at the time of last MRI was 34.8 years (range 30.0-51.2) and the median follow-up duration was 15.3 years (range 10.7-23.1). Visually apparent decrease in the bright STIR signal was observed in one or more PN affected areas in 12 patients (71%). The signal intensity change in PN was not uniform. Fading was more conspicuous in PN associated with the distal portion of the nerves, while PN in proximal/paraspinal locations often retained bright signal, most notably in patients with whole-body PN burden. Among the 5 patients without noticeable decrease in the bright STIR signal, 4 had diffuse PN type.

Conclusions: In the majority of adult NF1 patients (71%) who were followed for ≥10 years with STIR MRIs we observed decrease in signal intensity within some areas of their PN with advancing age. The diminished signal intensity difference between PN and surrounding tissues may present a challenge to monitoring PN size by volumetric MRI that relies on good demarcation between PN and healthy tissues. Understanding the basis for MRI changes at the tissue level would provide insight into the natural history of PN evolution.
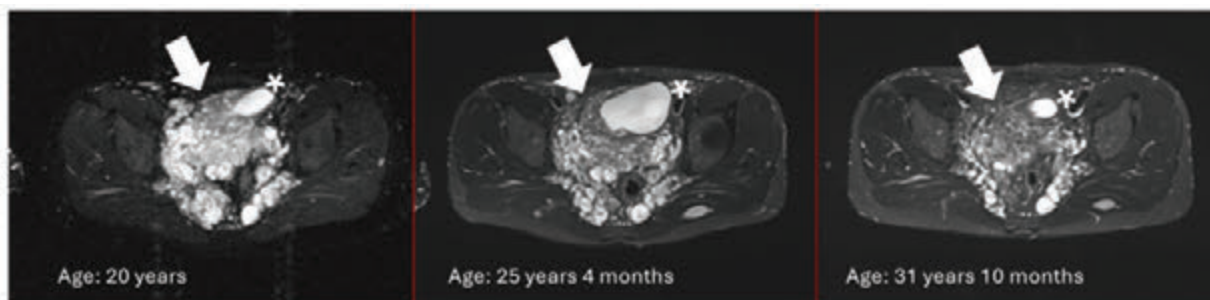
Figure: Example of a pelvic PN (white arrow) adjacent to the bladder (asterisk) on axial STIR MRI demonstrating decrease in signal intensity over time.

Full List of Authors: Eva Dombi[1], Andrea Baldwin[2], Anne Dufek[1], Joanne Derdak[1], Andrea M. Gross[1], Brigitte C. Widemann[1]
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<u>Platform</u>: Comparative Analysis of Validated Measures of Cognitive, Behavioral, Motor, and Physiological Impairments in a Pediatric Population of Neurofibromatosis Type 1

Saturday, 22 June, 13:30 – 13:45

Lindsey E. Aschbacher-Smith, MS, *Cincinnati Children's Hospital Medical Center, US*

Purpose: To establish brain-based, objective measures that reflect the severity of cognitive, behavioral, and motor impairments in neurofibromatosis type 1 (NF1) as outcome measures for clinical trials.

Background: Neurofibromatosis type 1 (NF1), an autosomal dominant disorder caused by mutation/loss of the neurofibromin gene, affects approximately 1 in 2,000 individuals. Approximately 50% of affected children exhibit impaired executive function, learning, and/or motor function. Currently, ordinal scales performed by subjective raters are used to evaluate these manifestations. This can impede accurate measurement of responses to potential therapeutics. Imaging and neurophysiological testing might provide valid, objective, and more reliable data reflecting relevant neuropathophysiological processes.

Methods: We recruited children ages 8-16 years with NF1. We assessed behavior, executive function, cognition, and motor development using validated scales and tests. We performed Transcranial Magnetic Stimulation (TMS) to evaluate inhibitory/excitatory motor physiology and Magnetic Resonance Imaging (Spectroscopy – MRS and Diffusion Tensor Imaging – DTI) to evaluate relevant compounds and white matter tractography in brain regions and pathways of interest. Spearman Rank Correlations and linear regression modeling were performed to explore associations between clinical assessments and brain measures.

Results: Data from twenty-five youth with NF1 (age mean 12.4, S.D. 3.0; 12 female) were analyzed. Poor executive function (ADHD scores +/- tests of visual attention (TOVA)) correlated with both impaired motor development (scores) and TMS measures of lower inhibition/excitation in the motor cortex. We also found evidence of positive associations between white matter tracts subserving motor control and executive function [DTI measures of quantitative anisotropy (QA)] and oxidative state/stress (MRS measures of glutathione concentration in the anterior cingulate region). Further, elevated choline concentrations, an indicator of inflammation or myelin turnover, correlated with several subdomains of impaired motor function. Conclusion: Measures of neuropathophysiological processes involving white matter tract integrity, oxidative stress/capacity, and cortical inhibitory/excitatory balance may reflect cognitive, behavioral, and motor impairments in children with NF1.

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Transition from Paediatric to Adult Care NF1, NF2 Schwannomatosis & Non NF2 Schwannomatosis

Saturday, 22 June, 13:45 – 14:15

Rosalie E Ferner, *Guy's and St. Thomas' NHS Foundation Trust, UK*

The transition from childhood to the adult world presents both challenges and opportunities for adolescents. It is a time of cognitive, physical and psychosocial change. Increasing independence from family emerges, social and romantic relationships evolve with peers and confidence in decision making develops.

For the individual with chronic disease, there is a gradual transfer from paediatric to adult clinical services, starting at around age 14 years, but varying according to individual needs. Our multi-disciplinary Transition Service supports our NF1, NF2 schwannomatosis and non NF2 schwannomatosis clinics. Paediatric and adult clinicians and clinical nurse specialists link with the clinical psychologist, social worker, physiotherapist and patient organisation to tailor care to the individual. Transition is a pivotal time to lay the foundations for integrated, patient focused healthcare.

The aim is to promote autonomy by teaching the young person and their family about the diagnosis, treatment and management of their disease. A healthy, safe lifestyle is encouraged with support from the clinic and community services. Age appropriate clinical and imaging surveillance and the role of genetic testing and counselling are explained. Literacy and communication skills are emphasised and gradual independence related to healthcare decisions is fostered.

Platform: Supporting Reproductive Choices and Decision-Making for Adults with Neurofibromatosis

Saturday, 22 June, 14:15 – 14:30

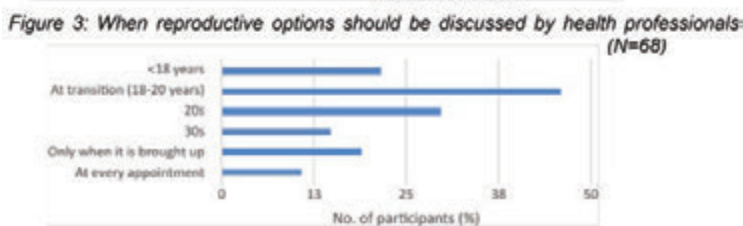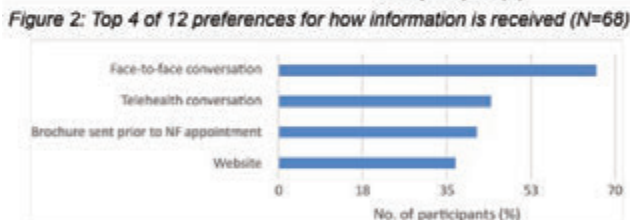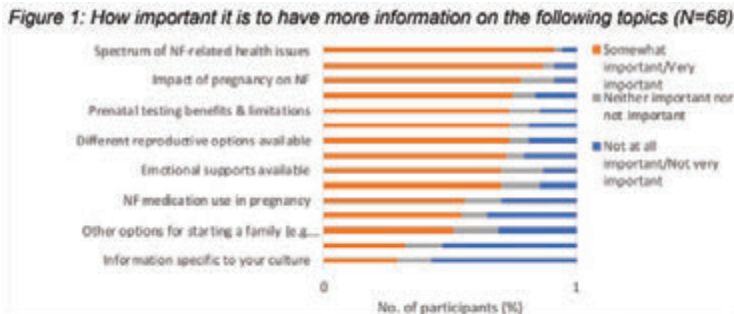**Jane Fleming, PhD**, *Royal North Shore Hospital, Australia*

Purpose: Individuals with Neurofibromatosis (NF) face unique reproductive choices when planning a family, however, there is a paucity of literature investigating the barriers and enablers to reproductive decision-making for individuals with NF: This mixed-method study explored the opinions and experiences of individuals with NF in Australia towards reproductive options to inform strategies and resources to support reproductive choice for this population.

Methods: Adults with NF and their partners were invited to participate in a focus group conducted by an independent facilitator. A semi-structured interview schedule was used to explore participants' opinions and experiences. Data was analysed using codebook thematic analysis. Findings were used to develop an anonymous survey for adults with NF and their partners. Invitations were distributed via support groups, social media and two Genetics services, and data analysed using descriptive statistics.

Results: Sixteen participants with NF1, *NF2*-related-schwannomatosis, and a partner participated in one of three focus groups. Three themes were identified 1) Barriers and Enablers; 2). Weighing up the options; 3). Recommendations. Many participants mentioned difficulty accessing information and services to support their reproductive planning, with financial cost also a barrier. Individual emotional struggles impacted family planning including anxiety; being ashamed, fearful, or overwhelmed; or uncertainty. Many felt family, friends, and health professionals (HPs) often dismiss health concerns. Many individuals with NF worried about the health of a child, others worried their neurofibroma burden would worsen during pregnancy (NF1), or that their disease would progress without medication (*NF2*-related-scwhannomatosis). However, for many, the desire to avoid passing on NF was paramount. Most participants wanted quality information and psychological support.

Sixty-eight people with NF and one partner completed a survey (57=NF1; 4=NF2, 3=other Schwannomatosis-related conditions; 4=Unsure), with 41.3% reporting they lacked, or were unsure, if they had sufficient information to consider whether, or not, to have children (Figure 1). Participants wanted more information, and to discuss reproductive options with HPs/support groups/peers (Figure 2). The perceived appropriate time for discussion varied widely, although 18.9% thought this should occur when initiated by people with NF (Figure 3). Preferred information delivery methods included: discussion with HPs face-to-face (66.2%) or via telehealth (44.1%); written information before appointments (41.2%) or online (36.8%).

Conclusions: Information and support for adults with NF navigating reproductive decision-making is an important, yet unmet need. These data highlight the importance of addressing these issues in clinic, with transition nominated as the preferred time. Based on preferences for receiving information, a targeted brochure and website is currently underway.

Figure 1: How important it is to have more information on the following topics (N=68)

Figure 2: Top 4 of 12 preferences for how information is received (N=68)

Figure 3: When reproductive options should be discussed by health professionals (N=68)

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KEYNOTE #3: UPDATE ON THE DIAGNOSIS AND MANAGEMENT OF CHRONIC NEUROPATHIC PAIN

Sunday, 23 June, 9:00 – 10:00

Andrew Rice, MD, *Imperial College London, UK*

This talk will take as its starting point the current International Association for the Study of Pain mechanistic definition of neuropathic pain: *"Pain directly caused by a lesion or disease of the somatosensory nervous system".*

After defining the condition and presenting some scene setting epidemiological data, Andrew Rice will describe a clinical algorithm which uses this definition as the basis of a step wise approach for increasing the diagnostic certainty (possible, probable, definite) of a case of neuropathic pain. This algorithm is structured to be compatible with the familiar sequential steps of a clinical consultation—history, physical examination and diagnostic tests. The use of symptom screening questionnaires will be discussed.

The speaker will then present the recent European Academy of Neurology, European Pain Federation and IASP Special Interest Group on Neuropathic Pain joint guidelines on neuropathic pain assessment. These were compiled using a comprehensive evidence appraisal process.

Moving on to clinical management the Andrew will discuss the evidence for benefit, harm and limitations of pharmacotherapy for pain neuropathic pain management. He will then give some examples of the novel drug targets being investigated for neuropathic pain and the opportunities for precision medicine.

Finally, Andrew will argue that the biopsychosocial model has been somewhat neglected in the field of neuropathic pain, in contrast to other areas of chronic pain management. Neuropathic pain management has perhaps excessively concentrated on biological/pharmacological aspects of the neuropathic pain syndrome whilst leaving the wider impacts of pain unaddressed. He will posit that that clinical outcomes might be further improved by a more holistic interdisciplinary approach which better addresses the mental, quality of life and social impacts of pain on an individual person. He will finish by arguing for matched as opposed to stratified models of care.

SCHWANNOMATOSIS

Session Co-Chairs: Masahiro Toda, MD, PhD, *Keio University, Japan;* Liyam Laraba, PhD, *University of Plymouth, UK;* Gareth Evans, MD, *University of Manchester, UK*

<u>Platform</u>: Characterization of the NF2-Associated Immunopeptidome

Sunday, 23 June, 10:55 – 11:10

Joseph Kissil, PhD, *Department of Molecular Oncology, The H. Lee Moffitt Cancer Center, US*

Purpose: To assess the utility of an immunotherapeutic approach in NF2-associated schwannomatosis.

Methods: To assess the utility of an immune-based therapeutic approach for NF2-associated schwannomas we characterized peptides associated with the major histocompatibility complex I (MHC-I), to identify antigens selectively expressed by *NF2*-deificent schwannoma cells. Towards this goal we utilized Schwannoma cell lines and primary tumor tissue from genetically engineered mouse models of NF2 and NF2 patients. The MHC-I associated peptide landscape was surveyed using affinity-based purification coupled with mass-spectroscopy analysis. Peptides were characterized for their ability to activate CD8+ T cells using a bone marrow derived dendritic cell (BMDC) vaccine approach followed by enzyme linked immune absorbent spot assay (ELISPOT). The prioritized peptides will be used to generate reagents to target these antigens, which will then be assessed their anti-tumor efficacy *in vivo*.

Results: Our preliminary characterization identified the repertoire of MHC-I associated peptides in a panel of NF2-deficient Schwann and schwannoma cells. Moreover, we identified peptides that are differentially presented in the context of *NF2* loss and are preserved across multiple *NF2*-deficient patient schwannoma cell lines and in mouse *Nf2*-deficient schwannoma cells. Significantly, select peptides were able to induce potent CD8+ T cell activation.

Conclusions: Our findings support the rationale and feasibility of our approach to assess an immune-based therapy approach in a genetically engineered model of NF2 and establish whether an immunotherapy-based approach should be evaluated as a therapeutic option for NF2 patients.

Additional Authors: Scott Troutman[1], Maria Rodriguez Gonzalez[1], Sepideh Mokhtari[2], Nam Tran[2] and Alex Jaeger[1]. Department of [1]Molecular Oncology and [2]Neuro-oncology, The H. Lee Moffit Cancer Center. Tampa, Florida, USA

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Platform: *LZTR1* and *SMARCB1* Mutations in Schwann Cells Induce Increased Pain Sensitivity Through Distinct Mechanisms

Sunday, 23 June, 11:10 – 11:25

Larry S. Sherman, PhD, *Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, US*

Purpose: Non-NF2 schwannomatosis patients typically present with intractable pain. A significant proportion of patients with schwannomatosis have mutations in the *SMARCB1* (also called *INI1, BAF47* and *SNF5*) and *LZTR1* genes. The purpose of this study was to determine how loss of SMARCB1 or LZTR1 from Schwann cells can influence pain sensitivity.

Methods: We generated mice with inducible, Schwann cell-targeted loss of either *SmarcB1* or *Lztr1* and characterized pain responses compared to age- and sex-matched control mice using a battery of pain assays. We generated conditioned media from these mice and from non-NF2 schwannomatosis patient-derived schwannomas with known *SMARCB1* or *LZTR1* mutations, and tested their effects on pain sensitivity *in vivo* and the expression and activity of pain mediators in sensory neurons *in vitro.* We also characterized the secretomes of mutant cells and tested the effects of agents that block elevated potential pain mediators.

Results: Both mouse lines demonstrate increased pain sensitivity. Dorsal root ganglion (DRG) neurons from mice with Schwann cell-targeted disruption of *Smarcb1* or *Lztr1* express elevated levels of TRPV1, a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin, and TRPA1, an ion channel that acts as a sensor for environmental irritants. Wild type DRG cells grown in *Smarcb1*- or Lztr1-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients expressed elevated levels of TRPV1 and TRPA1. Proteomic analysis demonstrated that the secretomes of *Smarcb1*-and *Lztr1*-mutant Schwann cells are distinct from each other and from wild type Schwann cells. These proteins are also elevated in human schwannomatosis schwannoma tissues. *Smarcb1* interacts with the promoters of these genes, including *IL6* and *CCL2,* and directly represses their transcription. How Lztr1 influences the Schwann cell secretome is unclear. Furthermore, agents that block at least some of these proteins can reverse the induction of TRPV1 in DRG cells treated with mutant Schwann cell conditioned media, alter calcium responses in neurons, and reduce pain responses to conditioned media in mice.

Conclusions: Collectively, these data indicate that loss of *Smarcb1* or *Lztr1* in Schwann cells leads to the increased transcription of factors that induce the expression of pain mediators in sensory neurons, and suggest a mechanism for schwannomatosis pain. Our studies further support blocking the activities of specific proteins secreted by mutant Schwann cells to treat Non-NF2 schwannomatosis pain.

Additional Authors: Fatima Banine[1], Steven Matsumoto[1], Kanon Yasuhara[1], Cristina Fernandez-Valle[2]. [1]Division of Neuroscience, Oregon National Primate Research Center, Oregon Health Science University, Beaverton, OR, USA and [2]Department of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA

<u>Platform</u>: NF2-Related Schwannomatosis: An Updated Genetic and Epidemiological Study

Sunday, 23 June, 11:25 – 11:40

Gareth Evans, MD, FRCP, *Department of Genomic Medicine, St Mary's Hospital, Manchester Academic Health Sciences Centre (MAHSC), Division of Evolution and Genomic Science, University of Manchester, Manchester, UK*

Background/Purpose: New diagnostic criteria for NF2-related schwannomatosis (NF2) have been published (Genet Med. 2022). An updated UK prevalence has been generated with an emphasis on the rate of *de novo* NF2 (50% rate is widely quoted).

Methods: The UK National NF2 database identifies patients meeting updated NF2 criteria from a highly ascertained population cared through England's highly specialized commissioned 4-centre NF2 service. Diagnostic prevalence was assessed on 01/02/2023.

	Popu-lation (millions)	NF2 patients living	Prevalence per 1000	1 in x	Hetero-zygotes	Familial	%familial of total living	*de novo*	Mosaic	%mosaic of *de novo*	Hetero-zygote, proven mosaic, or BVS	%familial
England	56.00	960	0.01714	58331	415	222	23.1	738	204	27.6	787	28.2
Wales	3.14	50	0.01594	62720	22	12	24.0	38	9	23.7	44	27.3
Scotland	5.45	51	0.00936	106863	21	6	11.8	37	14	37.8	43	14.0
N Ireland	1.90	21	0.01105	90476	14	5	23.8	16	3	18.8	21	23.8
Total	66.48	1082	0.01627	61445	472	245	22.6	831	230	27.7	895	27.4

Results: A total of 1082 living NF2 patients were identified on prevalence day (equivalent to 1 in 61,445). The table details the rates of *de novo,* familial and mosaic NF2. The proportion with inherited NF2 from an affected parent was only 23% in England. If those without a confirmed molecular diagnosis or bilateral vestibular schwannoma (BVS) are excluded, the rate of *de novo* NF2 remains high (72%).

Conclusion: This work confirms a far higher rate of *de novo* NF2 than previously reported, and highlights the benefits of maintaining patient databases for accurate counselling.

Additional Authors: Forde C,[1] Bowers NL,[1] Roberts N,[1] Burghel G,[1] King AT,[2] Lloyd SKW,[3] Rutherford SA,[2] Hammerbeck-Ward C,[2] Pathmanaban ON [2], Freeman SR,[3] Lavin T[4] Laitt R,[2#] Thomas O,[2#] Halliday D,[5] Afridi S,[6] Taylor A[7], Duff C,[8] Smith MJ[1]

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Targeting the Schwannoma-Neuron-Macrophage Crosstalk for the Treatment of Schwannomatosis and Associated Pain

Sunday, 23 June, 11:40 – 12:05

Lei Xu, MD, PhD, *Harvard Medical School, US*

In our study, we established patient-derived xenograft (PDX) models and dorsal root ganglia (DRG) imaging models. Using these novel models, we deciphered the cellular and molecular crosstalk between schwannoma (HMGB1)–neuron (CCL2)–macrophage (IL-6) in driving pain response, and identified EGF pathway as driver of SWN tumor progression. Our findings prompted the initiation of phase II clinical trial (NCT05684692) for pain relief in patients with SWN.

Sensitivity of Pathogenic Variant Detection in Schwannomatosis

Sunday, 23 June, 13:00 – 13:25

Miriam J. Smith, PhD, *University of Manchester, UK*

Purpose: Phenotypic overlap between *NF2*-related and non-*NF2*-related schwannomatosis (SWN) makes genetic diagnosis important for optimal clinical management in many cases. Current clinical genetic testing identifies approximately 90% of pathogenic variants in second generation *NF2*-related SWN, but is much lower for non-*NF2*-related- or sporadic SWN. We sought to determine the impact of additional genetic screening techniques and functional data on the detection and classification of heritable pathogenic variants that cause schwannomatosis.

Methods: We assessed the benefit of additional genetic testing methods such as karyotyping, long-read sequencing and RNA analysis, for detection and classification of pathogenic variants in people with schwannomatosis who had already undergone clinical testing using targeted next generation sequencing panels and multiplex ligation-dependent probe amplification.

Results: Use of additional genetic testing methods increased the sensitivity of detection of pathogenic variants in second-generation *NF2*-related SWN from 90% to 96%. In addition, RNA analysis and minigene assays provided evidence that helped to reclassify variants of uncertain significance in several cases of *NF2*-related and non-*NF2*-related SWN.

Conclusions: Karyotype analysis, genome sequencing and long-read sequencing are particularly valuable for the detection of large and/or complex structural variants, while RNA analysis is valuable for the accurate characterisation and classification of potential splice variants in non-canonical splice regions. However, there is still a high proportion of variants of uncertain significance identified in *LZTR1*-related SWN, making genetic diagnosis more challenging.

Platform: Effect of Bevacizumab on Intracranial Non-Target Meningiomas and Non-Vestibular Schwannomas in Persons with *NF2*-Related Schwannomatosis: An NF Clinical Trials Consortium Study (NF104)

Sunday, 23 June, 13:25 – 13:40

Scott Plotkin, MD, PhD, *Massachusetts General Hospital, Boston, MA, US*

Purpose: To assess the activity of bevacizumab in meningiomas and non-vestibular schwannomas in a patient cohort with *NF2*-related schwannomatosis (*NF2*-SWN).

Methods: The study cohort consisted of patients that were treated with bevacizumab for progressive NF2-associated vestibular schwannomas as part of a prospective NF Clinical Trials Consortium trial (NF104). Brain MRIs obtained at baseline and every 3 months until disease progression were analyzed retrospectively. Non-target intracranial meningiomas and non-vestibular schwannomas (NVS) were segmented on T1-weighted post-contrast sequences using a semi-automated segmentation software (3DQI) to calculate tumor volume. Radiographic response (RR) was defined as ≥20% decrease in volume compared to baseline, and progression was defined as a ≥20% increase in volume compared to baseline. RR rate was determined on a per-tumor (defined as proportion of tumors with RR) and per-patient (defined as proportion of patients with at least one tumor with RR) basis. Median progression-free survival (PFS) and PFS at 6, 12, and 24 months was determined for each tumor type.

Results: Forty-one meningiomas in 8 participants (4 women, median age 21.5 years) and 17 NVS in 7 participants (2 women, median age 21 years) were identified. Baseline tumor volumes are shown in Table 1. On a per-tumor basis, response rates were 15% for meningiomas and 24% for NVS (Figure 2). Only one NVS grew during treatment. On a per-patient basis, response rates were 38% for meningiomas and 57% for NVS. For meningiomas, median PFS, PFS-6, PFS-12, and PFS-24 were 14.2 months, 88%, 54%, and 22%, respectively (Figure 3). Median PFS, PFS-6, PFS-12, and PFS-24 were not reached in NVS (Figure 3).

	Meningiomas	Non-vestibular schwannomas
N	41	17
Baseline median tumor volume (IQR; cc)	0.95 (0.43-2.07)	2.08 (0.89-4.99)
Median % change from baseline at 6 months (IQR)	6% (-2%, 27%)	0% (-8%, 5%)
Median % change from baseline at 12 months (IQR)	16% (-2%, 30%)	0% (-26%, 12%)
Median % change from baseline at 24 months (IQR)	14% (4%, 27%)	0% (-29%, 11%)
Response rate (per-tumor)	15% (6/41)	24% (4/17)
Response rate (per-patient)	38% (3/8)	57% (4/7)

Table 1. Summary of growth characteristics of meningiomas and non-vestibular schwannomas.

Conclusions: A minority of non-target meningiomas and NVS met criteria for RR during treatment with bevacizumab. Median PFS was approximately one year for meningiomas and was not reached for NVS; this finding highlighting the heterogeneity of benefit for bevacizumab across multiple tumor types in *NF2*-SWN. Our data provide a reference standard for comparison of future novel therapeutics.

Full List of Authors: Vihang Nakhate, MD[1]; Ina Ly, MD[1], Alona Muzikansky[1], MA, Jaishri O. Blakeley, MD[2]; Jian L. Campian, MD, PhD[3]; D. Wade Clapp, MD[4]; Girish Dhall, MD[5]; Rakesh K. Jain, PhD[1]; Matthias A. Karajannis, MD, MS[6]; Roger Packer, MD[7]; James Tonsgard, MD[8]; Nicole J. Ullrich, MD, PhD[9]; Bruce Korf, MD, PhD[5]; Michael J. Fisher, MD[10]; Scott R. Plotkin, MD, PhD[1]
[1] Massachusetts General Hospital, Boston, MA; [2] Johns Hopkins University, Baltimore, MD; [3] Mayo Clinic, Rochester, MN; [4] Indiana University, Indianapolis, IN; [5] University of Alabama, Birmingham, AL; [6] Memorial Sloan Kettering Cancer Center, New York, NY; [7] Children's National Medical Center, Washington, DC; [8] University of Chicago, Chicago, IL; [9] Boston Children's Hospital, Boston, MA; [10] The Children's Hospital of Philadelphia, Philadelphia, PA.
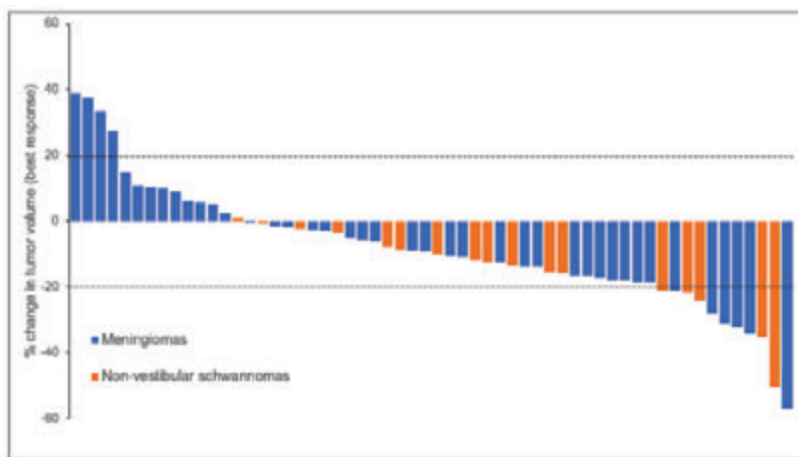

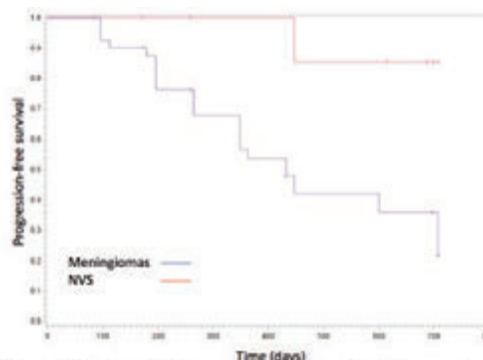Figure 2. Best radiographic response of meningiomas and non-vestibular schwannomas to bevacizumab (per-tumor analysis)

Figure 3. Kaplan-Meier curves for progression-free survival

Repurposing Anti-Retroviral Drugs to Treat NF2-Related Tumours

Sunday, 23 June, 14:20 – 14:45

Sylwia Ammoun, PhD, *Peninsula Medical School (Faculty of Health), University of Plymouth, UK*

We have identified a potential therapeutic target in NF2-related schwannomatosis (NF2) and sporadic Merlin-deficient schwannoma and meningioma tumors called Human Endogenous Retrovirus (HERV) type K. Genomic sequences of HERVs have been incorporated into human genome upon archaic germline retroviral infections that have been transmitted over the generations in Mendelian fashion and comprise 8% of the human genome. HERV type K (HERVK) is the most recently incorporated HERV which has capacity to transcribe and translate (1). HERVK proteins have been linked to tumorigenesis (2). Our recently published data demonstrate a strong overexpression of HERVK proteins in Merlin-negative patient-derived schwannoma and meningioma cells and tissues (3). We also found that HERVK proteins contribute to increased proliferation and survival of patient-derived schwannoma and meningioma cells *in vitro.* Ritonavir and Lopinavir are orally available retroviral protease inhibitors that are FDA approved for use in the treatment of Human immunodeficiency virus (HIV). Our translational work shows that they are effective in reducing patient-derived schwannoma and meningioma proliferation and survival *in vitro* by decreasing the expression of HERVK proteins and decreasing the activity of mitogenic ERK/cyclin D1 pathway. Both drugs were effective at concentrations that in clinical scenario in HIV patients and healthy volunteers caused only mild or no side effects. Ritonavir was more effective in meningioma compared to schwannoma and Lopinavir equally effective in both tumors at lower concentrations than Ritonavir. Lopinavir however requires to be administered in combination with Ritonavir.

Funded by Children's Tumour Foundation we are currently conducting a phase 0 (pharmacokinetic and pharmacodynamic) clinical trial in NF2 patients testing Ritonavir and Lopinavir. The aim of this study is to assess the delivery and molecular activity of orally administered Kaletra (100 mg Lopinavir and 25 mg Ritonavir) plus 200 mg Ritonavir/Norvir (twice daily) in peripheral subcutaneous schwannoma (CS) in NF2 patients. The primary objective is to determine the biological effect of the drugs at steady state concentration by investigation of molecular target inhibition in CS. The secondary objectives are to determine steady-state plasma and intra-tumoural (CS) concentration of the drugs, to assess a biomarker for treatment response by testing target inhibition in peripheral blood mononuclear cells (PBMC), to determine minimal biological effective dose, and to assess toxicity. This trial will allow faster insight into biological response to the drugs (Pharmacodynamics) and side effects before larger scale phase 2/3 trials.

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Platform: Characterization of a Novel *In Vitro* Model to Study Disease Mechanisms in *NF2*-Related Schwannomatosis and Testing Novel Therapeutics
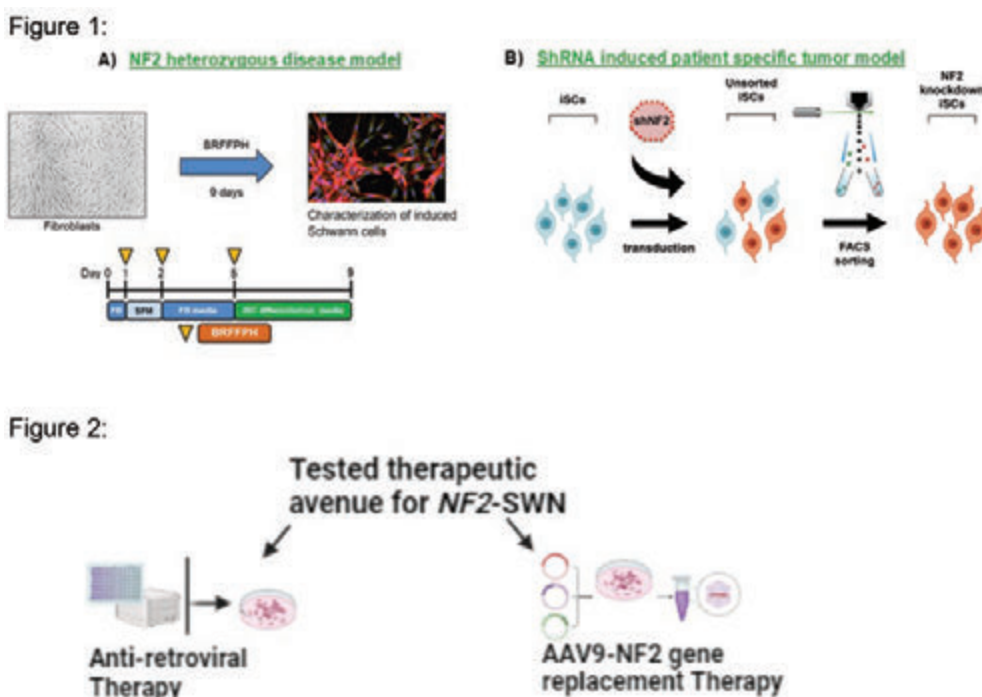
Sunday, 23 June, 14:45 – 15:00

Pipasha Biswas, MS, *The Ohio State University; Center for Gene Therapy, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, US*

Current treatment options for *NF2*-related Schwannomatosis (*NF2*-SWN) are very limited. Multiple surgeries and Radiation-like approaches are invasive, short-term solutions, and often cause further nerve damage. A major hurdle to developing novel treatments for *NF2*-SWN is the sparsity of reliable cellular and animal disease models that can represent underlying mechanisms for diverse NF2 patient mutations. To overcome this problem, we have developed a novel *in vitro* model system using patient skin fibroblasts which are readily available compared to fresh tumor samples. This disease model is robust and only requires treatment with Schwann cell-inducing factors for 9 days compared to other current models.

We successfully converted 4 healthy and 3 NF2 patient fibroblast cell lines using our novel *in-vitro* cellular model (Figure 1). Next, we verified the reduction in fibroblast (FB) marker expression alongside the expression of Schwann cell (SC) markers before testing with different therapies. Changes observed include overexpression of C-MYC and SOX2 (tumor pluripotent markers) that cause higher proliferation patterns compared to healthy control iSCs. As a next step, we have generated a patient-specific Schwannoma model (Figure 2) using LV-ShRNA. Moreover, we have tested seven different anti-retroviral drugs (ARD) as single or combination doses and AAV-NF2 gene therapy to reduce potential disease-relevant markers in heterozygous and patient-relevant shRNA tumor models.

We observed lower levels of NF2 expression in NF2 patient-derived iSCs compared to healthy/wild-type control cells, as revealed by immunofluorescence analysis. This finding was further evaluated by Western blot and qPCR analysis, which demonstrated reduced levels of NF2 protein and mRNA expression, respectively. Molecular characterization of healthy and NF2-mutant iSCs indicated reduced differentiation and maturation capacity in NF2 patient cell lines. This was evident through decreased expression of the Promyelinating SC marker Krox20 and mature myelinating SC marker MBP. These observations suggest that NF2 patient cells may remain in a more stem cell-like state, potentially due to the upregulation of C-MYC and SOX2. Even though the iSCs still contained one healthy copy of the NF2 gene at that point. Moreover, our patient-specific NF2 knockdown cell lines showed more stem cell marker expression, cell proliferation, and abnormal cell morphology compared to heterozygous iSCs-phenotypes. We determined that an ARD is capable of ameliorating the identified defects in heterozygous and shRNA-induced models.

Together, these models allow us to study the impact of losing either one or both copies of the gene using the same source cells and testing novel therapeutics.

Figure 1:
A) NF2 heterozygous disease model
B) ShRNA induced patient specific tumor model

Figure 2:
Tested therapeutic avenue for *NF2*-SWN
Anti-retroviral Therapy
AAV9-NF2 gene replacement Therapy

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BASIC / PRECLINICAL PLATFORM SESSION

Session Co-Chairs: Sheila Mansouri, PhD, *University Health Network, Canada;* Harish Vasudevan, MD, PhD, *University of California, San Francisco, US*

Platform: Alternatively Activated Macrophages are Associated with Faster Growth Rate in Vestibular Schwannoma

Sunday, 23 June, 15:00 – 15:15

Grace E. Gregory, BSc, *The University of Manchester, UK*

Background: Vestibular schwannoma (VS) are brain tumours that develop on the vestibulocochlear nerve causing sensorineural hearing loss, tinnitus, and imbalance. Previous studies comparing slow and fast-growing VS have yet to establish differences in their immune microenvironments with high dimensional imaging at the single cell level which will aid in assessing the viability of targetted immune therapies in these tumours.

Methods: This study provides the first use of high dimensional Hyperion Imaging Mass Cytometry and subsequent single cell analysis (Zanotelli and Bodenmiller) to determine the single cell counts per mm^2, neighbourhoods of 20 nearest cells (determined over 250 iterations, Schurch *et al.* 2020), and cell-cell spatial interactions (Weeratunga *et al.* 2023) between specific tumour and immune cell populations within 4 slow and 5 fast growing VS (volume change/year < 20% or ≥ 20%, respectively).

Results: Faster growing VS displayed an enriched expression of myeloid and exhausted T-cell markers and were more abundant in myeloid cells than slower growing VS, specifically in alternatively activated tumour associated macrophages (TAM, Fig 1). Neighbourhoods of nearest cells were used to determine that proliferative neighbourhoods within faster growing VS were composed of a significantly increased proportion of alternatively activated TAMs compared to slower growing VS (Fig 2). Alternatively activated TAMs were also found to be the most abundant proliferative cell type in VS, with a significantly higher abundance noted in faster growing VS (Fig 3). Finally, by assessing cell type associations it was identified that alternatively activated TAMs were sequestered by classically activated classically activated TAMs in slow but not in fast-growing VS.

Conclusions: Taken together, these data suggest that alternatively activated TAMs promote VS growth in proliferative neighbourhoods, while also actively proliferating themselves. The interactions between alternatively activated TAMs and neighbouring immune cell types indicates a role for targetted immune therapies in patients with VS, and to potentially to modulate the tumour immune microenvironment to promote classically activated TAM sequestration of alternatively activated TAMs.

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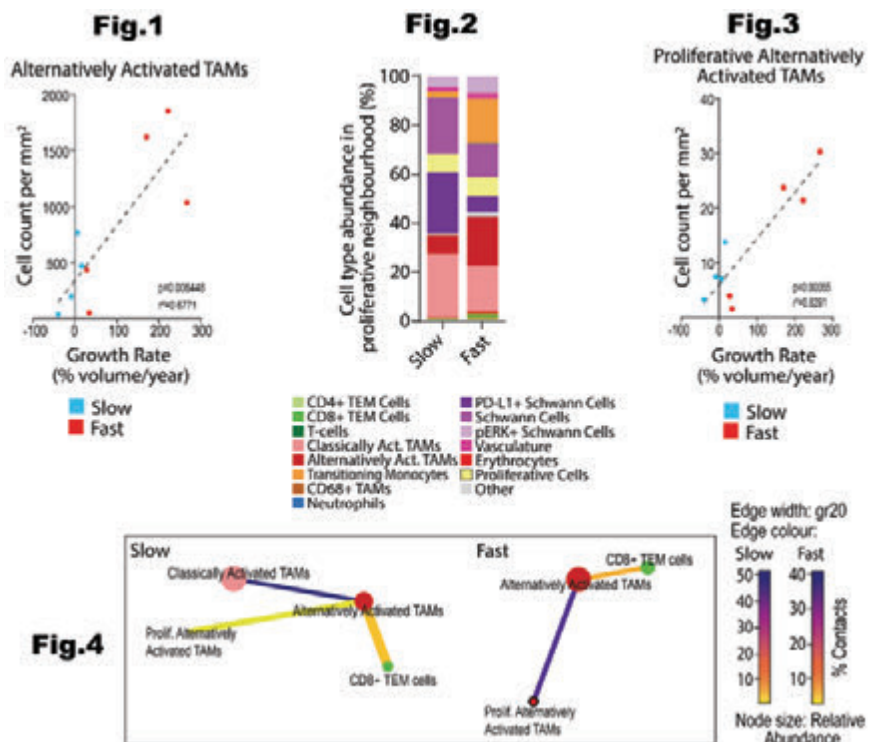
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Fig.1 Alternatively Activated TAMs

Fig.2

Fig.3 Proliferative Alternatively Activated TAMs

Fig.4

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Weeratunga P, Denney L, Bull JA, et al. Single cell spatial analysis reveals inflammatory foci of immature neutrophil and CD8 T cells in COVID-19 lungs. Nat Commun. 2023;14(1):7216. doi:10.1038/s41467-023-42421-0

Platform: Identification of Synthetic Lethality Targets Through Genome-Wide CRISPR Screen in *NF1*- and *NF1*/*SUZ12*-Deficient Human Schwann Cells and MPNST Cell Lines

Sunday, 23 June, 15:15 – 15:30

Julia Nikrad, PhD, *University of Minnesota, US*

Purpose: Synthetic lethality screens can reveal vulnerabilities in cells with cancer-specific mutations, expanding the range of therapeutic targets. Synthetic lethality occurs when simultaneous alterations in two or more genes lead to the loss of full cell viability, while the same alterations in either of the genes alone do not result in the loss of viability, a phenomenon with significant implications for cancer treatment. We hypothesized that *NF1*-null or *NF1*/*SUZ12*-null Schwann cells are selectively vulnerable to the loss of function of specific genes, that are not essential to isogenic *NF1*-proficient cells. To test this, we conducted genome-wide CRISPR/Cas9 knockout screens in *NF1*- and *NF1*/*SUZ12*-deficient human Schwann cells and malignant peripheral nerve sheath tumor (MPNST) cell lines.

Methods: Cas9-expressing *NF1* and *NF1*/*SUZ12* mutant Schwann cell lines were generated, alongside Cas9-expressing MPNST cell lines. We conducted high-throughput pooled CRISPR screens for synthetic lethality in these cells versus isogenic WT controls. Following negative selection, guide frequencies were analyzed to identify synthetic lethal candidate genes. Medium-throughput pipeline was developed for use in initial screening and validation of candidate synthetic lethal interactions. Additionally, drug response curves using available small molecule inhibitors are used to validate novel second-site synthetic lethal targets that may be druggable.

Results: Our CRISPR screens identified novel therapeutic targets that are synthetically lethal with *NF1* and/or *NF1*/*SUZ12* mutations. We found that some targets are unique to each genotype, while others are shared between different genotypes. Pathway analysis revealed synthetic lethal interactions involving ribosome biogenesis pathways, RNA processing (*NF1*-null), and DNA replication, cell cycle regulation (*NF1*/*SUZ12*-null). Thorough validation of targets involving both genetic manipulation and drug inhibition is ongoing.

Conclusion: CRISPR/Cas9 screens for synthetic lethality offer selective targeting of non-targetable cancer-associated mutations through inhibition of second-site targets. Our study identified potential synthetic lethal genes and pathways in *NF1*-deficient and *NF1*/*SUZ12*-deficient cells, with implications for selective cancer therapy.

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<u>Platform</u>: Targeting Granulocyte-Macrophage Colony Stimulating Factor Signaling in Plexiform Neurofibroma

Sunday, 23 June, 15:30 – 15:45

Jay Pundavela, PhD, *Cincinnati Children's Hospital Medical Center, US*

Plexiform neurofibromas (PNF) are peripheral nerve tumours that develop in patients with the genetic disorder Neurofibromatosis Type 1 (NF1). Apart from neoplastic Schwann cells, immune cells, including myeloid cells (macrophages and dendritic cells (cDC1 and cDC2)) and T lymphocytes, form an inflammatory environment to drive PNF formation. We have demonstrated that T cells are critical for PNF. It is unclear what factors mediate myeloid-lymphocyte interaction to foster the development of NF1 tumours.

The granulocyte-macrophage colony-stimulating factor (GM-CSF), encoded by the CSF2 gene, acts as a communication channel between infiltrating lymphocytes and myeloid cells in a state of inflammation. Studies have shown that Inhibition of GM-CSF signalling attenuates Nf1 mutant-associated blood disorder. Therefore, we posit that GM-CSF signalling plays a role in NF1-associated tumorigenesis.

Aim of the Study: To investigate the role of GM-CSF (CSF2) signalling in PNF development.

Methods: Generated neurofibroma mice (Nf1 f/f; DhhCre) with genetic loss of GM-CSF receptor subunits CSF2R-alpha (Csf2ra-/-; Nf1 f/f; DhhCre) or CSF2R beta-common (Csf2r-bc-/-; Nf1 f/f; DhhCre) to decipher the role of GM-CSF in PNF development.

Results: We identified by single-cell RNAseq analysis and flow cytometer of tumours from 7-month-old Nf1 f/f; DhhCre mice increased expression of GM-CSF receptors CSF2R-alpha and CSF2R beta-common in macrophage (CD11c-, CD11b+), cDC1 (CD11c+, Xcr1+) and cDC2 (CD11c+SIRPalpha+) dendritic cells. Genetic deletion of CSF2R-alpha and CSF2R beta-common moderately reduced the number and size of paraspinal tumours, associated with fewer Iba1+ macrophages, infiltration of CD3+ T-cells and CD11c+ dendritic cells (DC) observed by immunofluorescence. Loss of CSF2R-alpha or CSF2R beta-common receptors failed to improve survival and rescue the Remak Schwann cell-axon structure. The cytokine profile of CSF2R-alpha or CSF2R beta-common receptor knockout mice reveals altered levels of proinflammatory cytokines.

Conclusion: Our data show a partial role of GM-CSF in mediating myeloid-leukocyte interaction that fosters inflammation to drive neurofibroma development.

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<u>Platform</u>: A Platform for Rapid NF1 Patient-Derived Benign and Malignant Tumor Organoid Establishment and Screening

Sunday, 23 June, 15:45 – 16:00

Alice Soragni, PhD, *University of California, Los Angeles, CA, US*

Purpose: Neurofibromatosis Type 1 (NF1) is an autosomal dominant hereditary syndrome characterized by a wide range of clinical manifestations among affected individuals. This spectrum includes benign tumors such as cutaneous neurofibromas (cNF), plexiform neurofibromas (pNF), and optical pathway gliomas, as well as malignant forms like malignant peripheral nerve sheath tumors (MPNST). While the benign cNFs currently lack therapeutic options beyond surgical removal or desiccation—which may result in scarring or tumor regrowth if not entirely removed—malignant MPNSTs present as aggressive soft tissue sarcomas. These sarcomas have dismal 5-year survival rates and lack effective curative treatments. Given these challenges, there is a pressing need to expedite drug discovery efforts by developing tractable models that accurately represent the clinical, cellular, and molecular characteristics of NF1 tumors. To address this, we are focusing on the utilization of patient-derived tumor organoids. Organoids are promising tools for modeling tumor diversity and rapidly investigating patient-specific treatment response. Our overall goal is to accelerate the development of effective therapies and biomarkers of response for benign and malignant NF1 tumors.

Methods: We enrolled n=12 patients undergoing surgical cNF removal and n=6 undergoing primary or metastatic MPNST resection. We have performed detailed molecular and functional characterizations of both benign and malignant tumors. We performed high throughput screening with image-based and viability-based readouts.

Results: We determined best growth conditions to generate patient-derived organoids that recapitulate features of the tumor of origin. We have developed a robust pipeline to procure tissue from NF1 patients, expedite it to the lab and generate tractable organoid models from both benign (cNF) and malignant (MPNST) tumors. Tumor organoids are established in a geometry that facilitate high throughput drug screening, with results available within a week from surgery (Phan et al, 2019, Tebon et al, 2023). Screening results highlight both individual differences as well as shared vulnerabilities across tumor types (Al Shihabi et al, 2023; Nguyen et al, 2024).

Conclusion: Our study lays the groundwork for rapidly testing benign and malignant NF1 organoids established from a large cohort of NF1 patients, investigate their biology, and identify vulnerable pathways linked to specific genomic alterations.

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Platform: Towards an iPSC-Based MPNST Model: Impact of *NF1, CDKN2A* and *SUZ12* Inactivation in Cell Function and Identity

Sunday, 23 June, 16:00 – 16:15

Itziar Uriarte-Arrazola, MSc, *Hereditary Cancer Group, Germans Trias i Pujol Research Institute (IGTP), Spain*

Malignant Peripheral Nerve Sheath Tumor (MPNST) is a highly aggressive soft tissue sarcoma that appears in rare sporadic cases in the general population or with a much greater prevalence in the context of Neurofibromatosis type 1 (NF1). Mostly, MPNSTs in NF1 patients start developing with the complete inactivation of the *NF1, CDKN2A,* and *SUZ12/EED* (PRC2) tumor suppressor genes (TSGs), that characterizes the progression plexiform neurofibroma (PNF)-atypical neurofibroma (ANF)-MPNST. This study aims to elucidate the functional roles of these TSGs and the biological consequences of their loss in the progression towards an MPNST. For that, we employed genome editing to sequentially knockout the *CDKN2A* and *SUZ12* genes in an iPSC-based *NF1*(-/-) (1KO) neurofibromasphere model (Mazuelas et al. 2022). Newly edited TSG-KO lines were characterized and tested for sphere and tumor formation capacity.

The loss of *CDKN2A* in *NF1*(-/-) cells (2KO) did not alter proliferation capacity or ploidy stability. However, RNA-seq and gene enrichment analysis comparing 1KO vs 2KO cells, identified differences in nucleosome organization and potential ANF markers. 2KO cells were amenable to neurofibromasphere formation and after sciatic nerve engraftment, *NF1*(-/-) *CDKN2A* p14p16(-/-) but not *NF1*(-/-) *CDKN2A* p14(+/+) p16(-/-) cells generated neurofibromas *in vivo,* consistent with our genomic analysis of MPNSTs (Magallon et al. 2021). The presence of atypical histological features in these tumors is currently being analyzed.

The inactivation of *SUZ12* in 1KO and 2KO cell lines revealed an imposed biological order in TSG inactivation (*NF1-CDKN2A*-PRC2) since only iPSC-clones bearing the complete inactivation of *NF1, CDKN2A* p14 and p16 formed viable cell lines (3KO) after *SUZ12* inactivation. The most striking impact of PRC2 loss was the adoption of a mesenchymal-like identity already at neural crest (NC) stage, that was characterized by flow cytometry, immunocytochemistry, RNA-seq, methylome and ATAC-seq analyses. The 3KO cell lines exhibited loss of SOX10 and increase in SOX9 expression. Enrichment analysis of differentially expressed genes in 3KO vs 2KO cell lines depicted the downregulation of processes related to muscle differentiation and, remarkably, of glial differentiation. Consistently, we demonstrated experimentally *in vitro,* the lack of differentiation capacity towards Schwann cells of 3KO cell lines compared to 2KO cells. Contrarily, loss of PRC2 upregulated developmental processes, many related to neuron and axon development.

We are currently investigating how the new identity of 3KO cells is fixed epigenetically and the existing similarities and differences between 3KO NC cells with established MPNST cell lines.

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https://doi.org/10.1007/s00439-021-02296-x

Platform: Targeting Tumor Associated Macrophages to Fight MPNST

Sunday, 23 June, 16:15 – 16:30

Francesca Scantamburlo, MSc, *University of Padova, Italy*

Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) display significant immune cell infiltrates, including tumor associated macrophages (TAMs). These immune cells are emerging as important players in the growth of many cancers, especially in advanced stages. Yet, *the molecular crosstalk between MPNSTs and TAMs is lacking.* The aim of the project is to investigate the potential cross-talk between MPNSTs and macrophages infiltrating these peripheral nerve tumors with the final goal of identifying macrophage-specific metabolic actors that sustain neoplastic Schwann cell (SC) growth.

Methods: We use *in vitro* models of cell crosstalk mechanisms (co-cultures, conditioned media) employing MPNSTs and bone marrow derived macrophages (BMDMs). Through biochemical assays and transcriptome profiling we study the ability of MPNST cells to drive a TAM phenotype acquisition characterized by a specific metabolic state. Using Boyden chamber and Matrigel assays of tumor/endothelial cells co-cultured with macrophages we investigate the ability of MPNST-conditioned macrophages to tune MPNST invasion/migration and endothelial cell angiogenesis.

Results: We have found that conditioned media from MPNST cells drives up-regulation of a set of conventional M2-like TAM markers (mannose receptor CD206, metalloprotease 9 - MMP9, hypoxia inducible factor 1alpha - HIF1α, vascular endothelial growth factor - VEGF and macrophage galactose-type lectin 1 - MGL1), and of the metabolic enzymes glutamine synthetase (GLUL) and Arginase 1 (ARG1) in naive and M2 anti-inflammatory macrophages. Such MPNST-conditioned macrophages exert pro-angiogenic properties both *in vitro* and *in vivo* and sustain MPNST migration. By ablating the mitochondrial chaperone TRAP1, a key regulator in the metabolic rewiring of several cancer types, we find that these pro-tumoral functions of TAMs are impaired.

Conclusions: Our findings suggest a new crosstalk between MPNSTs and TAMs, in which macrophages exposed to conditioned media of tumor SCs acquire a pro-tumoral M2-like state. Such TAM phenotype enhances the malignant features of MPNSTs in a TRAP1-regulated way. The inter-cellular signaling unveiled by our study could be crucial in facilitating tumor maintenance, growth and invasion. Inhibition of TRAP1, a metabolic regulator previously identified as a target in MPNSTs, could be used as a therapeutic strategy to reverse this macrophage mis-education.

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Platform: Intratumoral Plasma Cells Are Required for a Durable Response to Anti-PDL1 Therapy in *De Novo* MPNSTs

Sunday, 23 June, 16:30 – 16:45

Joshua Lingo, BS, *University of Iowa, US*

Malignant peripheral nerve sheath tumors (MPNSTs) are deadly sarcomas that lack effective therapies. In immune competent mice bearing *de novo* MPNSTs, we reported that CDK4/6-MEK dual inhibition caused tumors to regress, delayed resistant tumor outgrowth, and improved survival relative to monotherapies. Drug-sensitive tumors that regressed contained plasma cells and increased CD8+ T cells. In other cancers, intratumoral plasma cells correlate with better patient response to immune checkpoint blockade (ICB). Impressively, CDK4/6-MEK inhibition sensitized MPNSTs to anti-PD-L1 ICB therapy with apparent cure (complete tumor ablation) in some mice. To test the importance of plasma cells in this setting, *de novo* MPNSTs were generated in *AID-/-; μS-/-* mice that selectively lack plasma cells. Tumor initiation rates were identical in wild-type and plasma cell deficient mice, indicating no role for plasma cells in MPNST formation. An absence of plasma cells also had no effect on the ability of dual CDK4/6-MEK inhibition to cause early regression and delayed outgrowth of *de novo* MPNSTs. By comparison, MPNSTs in plasma cell deficient mice were unresponsive to anti-PD-L1 monotherapy, unlike tumors in wild-type mice that exhibited delayed growth when compared to vehicle-treated tumors. Moreover, treatment with CDK4/6-MEK inhibitors no longer sensitized the tumors to anti-PD-L1 therapy. Indeed, the sustained response and curative potential of combination therapy targeting CDK4/6, MEK, and PD-L1 was completely lost in mice lacking plasma cells. Molecular and biological analyses of drug-treated MPNSTs from wild-type versus plasma cell deficient mice are ongoing. These results provide the first evidence in any tumor type that plasma cells are required for an effective response to ICB therapy, either alone or in combination with other anti-cancer agents. Such data highlight the potential value of intratumoral plasma cells in predicting patient response to immune-based therapies and underscore a need to better understand their role in tumor biology and immunotherapy.

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Platform: Persistence of Radial Glia Cells After Differentiation of NF2-Mutant Human Neuroepithelial Stem (NES) Cells

Monday, 24 June, 8:00 – 8:15

Jignesh Tailor, BMBCh, PhD, FRCSC, *Department of Neurological Surgery, Indiana University School of Medicine (IUSM), Indianapolis, Indiana, US*

Background: Ependymoma of the spinal cord (SP-EPN) occurs in up to 50% of patients with NF2-related schwannomatosis (NF2)[1,2]. These tumors are thought to arise from derailed *radial glia cells*[3,4]. To date, the only treatment option for clinically symptomatic late stage ependymoma is high-risk surgery. Treatments for *early-stage pre-neoplastic growths* do not currently exist. There is a <u>critical need</u> to define the molecular pathways in aberrant radial glia cells that lead to tumorigenesis and to identify new drug targets that halt the progression of these cells at an *early stage.* Interestingly, loss of heterozygosity of *NF2* gene is seen in spontaneous SP-EPN suggesting *NF2* gene alteration is a key driver of this disease[5-7]. However, *NF2*-dependent signaling disruption in RG and spinal ependymoma precursors is incompletely understood. Previously, we derived human neuroepithelial stem (NES) cell lines from embryonic hindbrain and induced pluripotent (iPS) cells[8,9]. These cells represent the human neural tube at 5-6 weeks gestation and can be differentiated towards spinal progenitors. They can be genetically modified to examine the molecular and cellular consequences of *NF2* mutations on progenitors of the developing brainstem/spinal cord.

Aim/Purpose: To determine the functional consequences of NF2-gene perturbation on differentiation of human neuroepithelial stem (NES) cells *in vitro* and *in vivo.*

Methods: CRISPR/Cas9 technology was used to generate NF2 knockouts in self-renewing human neuroepithelial cell lines (passage 20-30). Knockout of the NF2 gene was verified with western blot and sanger sequencing. The *in vitro* differentiation phenotype of NF2-mutant cells was compared with wild-type NES cells after growth factor removal. The *in vivo* phenotype was observed after transplantation in forebrain of 6-week-old NSG mice.

Results: We present preliminary data showing the generation of neuroepithelial stem (NES) cells with NF2 knockout following CRISPR-Cas9 editing of the NF2 gene. We observed persistence of pre-neoplastic radial glia-like cells after differentiation of *NF2* knockout neuroepithelial stem (NF2-/-) cells *in vitro* (figure 1) and the persistence of RG like cells *in vivo.* These cells display markers of of RG, such as expression of *SOX2, BLBP* and *GFAP.*

Conclusions: NF2-mutant NES cells (represented cells of 5-6 week human neural tube) fail to differentiate normally. The cells appear to persist as aberrant radial glial cells. This neuroepithelial cell-derived model of preneoplastic growth may be relevant to the study of molecular pathways underpinning ependymoma precursors in NF2-related schwannomatosis.

Figure 1:
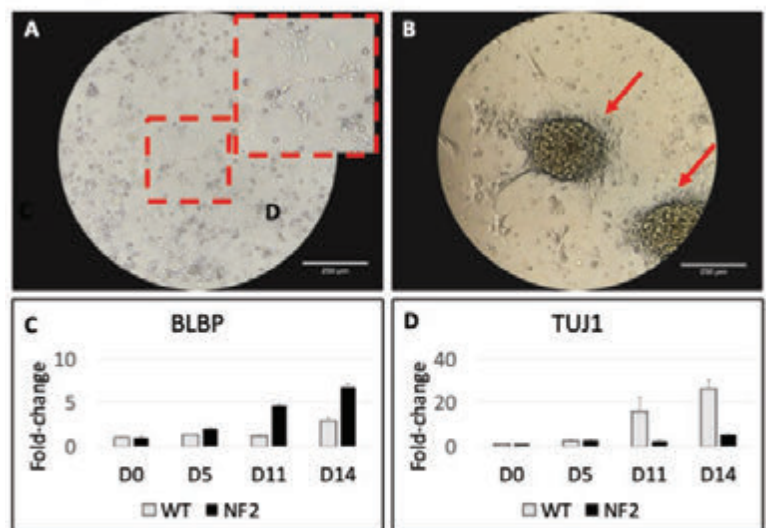
Figure 1. (A-B) *In vitro* differentiation of the WT and *NF2-/-* NES cells. WT cells remained in 2D monolayer and formed elaborate neuronal processes (A, inset). *NF2-/-* cells clustered into multiple spheres and continued to grow in spheres (B, red arrow). (C-D) *NF2-/-* fail to differentiate, as evidenced by lack of *TUJ1* expression after 14 days of differentiation by RT-PCR analysis (D). In contrast, *BLBP* expression increases in comparison to wild-type (C).

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<u>Platform</u>: RASopathies Influences on Neuroanatomical Variation in Children

Monday, 24 June, 8:15 – 8:30

Tamar Green, MD, *Department of Psychiatry & Behavioral Sciences, Stanford University, US*

Background: RASopathies are a group of disorders characterized by pathogenic mutations in the Ras-mitogen-activated protein kinase (Ras/MAPK) signaling pathway. Distinct pathogenic variants in genes encoding proteins in the Ras/MAPK pathway cause Noonan syndrome (NS) and neurofibromatosis type 1 (NF1), which are associated with increased risk for autism spectrum disorder (ASD) and attention deficit and hyperactivity disorder (ADHD).

Methods: This study examines the effect RASopathies (NS and NF1) has on human neuroanatomy, specifically on surface area (SA), cortical thickness (CT), and subcortical volumes. We compared structural T1-weighted images, using vertex-based analysis for cortical measures and Desikan ROI parcellation for subcortical volumes on children with RASopathies (n=91, mean age = 8.81, SD = 2.12) to sex- and age-matched TD (n=74, mean age=9.07, SD = 1.77).

Results: Compared to TD, RASopathies had convergent effects on SA and CT, exhibiting increased SA in the precentral gyrus, decreased SA in occipital regions, and thinner CT in the precentral gyrus. RASopathies exhibit divergent effects on subcortical volumes, with syndrome-specific influences from NS and NF1. Overall children with NS display decreased volumes in striatal and thalamic structures and children with NF1 display increased volumes in the hippocampus, amygdala, and thalamus.

Conclusions: Our study reveals the converging and diverging neuroanatomical effects of RASopathies on human neurodevelopment. The convergence of cortical effects on SA and CT indicates a shared influence of Ras/MAPK hyperactivation on the human brain. Therefore, considering these measures as objective outcome indicators for targeted treatments is imperative.

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Of note – this manuscript has been accepted for publication and a preprint is available here: https://pubmed.ncbi.nlm.nih.gov/38621478/

Platform: Trametinib in NF1-Related Plexiform Neurofibromas: Preliminary Results of One Year Follow-Up

Monday, 24 June, 8:30 – 8:45

D.C. Noordhoek, MD, *Department of Neurology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands*

Background: Plexiform neurofibromas (PN) are present in 50% of the patients with neurofibromatosis type 1 (NF1)[1]. They can cause pain, neurological deficit and cosmetic problems. Recent phase 2 clinical trials have shown promising results of treatment of inoperable PN with MEK inhibitors, especially in pediatric patients.[2,3] Our aim was to evaluate the effect of the MEK inhibitor trametinib in adult NF1 patients.

Methods: The TRAIN study is an open-label, single arm, phase 2 trial with trametinib 2 mg daily in 30 patients with NF1 and at least one symptomatic inoperable PN. The primary outcome measure is tumor volume decrease of the pre-defined target PN lesion(s) as measured by 3D volumetric analysis on 6-monthly MR imaging. A decrease of >20% in total tumor volume is considered treatment response. Secondary outcome measures are pain, safety and tolerability.

Results: Thirty patients have been enrolled between July 2020 and May 2023. The current median follow-up is 29 months (range 12-46 months). Average age at study registration was 44 years (range 19-67 years). Eleven patients (37%) discontinued study treatment within the first year: 8 patients due to adverse events, 2 because of disease progression and one patient because of a new contraindication for trametinib. Twenty-seven patients completed 12 months follow-up with MR imaging. The response rate was 48% within the first year of study treatment. Volume decrease ranged from: 21% to 57%. The remaining 52% of the patients had stable disease. The average Numeric Pain Rating Scale (NPRS) decreased from 5.2 at baseline to 3.5 at one year follow-up (p=0.027). The most common adverse events were acneiform rash, diarrhea, fatigue, asymptomatic anemia and hypoalbuminemia. Ten severe adverse events occurred of which 4 were related to study treatment (pneumonitis (n=1), rhabdomyolysis (n=1) and erysipelas requiring hospitalization (n=2)). All toxicity resolved after discontinuing treatment.

Conclusions: Approximately half of the adult NF1 patients with inoperable PNF showed a treatment response with trametinib within the first year. The average NPRS decreased significantly. However, adverse events are common and therefore more research is needed to select patients that will benefit the most from MEK inhibition.

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Platform: Patient-Reported Outcomes of Pain Severity and Pain Interference from ReNeu: Pivotal Phase 2b Trial of Mirdametinib in Adults and Children with Neurofibromatosis Type 1-Associated Plexiform Neurofibroma (NF1 PN)

Monday, 24 June, 8:45 – 9:00

Dusica Babovic-Vuksanovic, *Mayo Clinic, Rochester, MN, US*

Purpose: Patients with NF1 PN commonly experience pain, which negatively impacts their quality-of-life. We present patient and parent proxy-reported outcome analyses of pain severity and pain interference from ReNeu (NCT03962543), an open-label, multicenter, pivotal, phase 2b trial of mirdametinib (investigational, highly-selective, allosteric, CNS-penetrant, small-molecule MEK inhibitor) in adults and children (≥2y) with inoperable NF1 PN causing significant morbidities.

Methods: Fifty-eight adults and 56 children received mirdametinib ($2mg/m^2$ BID, 3-weeks-on/1-week-off 28d cycles). Change in pain severity (worst tumor pain; Numeric Rating Scale-11 [NRS-11]) and pain interference (Pain Interference Index [PII]) from baseline were assessed across the 24-cycle treatment phase; change from baseline at Cycle 13 was the prespecified secondary endpoint. NRS-11 scores (range: 0-10; higher scores=worse pain) were self-reported by patients aged ≥8 years. PII was both self-reported for patients ≥6 years and parent proxy-reported for patients 6-17 years (scores range from 0-6; higher scores=worse pain interference). Data were analyzed using mixed models for repeated measures. Clinically meaningful change (improvement) in NRS-11 was defined as a change from baseline ≤-1 point. Clinically meaningful change in PII was defined as a change from baseline <-0.8 points for adults and <-0.6 points for children (change from baseline <0.5*SD; SD calculated from respective baseline scale score data).

Results: Adults demonstrated improvement from baseline at Cycle 13 in NRS-11 and PII scores, with least-squares mean changes (SE) of −1.3 (0.2), $P<.001$ and −0.7 (0.2), $P<.001$, respectively. Children demonstrated improvement from baseline at Cycle 13 by self-report for NRS-11 (−0.8 [0.2]; $P=.003$) and PII (−0.5 [0.2]; $P=.017$) and by parent proxy-report for PII (−0.3 [0.1]; $P=.025$). Significant improvements in NRS-11 (Figures) and PII scores for adults and children began early (at Cycle 3 or 5) and were sustained through most timepoints during the treatment phase. Among adults with moderate-to-severe pain at baseline (NRS-11 score ≥4; n=29), early and sustained improvement in NRS-11 scores were reported ($P<.001$ all timepoints). From baseline to Cycle 13, clinically meaningful improvement in NRS-11 score was achieved by 79% (11/14) adults and 73% (8/11) children, and for PII was achieved by 58% (7/12) adults, 50% (5/10) children by self-report, and 60% (6/10) children by parent proxy-report (among patients who could have achieved a clinically meaningful change from baseline).

Conclusions: Adults and children with NF1 PN, including adults with moderate-to-severe pain, reported early, sustained, and clinically meaningful improvement in pain severity and pain interference over the course of mirdametinib treatment.
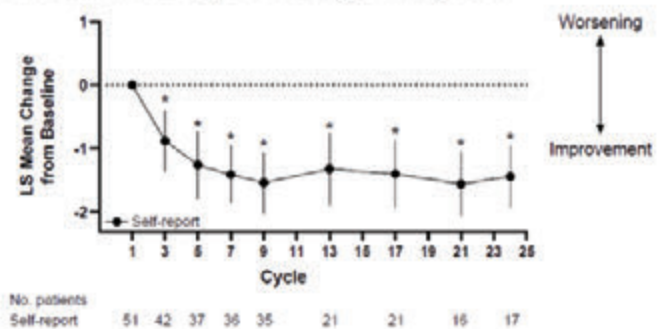
Figure 1: Least-Squares Mean Change From Baseline in Worst Tumor Pain (NRS-11) in Adults Estimated From MMRM Analysis Through all Cycles.

Vertical bars indicate 95% confidence intervals. *P<.05 for a statistically significant change from baseline. LSM, least-squares; MMRM, mixed model for repeated measures; NRS-11, Numeric Rating Scale-11.

Figure 2: Least-Squares Mean Change From Baseline in Worst Tumor Pain (NRS-11) in Children Estimated From MMRM Analysis Through all Cycles.
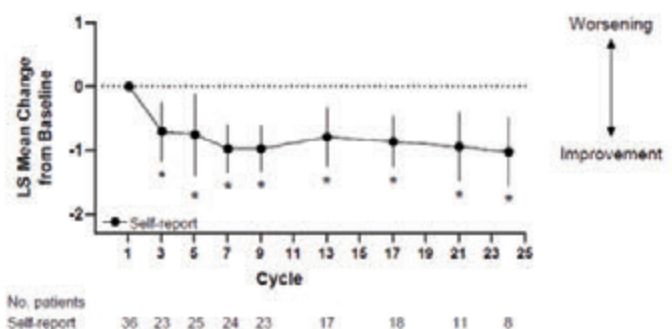
Vertical bars indicate 95% confidence intervals. *P<.05 for a statistically significant change from baseline. LS, least-squares; MMRM, mixed model for repeated measures; NRS-11, Numeric Rating Scale-11.

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KEYNOTE #4: REIMAGINING DRUGGABILITY USING CHEMOPROTEOMIC PLATFORMS

Monday, 24 June, 9:00 – 10:00

Daniel Nomura, PhD, *University of California, Berkeley, US*

The Nomura Research Group is focused on reimagining druggability using chemoproteomic platforms to develop transformative medicines. One of the greatest challenges that we face in discovering new disease therapies is that most proteins are considered "undruggable," in that most proteins do not possess known binding pockets or "ligandable hotspots" that small-molecules can bind to modulate protein function. Our research group addresses this challenge by advancing and applying chemoproteomic platforms to discover and pharmacologically target unique and novel ligandable hotspots for disease therapy. We currently have three major research directions. Our first major focus is on developing and applying chemoproteomics-enabled covalent ligand discovery approaches to rapidly discover small-molecule therapeutic leads that target unique and novel ligandable hotspots for undruggable protein targets and pathways. Our second research area focuses on using chemoproteomic platforms to expand the scope of targeted protein degradation technologies. Our third research area focuses on using chemoproteomics-enabled covalent ligand discovery platforms to develop new induced proximity-based therapeutic modalities. Collectively, our lab is focused on developing next-generation transformative medicines through pioneering innovative chemical technologies to overcome challenges in drug discovery.

NOVEL THERAPEUTICS

Session Co-Chairs: Amedeo Azizi, MD, *Medizinische Universitaet Wien, Austria;* Wade Clapp, MD, *Indiana University, US;* Laura Fertitta, MD, *Henri Mondor Hospital, CERENEF, France*

Targeting Schwann Cell-Tumor Microenvironment Interactions in NF1

Monday, 24 June, 10:15 - 10:45

Lu Q. Le, MD, PhD, *University of Virginia School of Medicine, US*

Neurofibromas are hallmark tumors of Neurofibromatosis type 1 (NF1). The tumor microenvironment (TME) and its interaction with *NF1*-mutant Schwann cells is known to play an important role in neurofibromagenesis. Part of the neurofibroma TME is a collagen-rich extracellular matrix (ECM) that makes up a large portion of the tumor mass. Identifying which cell types make the ECM and how the ECM interacts with Schwann cells to promote neurofibroma development is essential in preventing tumor growth. We investigated ECM enrichment during plexiform neurofibroma (pNF) development and identified TGF-β1 produced by the infiltrating immune cells as playing a key role in ECM dynamics. Indeed, TGF-β1 overexpression in the tumor microenvironment promoted pNF progression in vivo. Thus, targeting the TGFβ signaling pathway can hinder Schwann cell-tumor microenvironment interactions to restrain neurofibroma growth/ development. While plexiform neurofibromas are immune-rich tumors, when they transform to MPNST, they become "cold" tumors. We developed a strategy to harness the innate immune system, using cGAS-STING pathway activation, to turn MPNSTs into T cell-inflamed "hot" tumors, thus making them amenable to Immune Checkpoint Blockade, reducing MPNST growth via increased apoptosis. This strategy offers a potential novel treatment regimen for these malignant tumors in NF1.

<u>Platform</u>: Proteomics Reveals Potential Therapeutic Targets for Malignant Peripheral Nerve Sheath Tumors Based on Chr8q Status

Monday, 24 June, 10:45 – 11:00

Belinda B. Garana, PhD, *Pacific Northwest National Laboratory, US*

Purpose: Chromosome 8q (Chr8q) amplification in malignant peripheral nerve sheath tumors (MPNST) is associated with high-grade transformation in MPNST,[1] which has a five-year survival rate of only 20-50%.[2] Toward this end, this study characterizes the molecular changes that occur upon Chr8q amplifications to identify targeted treatment strategies.

Methods: We collected whole exome sequencing (WES), RNA-Sequencing (RNA-seq), global proteomic, and phospho-proteomic measurements from six MPNST patient-derived xenografts (PDX), with a varying degree of Chr8q amplification.[3] We ranked transcript, protein and phospho-site measurements by their correlation with Chr8q copy number as determined by the WES for the same sample, and then used pathway enrichment tools to identify over-represented pathways from the Human Molecular Signatures Database and kinase-substrate annotations from PhosphoSitePlus. We also used these correlation estimates to perform drug mechanism enrichment analysis, which enabled the prioritization of drug mechanisms of action that may be selectively toxic to distinct MPNST clone populations.

Results: Global proteomics analysis suggests decreased ARF signaling and increased expression of MYCBP and BCL2L2-PABPN1 in Chr8q-amplified samples. Phospho-proteomics analysis revealed increased activity of ATM kinase, which is also involved in the ARF-p53 circuit regulating tumor development.[4] Lastly, global proteomics suggested that PLK inhibitors may be more toxic to Chr8q-amplified MPNST PDX whereas MDM inhibitors may be more toxic to non-amplified clones.

Conclusions: By integrating WES, RNA-Seq, proteomics, and phospho-proteomics changes that occur upon MPNST progression, we identified potential roles of PLK, BCL2, and ATM in Chr8q-amplified MPNST as well as MDM inhibitors for non-amplified clones. This study suggests that combining PLK inhibitors with MDM inhibitors could enable effective whole-tumor treatment, which can be difficult to achieve with a single drug due to tumor heterogeneity.

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[3]University of Minnesota
[4]Johns Hopkins University

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Platform: Personalized MPNST Pre-Clinical Testing Using PDOX: Providing Treatment Possibilities to a Molecular Tumor Board

Monday, 24 June, 11:00 – 11:15

Sara Ortega Bertran, Biologist, Master in Genetics and Genomics, PhD Student, *Catalan Institute of Oncology (ICO-IDIBELL), Spain*

Malignant peripheral nerve sheath tumors (MPNST) are aggressive, locally invasive soft tissue sarcomas with a poor prognosis, depending mainly on the stage of the disease. Responding to the necessity of identifying new therapeutic possibilities, our laboratory has established a pre-clinical therapeutic platform comprising 25 patient-derived orthotopic xenograft (PDOX) MPNST mouse models. This platform serves three purposes: the evaluation of novel treatments, precision oncology strategies, and personalized treatments. The later allows exploring potential alternative therapies in case conventional treatment fails (Figure 1).

Our laboratory conducted a total of four cases of personalized treatment with the aim of informing a Molecular Tumor Board of additional treatment possibilities. Here we focus on the two pediatric cases performed in the last year. One involves a classic MPNST from an NF1 patient, bearing the inactivation of *NF1, CDKN2A,* and *PRC2.* Guided by the inactivation of these 3 tumor suppressors (TSGs) we conducted an *in vivo* precision oncology strategy using this patient MPNST PDOX, testing four co-treatments combining MEK, CDK, and BET inhibitors. We observed a decrease in tumor growth rate compared to vehicle in all treatments, especially in the combination of MEK inhibitors + BET inhibitor (Figure 2). None of the tested treatments elicited toxicity to the mice. The second case involved a sporadic case of MPNST with mutations in the *NF1,* PRC2, *Rb,* and *TP53* genes. Since this MPNST was not bearing the TSG inactivation signature borne by classic MPNSTs, we set up a mixed strategy consisting in the combination of a MEK inhibitor (Selumetinib) with various inhibitors related to the identified mutations (BETi, Wee1i, CDKi) or not (HDACi, and microtubule depolymerization inhibitor). We tested a total of five co-treatments, two of them generating a clear shrinkage of the PDOX. We observed an almost 60% decrease in tumor volume when combining Selumetinib with I-BET151 (BETi) (Figure 3), not producing a toxic effect in mice. The combination of Selumetinib + Docetaxel (inhibitor of microtubule depolymerization) also reduced the tumor volume by almost 45% but elicited a significant toxicity to treated mice. Currently the patient is receiving BET and MEK inhibitors in a compassionate-use basis, and its tolerability and effect is being monitored.

Figure 1:
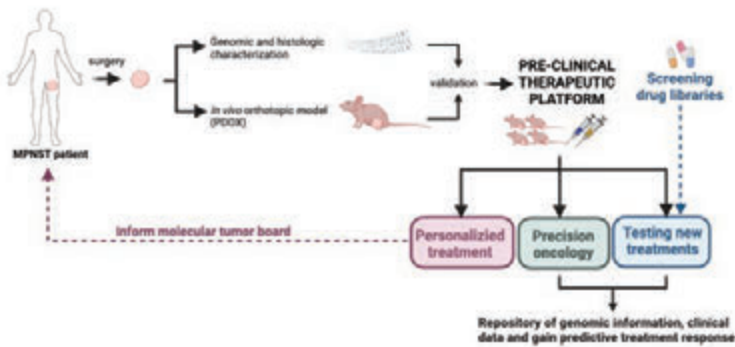
These results underscore the advantages of a pre-clinical therapeutic platform to provide a Molecular Tumor Board, additional treatment possibilities for their MPNST patients. In addition, our results emphasize the MEK-BET inhibitor combination as a promising treatment for MPNST patients.

Figure 1: Scheme of the purposes of the pre-clinical therapeutic platform.

Figure 2: Plot representing the increased tumor volume during the treatment in the *in vivo* testing of the four selected combinations in NF1-associated PDOX-MPNST mouse model.
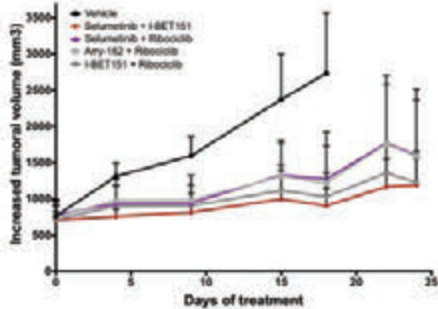
Figure 3: Plot representing the increased tumor volume during the treatment in the *in vivo* testing of the five selected combinations in sporadic PDOX-MPNST mouse model.

Figure 2:
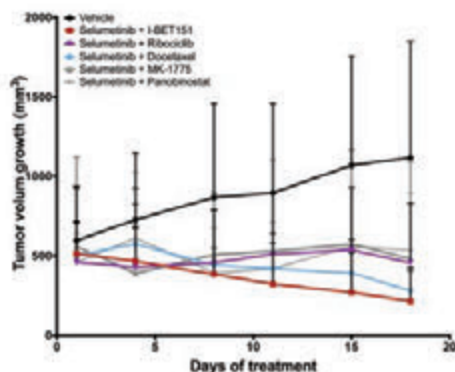
Full List of Authors: Sara Ortega-Bertran[1], Juana Fernández-Rodríguez[1], Miriam Magallón-Lorenz[2], Edgar Creus-Bachiller[1], Itziar Uriarte-Arrazola[2], Helena Mazuelas[2], Alicia Castaneda[3], Moira Garraus[3], Meritxell Carrió[2], Bernat Gel[2], Alberto Villanueva[1], Héctor Salvador[3], Eduard Serra[2], and Conxi Lázaro[1]

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HS has served on advisory boards for AstraZeneca/Alexion. Other authors report no conflicts of interest.

Figure 3:

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<u>Platform</u>: Combined Efficacy of SOS1 and KRAS^{multi} Inhibitors in Malignant Peripheral Nerve Sheath Tumors

Monday, 24 June, 11:15 – 11:30

Özlem Yüce Petronczki, PhD, *Böhringer Ingelheim RCV GmbH & Co, Vienna, Austria*

Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are a type of highly invasive and aggressive soft tissue sarcomas that originates from Schwann cells and is a primary cause of mortality in patients with Neurofibromatosis Type 1 (NF1). NF1 is a genetic condition caused by mutations in NF1 gene, which leads to elevated RAS signaling. Therefore, individuals with NF1 are prone to the development of neurofibromas which are typically benign and called Plexiform Neurofibromas (PNFs). However, they can transform into MPNSTs. The exact mechanisms that cause this transformation are not entirely known. With limited effective targeted therapies available, surgical removal remains the primary treatment option. However, due to tumor size and location, surgery may not always be feasible.

We investigate the therapeutic capabilities of two new, potent, and orally bioavailable small molecule inhibitors targeting SOS1 (BI 1701963) or KRAS (KRAS$_{multi}$ inhibitor BI 3706674), as treatment options for MPNSTs. The KRAS$_{multi}$ inhibitor BI 3706674 forms a non-covalent bond with KRAS in its GDP-bound, inactive state, thereby interfering with KRAS signaling. The SOS1i BI 1701963 blocks the interaction of KRAS and its exchange factor SOS1. Therefore, we hypothesize that both inhibitors will prove effective in NF1 models by reducing activate KRAS levels.

In this study, we carried out a range of *in vitro* and *in vivo* tests to assess the effectiveness of the inhibitors BI 1701963 and BI 3706674, both individually and in combination. The studies were done in comparison to the MEK inhibitor trametinib. The *in vitro* results showed a synergistic inhibitory effect on cell proliferation when BI 1701963 and BI 3706674 inhibitors were combined under both in 2D and 3D growth conditions. This combination led to suppression of the RAS pathway, specifically the MAPK and PI3K signaling cascade, and to an increase in apoptosis. The *in vivo* efficacy of either BI 1701963 or BI 3706674 alone or in combination was evaluated in MPNST mouse models. When used in combination, we observed response ranging from significant tumor growth delays to tumor regressions in several cell line- or patient-derived models. In-depth pharmacodynamic studies are ongoing.

In summary, this study highlights the combined effect of SOS1 (BI 1701963) and KRAS (BI 3706674) inhibition in the treatment of MPNSTs. The combination therapy of these novel therapeutics could potentially represent a promising new treatment approach for patients suffering from MPNSTs.

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Platform: Vertical Inhibition of ERK Signaling is Effective in Preclinical Models of Malignant Peripheral Nerve Sheath Tumors

Monday, 24 June, 11:30 – 11:45

Jiawan Wang, PhD, *Division of Pediatric Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, and Department of Oncology and Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD, US*

Background: Treatment for patients with malignant peripheral nerve sheath tumor (MPNST) remains an urgent clinical need despite decades of efforts. The most recurrent genomic alterations underlying the pathogenesis of MPNST are loss of function of the RAS-GAP (GTPase-activating protein) neurofibromin (*NF1,* 90%), *CDKN2A* (60-80%), PRC2 (70-90%), and *TP53,* as well as gain of chromosome 8 (80%), yet molecular targeting of these alterations represents a unique challenge. We have reported the anti-tumor activity of multiple combinatorial strategies including co-targeting MEK+mTOR, MEK+MET, MEK+SHP2 and SHP2+CDK4/6 in traditional and patient-derived models of NF1-MPNST, and some of these approaches have promising translational potential for the treatment of this disease.

Purpose: We sought to explore the activity of clinically available agents and rational combinations in NF1-MPNST, using treatment regimens already in clinical development for several other types of cancer driven by hyperactive RAS-ERK signaling.

Methods: Traditional and patient-derived NF1-MPNST cell lines were used for the *in vitro* evaluation of ERK signaling and cell growth in response to loss/gain of function of specific driver genes or drug treatment, using active RAS pull down, immunoblotting, Incucyte real-time imaging, cell proliferation assay, colony formation assay, RNA sequencing, as well as genetic manipulation such as lentivirus-based shRNA-mediated constitutive knockdown and doxycycline-inducible gene expression cell system. Cell line and patient-derived xenograft models were assessed for *in vitro* and *in vivo* efficacy of single agents and their combination.

Results: Here we demonstrate that NF1 negatively regulates RAS/ERK signaling through C/BRAF, not ARAF, and further that the cell growth and ERK signaling of NF1-MPNST are more dependent on C/BRAF than ARAF. Genetic ablation or pharmacological inhibition of C/BRAF achieves combination benefit when combined with a MEK inhibitor through more potent suppression of ERK and cell cycle signaling, relative to single agents, in both traditional and patient-derived, as well as MEK inhibitor-resistant models of NF1-MPNST, *in vitro* and *in vivo*.

Conclusions: Combined inhibition of B/CRAF and MEK using clinically available agents represents a viable therapeutic approach in NF1-MPNST and can be readily translated into the clinic for patients with NF1-deficient tumors.

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Platform: Combined MEK and Histone Deacetylase Inhibition Exploits a Targetable Vulnerability in Polycomb Repressor Complex 2 Deficient Malignant Peripheral Nerve Sheath Tumors

Monday, 24 June, 11:45 – 12:00

Kyle B. Williams, PhD, *Department of Pediatrics, Masonic Cancer Center, University of Minnesota Twin Cities, US*

Malignant peripheral nerve sheath tumors (MPNST) are among the deadliest soft tissue sarcomas and a leading cause of mortality for people with neurofibromatosis type 1 (NF1). ~80% of MPNSTs harbor biallelic loss of function mutations in the polycomb repressor complex 2 (PRC2) genes SUZ12 or *EED*. PRC2 regulates chromatin accessibility by writing repressive trimethylation marks on histone H3 at lysine 27 (H3K27TriM). PRC2 mutations result in epigenetic homeostasis dysregulation and have been identified in many cancers, contributing to therapy resistance and immune evasion. We have identified this altered epigenetic state as a targetable vulnerability in PRC2-deficient MPNST.

Methods: Using CRISPR/Cas9, we created immortalized human Schwann cell lines lacking *NF1* or *NF1* and *SUZ12* (mimicking the genetics of the majority of MPNSTs). Using a targeted epigenetic drug library, we identified compounds showing selective lethality towards *NF1/SUZ12* double mutant cells. Here we describe the identification of these novel drugs, *in vivo* testing in models of MPNST, and design of an early phase clinical trial using combined HDAC and MEK inhibition.

Results: We identified drugs capable of further perturbing chromatin homeostasis, such as HDAC inhibitors (HDACi). Characterization studies showed *NF1/SUZ12* mutant Schwann cells recruit more HDACs to their chromatin, possibly to reduce global transcription and overcome the lack of PRC2 activity. When HDACi was tested in combination with MEK inhibition (MEKi) strong synergy was observed against *NF1/SUZ12* deficient human Schwann and MPNST cell lines. To elucidate the mechanisms driving this synergy, transcriptome and proteomic analyses of MPNST cells treated with MEK and HDAC inhibitors (alone and in combination) were conducted.

These clinically interesting candidates were tested as single agents and in combination using *in vivo* MPNST models. The MEKi/HDACi combination showed striking synergy across all models tested, including MPNST cell lines and patient derived xenografts. We observed durable responses in survival studies and the ability to dramatically shrink large established tumors was observed.

Conclusions: Our results implicate targeting of epigenetic homeostasis, in combination with MEKi, as a major vulnerability of MPNSTs deficient for PRC2 activity. Moreover, other RAS driven cancers harboring loss of PRC2 activity might respond to this treatment approach. These results have led us to design a phase 0 "window of opportunity" clinical trial for the treatment of PRC2 deficient MPNSTs with combination therapy using the MEK inhibitor mirdametinib and the HDAC inhibitor vorinostat. 4-5 patients, with histology confirmed H3K27TriM negative MPNST, will be enrolled on a single cycle of mirdametinib and vorinostat before moving on to standard of care. Research biopsy samples and imaging studies will be used to evaluate the pharmacodynamic activity of the drugs on target and monitor any possible effects on tumor size and proliferation. At the time of this submission, this trial has received "Study May Proceed" status from the FDA and we are awaiting IRB approval.

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<u>Platform</u>: MEK/SHP2 Inhibition Prevents Congenital Pseudarthrosis Of The Tibia Caused by NF1 Loss in Schwann Cells and Skeletal Stem/Progenitor Cells

Monday, 24 June, 13:25 – 13:40

Céline Colnot, PhD, *Univ Paris Est Creteil, INSERM, IMRB, Creteil, France*

Neurofibromatosis type 1 (NF1) is a genetic disorder caused by mutations in the *NF1* gene, encoding neurofibromin a negative regulator of RAS. NF1 is characterized by a wide range of symptoms, including tumors (neurofibromas), skin hyperpigmentation (café-au-lait macules) and skeletal manifestations. The most severe form of skeletal manifestations, congenital pseudarthrosis of the tibia (CPT), is marked by tibial bowing leading to spontaneous tibial fracture and fibrous non-union. While NF1 neurodermatological manifestations are known to arise from *NF1* biallelic inactivation in Schwann cells and melanocytes, the cellular origin of CPT remains unknown. Recently, analysis of the *Prss56-Nf1 KO* mice, where *Nf1* is inactivated in boundary cap derivatives revealed that boundary-cap cells, a transient population of neural-crest derivatives, is the population responsible for both plexiform/cutaneous neurofibromas and skin hyperpigmentation. These results raised the question of a possible common origin of all NF1 symptoms.

In our study, we unraveled the cellular origin and pathogenic mechanisms of CPT through analyses of bone samples from CPT patients and *Prss56-Nf1 KO* mice. We showed that CPT is associated with *NF1* biallelic inactivation in human periosteum, the tissue covering the outer surface of bones and crucial for bone repair. *NF1* biallelic inactivation was detected in skeletal stem/progenitor cells (SSPCs) and Schwann cells in pathological periosteum. In parallel, we described a pseudarthrosis phenotype in *Prss56-Nf1 KO* mice, resulting from *Nf1* loss in both SSPCs and SCs in the periosteum. *Nf1* KO SSPCs fail to undergo chondrogenic differentiation leading to fibrogenic differentiation. More strikingly, *Nf1* KO SCs are the main driver of fibrotic accumulation in CPT as they acquire a pro-fibrotic function and promote fibrotic fate of wild-type SSPCs via TGFβ. To target both *NF1*-deficient Schwann cells and SSPCs, we combined MEK and SHP2 inhibitors and showed that combined in vivo treatment prevents fibrous non-union in the Prss56-Nf1 KO mouse model. Overall, our study deciphers the mechanisms of CPT and shows that CPT is caused by boundary cap derived SCs and SSPCs. We demonstrated the efficacy of combining MEK and SHP2 inhibitors to counteract the pathogenic action of Nf1-deficient SCs and SSPCs, opening new promising therapeutic strategies for the treatment of CPT.

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Platform: A Randomized Double-Blind, Vehicle-Controlled, Phase 2b Study of NFX-179 (Nedometinib) Topical Gel for the Treatment of Cutaneous Neurofibromas in Neurofibromatosis Type 1

Monday, 24 June, 13:40 – 13:55

Kavita Sarin, MD, PhD, *Stanford University Medical Center, Department of Dermatology, US*

Background: Cutaneous neurofibromas (cNFs) are benign skin tumors driven by uninhibited activation of the Ras pathway and cause significant morbidity in persons with neurofibromatosis type 1 (NF1). Currently, there are no FDA-approved therapeutics for cNFs.

Purpose: This study evaluated the safety and efficacy of NFX-179 Topical Gel, a metabolically labile topical MEK inhibitor, in reducing cNF size.

Methods: A multicenter, randomized, double-blind, vehicle-controlled Phase 2b clinical trial of NFX-179 Gel was conducted across 24 centers in the United States. 199 participants were randomized to three arms: NFX-179 Gel 1.5%, NFX-179 Gel 0.5% or vehicle applied once daily to 10 target cNFs for six months. The primary efficacy endpoint was the percent of subjects with a $\geq$ 50% reduction in cNF volume in 5 or more of the 10 treated cNFs.

Results: Treatment with NFX-179 Gel resulted in dose-dependent responder rates of 44.2% in the NFX-179 Gel 1.5% arm, 34.6% in the NFX-179 Gel 0.5% arm, and 24.1% in the vehicle arm (Figure 1). NFX-179 Gel 1.5% achieved a statistically significant improvement over vehicle in reducing cNF volume ($p = 0.03$ chi-square test). Secondary endpoints, including the height reduction responder rate also achieved statistically significant improvement over vehicle (39.5% in the NFX-179 Gel 1.5% arm vs 10.3% in the vehicle arm, $p = 0.0005$, chi-square test, Figure 2). A Patient Global Impression of Change (PGI-C overall) 7- point scale was administered after 6 months of treatment. 49.0% of tumors were rated by subjects as "better" overall in the NFX-179 Gel 1.5% arm versus 21.1% of tumors in the vehicle arm ($p < 0.0001$, Figure 3). PGI-C responders, defined as subjects who reported PGI-C overall improvement and considered the improvement to be meaningful for five or more of the ten treated tumors, were also higher in the NFX-179 Gel 1.5% Arm versus vehicle (37.2% vs 19.0%, $p = 0.040$, chi-square test). The most frequent adverse events were cutaneous, including rash, erythema and dermatitis at the application site and were mostly mild to moderate. Mean plasma drug concentrations at steady state were $<$ 1 ng/mL and no drug-related serious AE were observed during the course of the study.

Conclusion: Topical treatment with NFX-179 Gel 1.5% achieved a statistically significant and meaningful reduction in cNF size with minimal systemic exposure. This is the first Phase 2b clinical trial to report positive results in the treatment of cNFs and supports the progression of the NFX-179 Gel program to Phase 3 development.

Figure 1

Figure 2

Figure 3

Patient scoring of "**overall change** from baseline" at 26 weeks per tumor
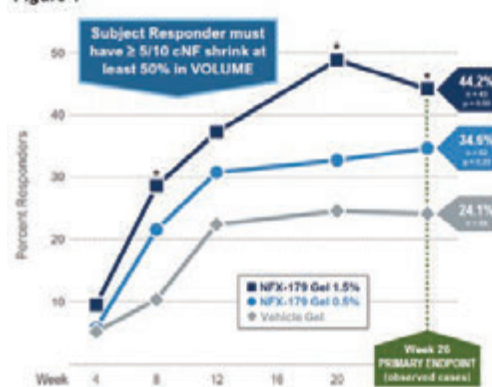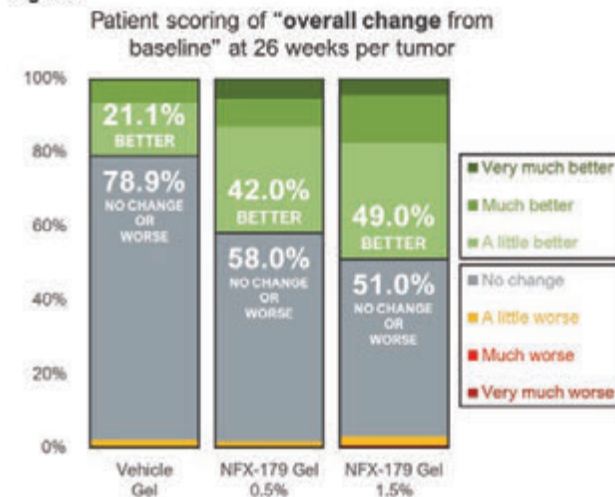
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<u>Platform</u>: Targeted Exon Skipping of *NF1* Exon 52 as a Mutation-Specific Therapeutic for Neurofibromatosis Type 1

Monday, 24 June, 14:05 – 14:20

Cameron Church, *University of Alabama at Birmingham, Birmingham AL, US*

Modification of pre-mRNA splicing using antisense oligonucleotides (ASOs) can be used to skip one or more exons carrying pathogenic DNA sequence variants. Our previously published data indicates *NF1* exon 52 is a good target for exon skipping; a cDNA screen indicates an NF1 isoform lacking exon 52 maintains both high neurofibromin expression and the ability to suppress Ras activity. To determine the essentiality of exon 52, we have developed a novel mouse model with deletion of exon 52 (DelE52). Mice null for *NF1* exon 52 show no gross abnormalities or histological lesions. We have established an aging cohort of DelE52 mice that are currently between 1-18 months and show no evidence of tumor development. This provides further proof-of-concept that inclusion of exon 52-encoded amino acids is not essential for neurofibromin function. To develop an exon skipping approach, we designed antisense phosphorodiamidate morpholino oligomers (PMOs) to skip exon 52. Our lead PMO is able to restore NF1 expression and Ras-suppression in a cell line with pathogenic variation in exon 52 with a low IC_{50} of 45nM. This oligo sequence is currently being cloned into a U7 snRNA AAV9 expression construct with 2A-linked GFP-luciferase bioreporter for easy biodistribution analysis. Further, we have created a novel humanized exon 52 mouse model containing pathogenic variant R2550X in exon 52 (hR2550X) for pre-clinical ASO testing. Preliminary testing of the hR2550X model in conjunction with a floxed allele and CAGG-Cre inducible NF1 loss with VIVO-PMOs shows RNA skipping efficiency in multiple tissues. Hence, exon skipping holds therapeutic potential for intragenic NF1 exon 52 mutations.

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<u>Platform</u>: Targeting the Tumor Microenvironment to Improve Immunotherapy

Monday, 24 June, 14:20 – 14:35

Lei Xu, MD, PhD, *Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, US*

Purpose: Reprogramming the suppressive immune microenvironment in tumors remains a major challenge in immunotherapy. Here, we explore the potential of anti-vascular endothelial growth factor (anti-VEGF) treatment to normalize the tumor microenvironment and enhance the effectiveness of immune checkpoint inhibitor (ICI) therapy.

Methods: Two mouse VS cell lines were implanted into the cerebellopontine angle (CPA) of syngeneic immune-competent mice. We evaluated the efficacy of combined ICI (anti- PD-L1) and anti-VEGF treatment on schwannoma tumor progression. Mechanistic investigations focused on the effects of combined anti-VEGF treatment on anti-PD-L1 drug delivery and the intratumoral infiltration and function of immune cells.

Results: We found that combined anti-VEGF treatment enhances anti-PD1 efficacy. We demonstrate that anti-VEGF treatment, via normalizing tumor vasculature and perfusion, increases anti-PD1 drug delivery and immune effector CD8 T cell and NK cell intratumoral infiltration. Furthermore, we found that anti-VEGF treatment upregulates NK cell activating receptor NKG2D expression, thereby enhancing NK cell anti-tumor cytotoxicity. Importantly, using patient VS tissues, we demonstrate that anti-VEGF treatment activates NK cells in human tumors.

Conclusions: These results suggest that anti-VEGF treatment effectively reprograms the tumor microenvironment from immune-suppressive to immune-stimulatory, unleashing the anti-tumor activities of CD8 T cells and NK cells. This study underscores the potential of combining ICI and anti-VEGF treatment as a promising approach in VS treatment.

Full List of Authors: Zhenzhen Yin, Limeng Wu, Jie Chen, Yao Sun, Simeng Lu, Scott R. Plotkin, and Long-Sheng Chang, Lei Xu

CLINICAL PLATFORM SESSION

Session Co-Chairs: Katrina Morris, MD PhD, *Concord Repatriation General Hospital, Australia;* Thorsten Rosenbaum, MD, PhD, *Sana Hospital Duisburg, Germany*

Platform: Clinical Features Predictive of Plexiform Neurofibroma Response to Mitogen-Activated Kinase Inhibitor

Monday, 24 June, 15:00 – 15:15

Chelsea Kotch, MD MSCE, *Children's Hospital of Philadelphia, Philadelphia, PA, US*

Background: MEK inhibitors (MEKi) are changing the approach to treatment for plexiform neurofibroma (PN), with multiple MEKi resulting in high rates of objective response. However, despite these successes, a subset of PN fail to demonstrate reductions in tumor volume and little is known about phenotypic or imaging features associated with treatment response.

Methods: We performed a retrospective cohort analysis integrating clinical trial demographic, imaging, and outcomes data (NCT01362803, NCT02407405, NCT02096471, NCT03231306, NCT03363217) to identify baseline clinical and imaging features associated with response of PN to MEKi. Participants were evaluable for response if they completed at least two cycles of therapy and had a follow-up magnetic resonance image (MRI) evaluation at first timepoint per clinical trial schedule of assessments. Partial response (PR) was defined as ≥20 percent reduction in tumor volume from baseline imaging at clinical trial enrollment.

Results: Of 232 eligible participants, adequate clinical trial imaging and outcomes data was available for 223 participants. 184 participants had imaging with a central response evaluation and represented the primary study cohort. In the primary cohort analysis, the median age at clinical trial enrollment was 15.2 years with a median tumor volume of 488 milliliters. The median time to PR was 8 cycles and 118 (64%) participants achieved a PR. Of the participants that achieved a PR, 29 (25%) achieved >30% reduction in volume from baseline. Thirty-five participants (19%) required a dose reduction prior to 6 cycles of therapy due to toxicity. In univariate analysis, younger age and lower body surface area (BSA) were significantly associated with PR while female sex and typical PN appearance on imaging (versus multi-nodular/nodular) approached significance. In multivariable analysis, only lower BSA was significantly associated with PR while typical PN appearance approached significance. In the multivariable analysis of participants less than 16 years of age treated per BSA-based dosing, lower BSA was the only feature significantly associated with PR. In a multivariable analysis restricted to patients >18 years of age, lower BSA demonstrated a trend in measure of association but was limited due to small sample size. In the expanded analysis of all 223 participants, lower BSA and typical PN appearance were significantly associated with PR.

Conclusion: Typical appearance of PN on MRI and lower BSA were associated with partial response to MEK inhibitors. To prospectively explore these findings, future studies of MEK inhibitor for PN should integrate analyses of clinical features and tumor pharmacokinetic-pharmacodynamic evaluations.

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<u>Platform</u>: Genetic Influence, Individual Genetic Profiling and Targetable Treatment in Patients with NF2-Associated Vestibular Schwannomas

Monday, 24 June, 15:15 – 15:30

Isabel Gugel, MD, PhD, *Department of Neurosurgery, Centre of Neurofibromatosis, Centre of Rare Disease, University Hospital Tübingen, Tübingen, Germany*

Objective: To investigate the relationship between genetic alterations (*NF2* mutation type, VEGF expression, etc.) and clinical parameters (growth rate, hearing status, tumor load) in primary operated vestibular schwannomas (VS) in patients with neurofibromatosis Type 2 (NF2) related schwannomatosis. The response of the therapy (surgery, bevacizumab) as well as possible novel genes and pathways that can be individually targeted will also be investigated.

Methods: 16 operated tumors in 8 NF2 patients with detailed long-term follow-up data (3D-volumes, pure-tone, and speech audiometry, auditory evoked potentials) and clinical parameters (e. g. tumor load) as well as their *NF2* mutation type were included. 3D volumetric data sets were used, and the growth rate was calculated by a linear regression model. After DNA isolation of paraffin-embedded samples, whole-exome sequencing (WES) was performed for all tumors. Signaling pathway analysis was completed to assess response to treatment and to identify potential targeted genes and/or pathways.

Results: The mean age at the time of diagnosis was 11 ± 5 (range 1-16) years and at the time of first surgery was 17 ± 4 (range 11-23) years. All tumors received (externally) off-label bevacizumab treatment before or after surgery with an initial dose of 5 mg/kg body weight every 2 weeks and adaptive dose reduction if radiological and clinical response was positive. Three tumors were operated on multiple times at different treatment intervals (with and without bevacizumab). A total of approximately 400 datasets of 3D volumetry and hearing parameter were collected and included. Patients with concomitant spinal ependymomas showed faster VS growth rates compared to patients with associated peripheral nerve schwannomas with lower VS growth rates. Six patients had truncating mutations (frameshift or nonsense) and two patients exhibited deletions of the *NF2 gene.*

Conclusions: The response of neoadjuvant or adjuvant bevacizumab treatment for the treatment of NF2-associated VS is heterogenous, worse in surgically reduced (small) tumors, and in young patients. Spinal tumor load appears to have a stronger negative influence on VS growth rate than the protective positive influence of peripheral tumor load. WES analysis in primary operated tumors seems to be an opportunity for individualized targeted treatment, which can be validated and simulated in vitro models.

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Platform: ReNeu: A Pivotal Phase 2b Trial of Mirdametinib in Children and Adults with Neurofibromatosis Type 1 (NF1)-Associated Symptomatic Inoperable Plexiform Neurofibroma (PN)

Monday, 24 June, 15:30 – 15:45

Christopher L. Moertel, MD, *University of Minnesota, Minneapolis, MN, US*

Purpose: ReNeu (NCT03962543) is an open-label, Phase 2b study evaluating the efficacy and safety of the oral, selective MEK1/2 inhibitor mirdametinib in adult and pediatric patients aged ≥ 2 years with symptomatic inoperable NF1 PN.

Methods: Mirdametinib was administered as a capsule or dispersible tablet (a formulation particularly for patients with difficulty swallowing; $2mg/m^2$ BID, max 4mg BID) without regard to food in 3wk on/1wk off 28-d cycles. The primary endpoint was confirmed objective response rate (ORR; percentage of patients with MRI-assessed $\geq 20\%$ reduction of target PN volume by blinded independent central review [BICR] within the 24-cycle treatment phase); minimum clinically relevant ORR (null) was defined as 23% for adults and 20% for children. Patients could continue treatment in a long-term follow-up (LTFU) phase. Duration of response (DoR), time to response (TTR), change from baseline in target PN volume, pain severity, pain interference, health-related quality of life (HRQoL), and safety were also assessed.

Results: All 114 patients (58 adult, 56 pediatric) received mirdametinib. As of the 20-Sept-2023 data cutoff (DCO), BICR-confirmed ORR was 41% (95% CI, 29-55; $P<0.001$ vs null) in adults and 52% (95% CI, 38-65; $P<0.001$ vs null) in pediatric patients. Two adults and 1 pediatric patient also had a confirmed response in the LTFU. Median (range) target PN volumetric best response from baseline was -41% (-90, 13) and -42% (-91, 48) in adult and pediatric patients, respectively. As of DCO, median treatment duration was 22 mo for both cohorts and median DoR was not reached. Median (range) TTR was 7.8 (4-19) mo in adults and 7.9 (4-19) mo in pediatric patients. Adult and pediatric patients had statistically significant improvements from baseline to Cycle 13 in key pain severity, pain interference, and HRQoL measures. Most frequent ($\geq 35\%$ patients) adverse events (AEs) were dermatitis acneiform, diarrhea, nausea, and vomiting in adults and diarrhea, dermatitis acneiform, and vomiting in pediatric patients. Grade ≥ 3 treatment-related AEs were experienced by 16% and 25% of adult and pediatric patients, respectively, and 22% and 9%, respectively, discontinued due to AEs.

Conclusions: In ReNeu, the largest multicenter NF1 PN trial reported to date, mirdametinib demonstrated significant clinical activity, significant improvements in pain symptoms and HRQoL, and manageable safety in adults and children. Together with a dispersible tablet formulation, these results underscore mirdametinib's potential to become an important new treatment option for NF1 PN patients across all ages.

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Platform: Demographic and Disease Predictors of Executive Functions in Children and Adolescents with Neurofibromatosis Type 1: Results from the Largest International Dataset

Monday, 24 June, 15:45 – 16:00

Xiaoli Zong, PhD, *Florida State University College of Medicine, US*

Purpose: This study aims to explore the age trends of executive functions (EF) in children and adolescents with neurofibromatosis type 1 (NF1) and how these age trends vary across demographic and NF1-related disease factors.

Methods: Using integrative data analysis, individual-level data of 1,294 children and adolescents with NF1 (ages 3-18 years) were combined from nine institutions (Table 1). Parent-rated EF was assessed with age-appropriate versions of Behavior Rating Inventory of Executive Function. Time-varying effect modeling (TVEM) was used to delineate the age trends of EF for the entire sample and within subgroups of child sex, parental education, NF1 heritability, and plexiform neurofibromas. TVEM is a nonparametric statistical technique that can flexibly estimate the associations between variables as continuous functions of time. This technique imposes no constraints on the shape (e.g., linear, quadric) of the associations and produces curvilinear estimates of the intercepts and slopes with 95% confidence intervals (CIs), and these estimates are typically summarized graphically. Significant deviations from the normative group are indicated by 95% CIs that do not include the norm mean of 50. Significant age or group differences are indicated by non-overlapping 95% CIs between specific ages or groups.

Results: Standard scores of inhibitory control, flexibility, emotional control, working memory, and planning/organization problems in children and adolescents with NF1 were generally higher than the norm means across ages 3-18. Working memory problems were clinically mildly elevated between ages 4-8 and 11-17 (Figure 1). These age patterns varied across parental education and NF1 heritability, but not child sex. Children with high (vs. low) parental education exhibited fewer EF problems in all five domains (see Figure 2 as an example) across early childhood to adolescence (from age 3 to ages 10-13). Children with sporadic (vs. familial) NF1 exhibited fewer problems in inhibitory control (at ages 5-12), flexibility (at ages 4-10; Figure 3), working memory, (at ages 3-13), and planning/organization (at ages 3-11) across early childhood to adolescence.

Conclusions: Using an innovative statistical approach, this study revealed the age trends of parent-reported EF between ages 3 to 18 in a large sample of children and adolescents with NF1. The findings demonstrated the vulnerability for EF difficulties in children with NF1 from childhood to adolescence, particularly in early adolescence. The results also highlighted heterogeneity within children with NF1 by showing variations in age trends of EF across demographic and disease-related factors. These findings can help establish the norms for EF development in children and adolescents with NF1 and contribute to the design of intervention and prevention programs tailored to different developmental stages and diverse demographic and disease-related characteristics.

Figure 1. Age Trends of Working Memory Problems
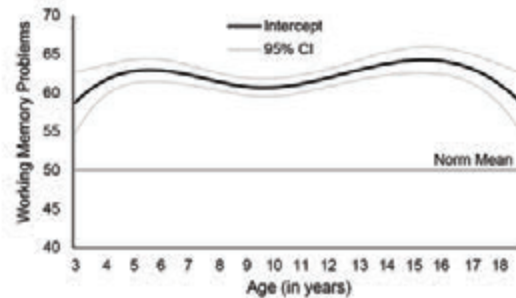
Note. Significant deviations from the normative group are indicated by 95% confidence intervals (CIs) that do not include the norm mean of 50. Significant age differences are indicated by non-overlapping 95% CIs between specific age points.

Figure 2. Age Trends of Inhibitory Control Problems for Different Parent Education Groups
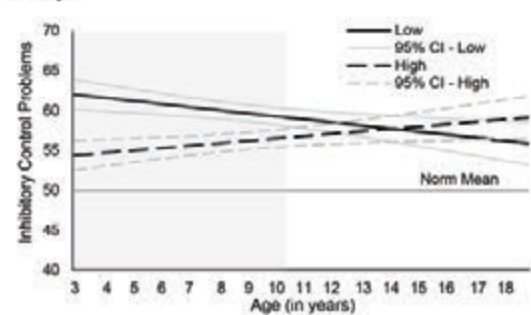
Note. Significant deviations from the normative group are indicated by 95% confidence intervals (CIs) that do not include the norm mean of 50. Significant age differences are indicated by non-overlapping 95% CIs between specific age points. Significant group differences at a specific age point are indicated by non-overlapping 95% CIs between groups. The shaded grey area represents the age range where significant group differences were observed.

Figure 3. Age Trends of Flexibility Problems for Different NF1 Heritability Groups
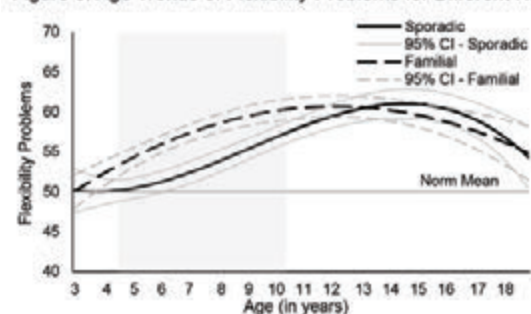
Note. Significant deviations from the normative group are indicated by 95% confidence intervals (CIs) that do not include the norm mean of 50. Significant age differences are indicated by non-overlapping 95% CIs between specific age points. Significant group differences at a specific age point are indicated by non-overlapping 95% CIs between groups. The shaded grey area represents the age range where significant group differences were observed.

Table 1. Characteristics of Data for Each Site and the Combined Sample

Characteristic	National Cancer Institute, USA	University of Wisconsin–Milwaukee, USA	Children's National Hospital, USA	Children's Hospital Colorado, USA	Murdoch Children's Research Institute, Australia	Children's Hospital at Westmead, Australia	Washington University in St. Louis, USA	University of California, Los Angeles, USA	University of Texas, MD Anderson Cancer Center, USA	Combined
Recruitment population	Convenience sample enrolled in a natural history study recruited nationally (clinicaltrial.gov number: NCT00924196)	Convenience sample from NF1 clinic	Convenience sample recruited through patients seen for clinical evaluation in a pre-clinical appointment survey from a pediatric neuropsychology clinic	Convenience sample recruited through patients seen for outpatient neuropsychological evaluation	Convenience sample recruited on a sequential basis from a multidisciplinary NF1 Clinic	Convenience sample recruited on a sequential basis from a neurogenetics clinic	Convenience sample from NF1 outpatient clinic	Referrals from NF1 clinics across Southern California and lab website	Clinical referrals through the NF1 clinic	NA
Executive function measurement	BRIEF-Preschool, BRIEF	BRIEF-Preschool, BRIEF, BRIEF2	BRIEF-Preschool, BRIEF, BRIEF2	BRIEF-Preschool, BRIEF, BRIEF2	BRIEF-Preschool, BRIEF, BRIEF2	BRIEF-Preschool, BRIEF	BRIEF2	BRIEF	BRIEF- Preschool, BRIEF, BRIEF2	NA
Total N.	246	231	227	163	102	92	85	82	66	1294
Inhibitory Control N.	246	231	226	163	101	92	85	82	66	1292
Flexibility N.	246	231	224	163	102	66	84	82	66	1264
Emotional control N.	246	231	225	163	102	92	85	82	66	1292
Working memory N.	246	231	227	163	102	92	84	82	66	1293
Planning/organization N.	245	231	223	163	102	92	84	82	66	1288
Age, median (range), y	11.30 (3-18)	5.83 (3-13)	9.55 (3-18)	11.46 (3-18)	8.67 (3-15)	8.21 (3-16)	9.67 (5-17)	12.79 (7-18)	10.30 (3-18)	9.42 (3-18)
Sex, N.	246	231	222	163	101	92	85	82	66	1288
Male, N. (%)	142 (57.7)	143 (61.9)	121 (54.5)	74 (45.4)	63 (62.4)	42 (45.7)	41 (48.2)	33 (40.2)	34 (51.5)	693 (53.8)
Female, N. (%)	104 (42.3)	88 (38.1)	101 (45.5)	89 (54.6)	38 (37.6)	50 (54.3)	44 (51.8)	49 (59.8)	32 (48.5)	595 (46.2)
Race, N.	246	231	107	160	0	0	0	82	61	887
White, N. (%)	191 (77.6)	184 (79.7)	54 (50.5)	138 (86.3)	0	0	0	66 (80.5)	41 (67.2)	674 (76.0)
Racial minorities, N. (%)	55 (22.4)	47 (20.3)	53 (49.5)	22 (13.8)	0	0	0	16 (19.5)	20 (32.8)	213 (24.0)[a]
Parent education, N.	236	231	9	148	99	57	43	77	0	900
Low,[b] N. (%)	80 (33.9)	53 (22.9)	1 (11.1)	66 (44.6)	42 (42.4)	17 (29.8)	18 (41.9)	11 (14.3)	0	288 (32.0)
High,[b] N. (%)	156 (66.1)	178 (77.1)	8 (88.9)	82 (55.4)	57 (57.6)	40 (70.2)	25 (58.1)	66 (85.7)	0	612 (68.0)
NF1 heritability, N.	226	228	0	159	102	78	84	0	60	937
Sporadic, N. (%)	142 (62.8)	145 (63.6)	0	105 (66.0)	64 (62.7)	44 (56.4)	43 (51.2)	0	42 (70.0)	585 (62.4)
Familial, N. (%)	84 (37.2)	83 (36.4)	0	54 (34.0)	38 (37.3)	34 (43.6)	41 (48.8)	0	18 (30.0)	352 (37.6)
Plexiform neurofibromas, N.	226	61	0	162	76	18	0	0	66	609
No, N. (%)	25 (11.1)	42 (68.9)	0	138 (85.2)	58 (76.3)	14 (77.8)	0	0	47 (71.2)	324 (53.2)
Yes, N. (%)	201 (88.9)[c]	19 (31.1)	0	24 (14.8)	18 (23.7)	4 (22.2)	0	0	19 (28.8)	285 (46.8)

Note. NA = not applicable. N. = valid number of participants. NF1 = neurofibromatosis type 1. BRIEF = Behavior Rating Inventory of Executive Function.
[a] Among racial minority groups, there were Black/African American (n = 71, 8.0%), Latino/x (n = 42, 4.7%), Asian (n = 23, 2.6%), Native American (Pacific Islander/American Indian) (n = 7, 0.8%), mixed/biracial (n = 20, 2.3%), and others (n = 50, 5.6%). [b] Low = high school or lower education. High = some college or higher education. [c] Most patients enrolled in this study had a plexiform neurofibroma due to the referral pattern for treatment studies at the National Cancer Institute, USA.

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Platform: Characteristics and Association of Pain and Quality of Life at Baseline and with Treatment in Children with Neurofibromatosis Type 1 and Plexiform Neurofibromas Enrolled on a Phase II Trial of Selumetinib

Monday, 24 June, 16:00 – 16:15

Pam Wolters, PhD, *Pediatric Oncology Branch (POB), National Cancer Institute (NCI), US*

Background: PNs can cause pain, functional impairment, disfigurement, and impaired quality of life (QOL). We prospectively assessed clinical benefit in children with NF1 and PNs using patient reported outcome (PRO) measures in a phase II trial of the MEK 1/2 inhibitor selumetinib (SPRINT, NCT01362803). The current analyses examined the characteristics and relation of pain and QOL at the baseline and follow-up evaluations.

Methods: Children 2-18 years old with NF1, inoperable PNs, and ≥1 clinically significant PN morbidity were enrolled (stratum 1). Children (≥8) and parents (of children ≥5) prospectively completed PRO measures every 2-4 cycles (1 cycle=28 days) to 1 year then annually to 4 years to assess child self-reported (SR) PN-related tumor pain intensity of a physician-selected tumor (Numeric Rating Scale [NRS-11]; 0=no pain to 10=worst pain), child SR and parent-reported (PR) pain interference in daily life (Pain Interference Index [PII]; 0=none to 6=completely), and QOL (PedsQL generic; 0-100, higher scores=better QOL). Parents rated PN visibility and NF severity as mild, moderate, or severe. Nonparametric tests were used, and alpha was set at 0.05.

Results: Fifty children (Mean*[M]* age=10.3 years, range=3-17; 60% male, 84% White; 90% not Hispanic or Latino/a/e; *M* years of parent education=14.8, range=9-19) enrolled at 4 sites. At baseline, 33 children ≥8 years (*M* age=12.4) completed SR PRO measures and all 50 caregivers completed PR measures. On the NRS-11, 70% of children rated their tumor pain intensity (*M*=3.0) as mild (31%), moderate (24%), or severe (15%). There was no difference in tumor pain intensity, pain interference, or total QOL by age, parent education, or biological sex. Parents' ratings of their children's QOL (*M*=60.8) were significantly poorer than their child's ratings (*M*=73.9; $p<0.001$), but there was no difference in their pain interference scores ($p=0.492$). Children taking pain medication regularly (n=21) had greater SR tumor pain intensity ($p=0.023$), PR pain interference ($p<0.001$), and poorer SR QOL ($p=0.045$), and children with an anxiety diagnosis (n=9) had higher PR pain interference and poorer PR QOL than those without. Pain and QOL scores did not differ by PN visibility ratings, but SR pain interference ($p=0.014$) and PR QOL ($p=0.004$) were significantly worse between NF1 severity groups (severe vs. mild). Greater SR tumor pain intensity (all $p<0.05$) and pain interference (all $p<0.01$) were associated with poorer SR physical, social, and emotional function and total QOL. With selumetinib, SR tumor pain intensity, and SR/PR pain interference and total QOL scores improved significantly from baseline to follow-up evaluations. At pre-cycle 13, change in SR tumor pain intensity was significantly associated with change in school function ($p<0.01$), and SR/PR pain interference was significantly associated with change in physical and school function and total QOL (SR all $p<0.05$; PR all $p<0.01$).

Conclusions: Child and parent baseline PRO evaluations suggest that children with NF1 and symptomatic PNs have significant pain, despite taking pain medication, that affects their QOL. Treatment with selumetinib is associated with less pain and improved QOL, with changes in pain related to changes in some QOL domains. Given the relation among pain, anxiety, and QOL, helping children to manage their pain and cope with anxiety through medical and psychological interventions also may improve their QOL.

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<u>Platform</u>: Non-Invasive High Intensity Focused Ultrasound (HIFU) Treatment of Cutaneous Neurofibromas

Monday, 24 June, 16:15 – 16:30

Sirkku Peltonen, MD, PhD, *University of Helsinki and Helsinki University Hospital, Finland*

Purpose: Improve the safety and efficacy of high intensity focused ultrasound (HIFU) for treatment of cutaneous neurofibromas (cNFs).

Methods: This study was a continuation (Phase B) of a previous study on HIFU treatment of 147 cNFs in 20 NF1 patients (Peltonen et al 2024, in press). Seven patients from this study were included in Phase B. A single treatment session was performed using a 20 MHz HIFU-device with integrated dermoscopic guidance and a handpiece with a focus depth of 2.3 mm below the skin surface. Dosing was modified from 0.7 J/dose at 250 ms/dose in Phase A to 0.9 J/dose at 500 ms/dose to promote thermal effects over mechanical tissue destruction, and thereby obtain a gentler therapy without superficial skin erosions. Doses, 13-25 per tumor, median 18 were applied with an interval of 1-2 seconds approximately 1 mm between each other to fully cover the tumor including a 1 mm margin. Local anesthesia was not used. Post-treatment effects were evaluated immediately and at follow-up visits.

Results: Seven patients and 54 cNFs (diameter 2-5 mm, median 5.0 ± 0.85 mm) were included. The data analysis compared Phase A and B. The immediate and short-term biological responses were local flare and edema. Pain during the treatment was graded by patients between 1 and 8 (median 5) on a 0-10-scale. Mild side effects were reported post-treatment in six cases, and in two cases at the 1-week follow-up, while all other assessments observed no side effects. Pigment changes were observed in some cases and were apparently related to a combination of skin type and the creation of superficial erosions. At the 6-month follow-up, participants rated the treatment as "very satisfying" with no scarring or serious adverse events. The primary safety endpoints were thus fulfilled.

Visual assessment after six months showed reductions in size for 44% of tumors and 26% were no longer visible or substantially reduced. The efficacy was somewhat lower than that obtained in Phase A with 26% and 49% respectively. The effect was noted to correspond to superficial erosions which in turn may cause dyspigmentation.

Conclusions: HIFU presents a rapid, well-tolerated, and precise non-invasive option for cNF treatment. Improvement of tumor appearance can be obtained in 70-76 % of treated tumors, with possibilities to adjust settings to balance efficacy with risks for superficial erosions and dyspigmentation. The method supports repeated treatment and ongoing management of smaller growing tumors.

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Platform: Overcoming Barriers to Breast Screening in NF1

Monday, 24 June, 16:30 – 16:45

Yemima Berman, BMBS FRACP BSc Hons PhD, *Royal North Shore Hospital / University of Sydney, Australia*

Purpose: This study aimed to 1) Explore clinical and imaging modality factors impacting radiological interpretation of breast surveillance in young women with neurofibromatosis type 1. 2) Develop educational resources to support women with Neurofibromatosis 1 (NF1) considering surveillance.

Young women with NF1 (<50 years) have a five-fold increased risk of breast cancer compared to women without NF1. Guidelines recommend annual breast surveillance from age 30. We explored factors impacting the radiological interpretation in women with NF1 to identify the optimum breast imaging modalities. Due to co-morbid cognitive deficits, cancer worry and non-attendance, we designed and evaluated resources to support women considering surveillance.

Methods: Women with NF1 (30-49 years) were offered magnetic resonance imaging (MRI), Mammogram (MMG) and ultrasound (US). Data was compared to an age-matched *BRCA1/2* pathogenic variant (PV) control group. 2) Three resources were developed to include NF1-specific surveillance information. Women who had surveillance, and clinicians, were invited to review the resources via interview or survey-based methodologies.

Figure 1: Example of MMG and MRI images for a participant with NF1 who exhibited marked cNF burden on both breasts. **a).** 2D MMG CC view both breast (synthetic 2D derived from tomosynthesis); **b).** 3D MMG MLO view both breast (synthetic 2D derived from tomosynthesis); **c).** Axial T2 weighted STIR sequence (on the left) Axial post contrast T1 weighted MRI image (on the right). Orange arrow pointing to cutaneous neurofibroma (cNF)
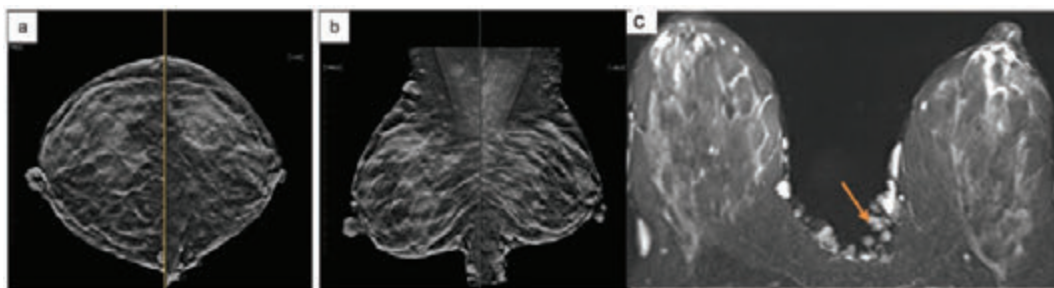
Results: 1) Twenty-seven women with NF1 undertook breast MRI, MMG and US. 19 BI-RADS 3/4 lesions were detected in 14 women, mostly by MRI and US (95% and 79%), with 11% identified using MMG. Biopsy rates were comparable to *BRCA* PV carriers (n=59, P=0.311). No breast cancer was identified.

Breast density was similar between *NF1* and *BRCA* PV cohorts (P=0.45); however, moderate/marked BPE was significantly higher in the NF1 cohort (P=0.002). Radiologists had higher confidence in interpreting 3D MMG versus 2D MMG images (P<0.001), which decreased with increasing breast density. CNFs did not impact breast MRI and MMG interpretation.

Figure 2: Clinician diagnostic confidence with 3D compared to 2D MMG images ***P < 0.001. Association between clinician diagnostic confidence in interpretation of 2D and 3D MMG images
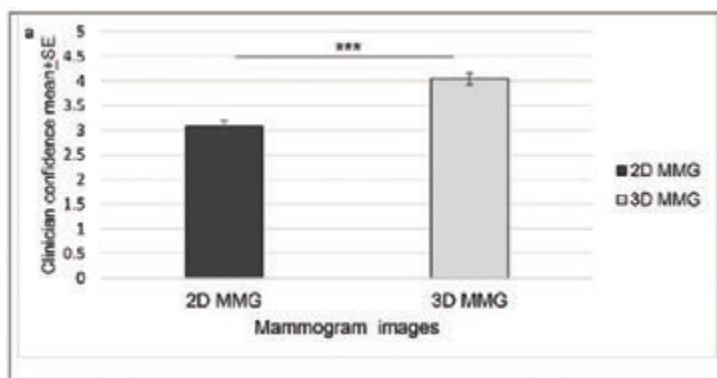
2) Feedback from nine patients was obtained by qualitative interview; 10 patients and 21 clinicians evaluated a website and animation by survey. Patients (>85%) rated design aspects of animation/website as 'good/very good', with amount (>90%) and length (>70%) of information 'about right'. HPs rated amount of information and design aspects slightly lower; 63% rated the animation 'too long'. Resources were modified to clarify content, reduce readability and length (animation), provide more information about NF1, breast cancer, and support, and improve design.
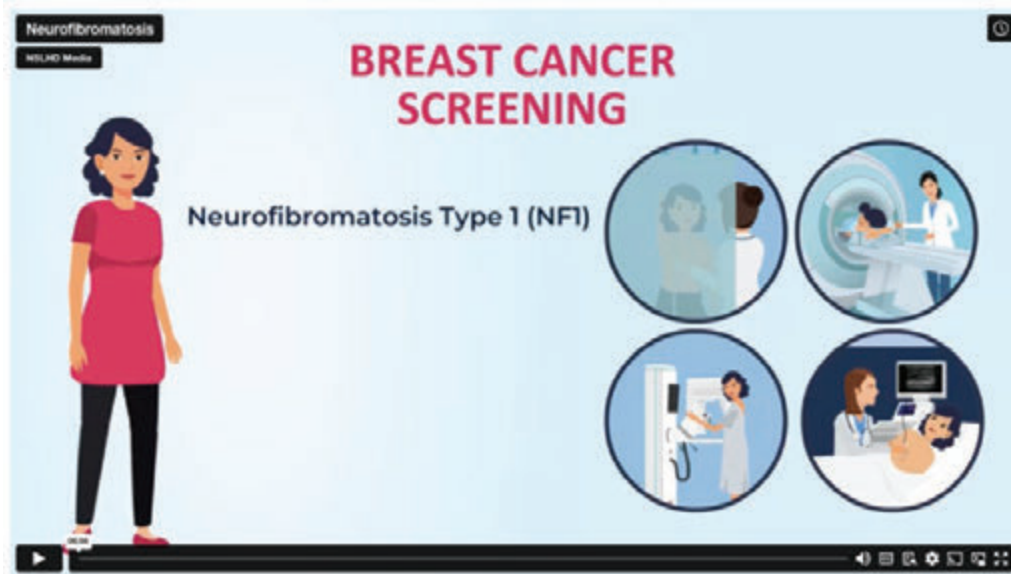
Conclusion: Although cNFs did not affect clinician confidence in 3D MMG interpretation, increasing breast density impeded 2D/3D MMG confidence. Given that most women with NF1 demonstrated breast density (BI-RADS 3C/4D), MRI was the preferred surveillance mode. For those with high breast density and high cNF breast coverage, 3D MMG is preferred if MRI is unavailable. Resources for those considering surveillance have been developed and endorsed by relevant stakeholders: https://www.nslhd.health.nsw.gov.au/genetics/Pages/Our-NF-clinic.aspx

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Platform: Incidence of Ophthalmic Complications in NF-1 Patients Treated with MEK Inhibitors

Monday, 24 June, 16:45 – 17:00

Zsila Sadighi, MD, *The University of Texas MD Anderson Cancer Center, Houston, TX, US*

Purpose: MEK inhibitors (MEKi) offer a novel and promising therapeutic approach for addressing symptoms of Neurofibromatosis type 1 (NF1), such as plexiform neurofibromas and optic gliomas. There are potential ophthalmic complications, such as MEKi-induced retinopathy (MEKAR), therefore, individuals undergoing MEKi treatment typically have regular ophthalmologic assessments. Our research seeks to evaluate the importance of these routine screenings in a primarily pediatric NF1 cohort receiving MEKi, focusing on the incidence of ocular adverse events (OAE).

Methods: We performed an IRB approved retrospective study of 45 patients diagnosed with NF1 who underwent treatment with MEKi. The study included individuals who had both baseline assessments and follow-up examinations post the initiation of MEKi therapy. Each evaluation consisted of a comprehensive eye examination, including visual acuity, dilated fundoscopic examination, optical coherence tomography (OCT) of the macula and nerve fiber layer, as well as Humphrey visual field testing.

Results: Of 45 patients reviewed, 26 patients met inclusion criteria. Sixty-two percent of patients were male with an average age at treatment initiation of 13 years. Median age was 15 years with range of 2 to 23 years old. Plexiform neurofibromas were the most common indication for MEKi (n=22). Selumetinib was the most commonly used MEKi (n=19, 77%), followed by trametinib (n=6, 23%), and mirdametinib (n=1, 4%). Only two patients had to switch to different MEKi. Patients were followed for a mean of 413-days after starting the treatment (range 103-1122 days) for an average of 3.85 ophthalmology exams per patient. No retinopathy was observed at each of the 3–6 month follow-ups. Lisch nodules were seen in 69% of patients. Around 15% of patients experienced dry eye symptoms with none of the patients requiring artificial tears for management. Pre-existing optic neuropathy was present in 7 patients. No dose adjustments were needed due to OAEs. One patient's visual acuity decreased temporarily but returned to baseline and the patient remains on MEKi. No patient death was reported.

Conclusions: Regular ophthalmologic assessments are crucial for NF1 patients undergoing MEKi treatment. Consensus guidelines on frequency of ophthalmologic screening during MEKi treatment are not well reported. In our cohort no patients had MEKAR at regular 3–6 month follow ups. In addition to baseline screening, future larger cohort studies are needed to confirm if decreasing the frequency of retinal exams is safe while on MEKi to inform future clinical screening guidelines for NF1 patients on MEKi.

Disclaimer: The results of this study will be presented in The North American Neuro-Ophthalmology Society (NANOS) conference, March 2024

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Platform: Predicting the Clinical Phenotype in *NF2*-Related Schwannomatosis Patients

Monday, 24 June, 17:00 – 17:15

Marica Eoli, *Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy*

Different genetic scores have been proposed to predict the phenotype of *NF2* related Schwannomatosis (NF2 -SCHW MIM # 101000). The UK NF2 Reference Group proposed the Genetic Severity Score (GSS) based on the type and location of *NF2* germline variant observed (Halliday D et al doi:10.1136/jmedgenet-2017-104519). A new score FGSS was proposed based also on a functional assay of Merlin and its downstream pathways and including patients carryng Ring 22 chromosome (Catasus N et al 2021 doi:10.1136 jmedgenet-2020-107548).

81 patients (48 females and 33 males, median age 41;16-75) were identified by scanning the electronic NF2-SCHW patient database at Fondazione IRCCS C. Besta. The median follow-up was 14 yrs. Clinical diagnosis was established following: https://doi.org/10.1016/j.gim.2022.05.007

All patients underwent *NF2, SMARCB1* and *LZTR1* mutational screening by NGS and MPLA analysis using blood DNA, DNA tissue from two different tumours (7 cases) and or DNA from olfactory mucosa brushing (6 cases).

According to the new nomenclature 57 patients were classified as *NF2* related Schwannomatosis and 24 as NOS Schwannomatosis. All patients underwent brain and spinal MRIs with gadolinium, full neurological, ophthalmic, and audiological assessment.In 24 patients the diagnosis was presumed and 7 patients confirmed mosaic. *NF2* gene pathogenic variants were found in 57 subjects (70%), in most cases *NF2* point mutations, but in 7 cases a whole gene deletion was observed, in 2 a ring *NF2* chromosome 22 and in 7 cases a mosaic *NF2*-SCH were found. In 24 (30%) patients no pathogenic variant was found.

We stratified all cohort following GSS and FGSS. The disease outcome differed significantly depending both on clinical and genetic factors A strong negative correlation was observed between both genetic scores and age at first symptoms and age at diagnosis. A similar incidence of intracranial meningioma was observed in GSS groups 2A mild and 2B moderate and FGSS in groups 1, 3, 5. A high incidence of bilateral VS was found in GSS group 2A mild and in FGSS group 3. The percentage of spinal schwannoma did not differ between GSS 2A, 2B, 3 groups and FGSS 4, 5, 6 groups.

GSS and FGSS showed significant correlation with several measures, allowing stratification of patients with severe and very mild disease. However, there is a grey area when considering patients with moderate phenotypes. Mosaic patients have shown a high clinical variability.

Large cohorts of NF2-SCHW patients are needful to identify more accurate scores.

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KEYNOTE #5: ENGINEERING SERENDIPITY: AI'S RAPIDLY EXPANDING ROLE IN RESEARCH AND CARE

Tuesday, 25 June, 9:00 – 10:00

Casey Greene, PhD, *University of Colorado, US*

Artificial Intelligence (AI) and Machine Learning (ML) are revolutionizing the healthcare landscape, offering unprecedented opportunities to enhance patient outcomes and accelerate research. This presentation aims to provide a balanced discussion of AI/ML in clinical settings and research settings. I will discuss how lessons from the genome-wide association study era of human genetics point to potential but also the long road to implementation in patient care settings. I'll discuss how these methods can advance research and answer questions, particularly in the context of rare diseases. I will also address the broader implications of AI in research and practice including ethical considerations.

AI / MACHINE LEARNING, BIOMARKERS

Session Chair: Said Farschtschi, MD, *University Medical Center Hamburg-Eppendorf, Germany;* Ina Ly, MBBS, *Massachusetts General Hospital, US;* Eduard Serra, PhD, *Germans Trias i Pujol Research Institute, Spain*

Automatic Detection and Differentiation of Neurofibromas in NF1 – Current Status of Radiomics- and Deep Learning-Based Applications for MRI Image Analysis

Tuesday, 25 June, 10:45 – 11:00

Inka Ristow, MD, *Department of Diagnostic and Interventional Radiology and Nuclear Medicine and Nuclear Medicine, University Medical Center Hamburg-Eppendorf, Germany*

Purpose: 1) To evaluate the value of an MRI-based radiomics machine learning classifier to differentiate between benign, atypical, and malignant peripheral nerve sheath tumors in NF1.

2) To evaluate a whole-body MRI anatomy-informed automatic tumor segmentation approach for neurofibroma in NF1.

Methods: 1) For the radiomics study, 117 benign, 17 malignant, and 8 atypical neurofibromas from 36 NF1 patients were included. 107 features per lesion were extracted using PyRadiomics and applied for benign versus malignant differentiation. A 5-feature signature was defined based on the most important features and tested for signature-based benign versus malignant classification (random forest, leave-one-patient-out evaluation). In the second step, signature feature expressions were evaluated for radiomics-based classification of these three entities.

2) For the neurofibroma segmentation study, manually segmented whole-body STIR MRI datasets were randomly split into training (70 MRIs), validation (12 MRIs), and test subset (11 MRIs). T1-weighted images were included to account for anatomical structures. T1 and STIR images were rigidly registered to compensate for a patient positioning. TotalSegmentator was trained using an unsupervised domain adaptation method. The adapted model segmented key anatomical features in the T1 data, including body surface and internal organs. STIR images were divided into four anatomical zones (head-neck, chest, abdomen, and legs) based on the landmarks identified from the anatomy segmentation. Four Dynamic UNets (DynUNets) were trained. The segmentation was performed patch-wise. Masking was applied with a morphologically eroded body outline to exclude superficial cutaneous neurofibroma.

Results: 1) The mean AUC for the radiomics-based benign versus malignant differentiation was 0.94, corresponding to a correct classification of on average 16/17 malignant and 114/117 benign tumors (sensitivity 94%, specificity 97%). Exploratory analysis with the atypical tumors revealed intermediate radiomic feature expression.

2) By adapting TotalSegmentator for WB-MRI segmentation and employing dedicated Dynamic UNet models across four anatomical zones, we achieved substantial improvements of 20% in terms of the Dice coefficient (0.54 ± 0.06) on a test set, particularly in test cases with high tumor burden.

Conclusions: 1) Machine learning using MRI-based radiomics characteristics allows sensitive and specific differentiation of benign and malignant peripheral nerve sheath tumors. Feature expression of atypical tumors clustered in-between benign and malignant tumor feature expressions, which illustrates biological plausibility of the considered radiomics characteristics.

2) We introduce an anatomy-informed pipeline for whole-body neurofibroma segmentation, enhancing preliminary delineation accuracy. Future efforts will focus on integrating this pipeline into a semi-automated workflow to streamline MRI-based tumor segmentation.

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<u>Platform</u>: Machine Learning and High-Content Imaging for Modeling Neurofibromin in Schwann Cells

Tuesday, 25 June, 11:15 – 11:30

Gregory P. Way, PhD, *University of Colorado Anschutz Medical Campus, US*

Purpose: We aim to characterize cell morphology signatures of neurofibromin in Schwann cells, which we can use in future large-scale drug screens to identify drugs that make NF1 patient cells look healthy.

Methods: We optimized and applied a multiplex, high-content fluorescence microscopy assay (called Cell Painting[1]) to three isogenic Schwann cell lines with different *NF1* genotypes (*NF1*^{+/+}*, NF1*^{+/-}*,* and *NF1*^{-/-}). We used Cell Painting to mark Schwann cell nuclei, endoplasmic reticulum (ER), mitochondria, and actin (Figure 1). We and others have shown that Cell Painting quantifies cell health phenotypes[2], compound mechanism of action[3], toxicity[4], cancer cell resistance[5], and provides new information not captured by molecular readouts like gene expression[6]. We imaged a total of 20,609 cells and measured 1,535 morphology features, which represent various organelle shapes and intensity patterns. Using machine learning best practices, we trained an XGBoost ensemble decision tree[7] to predict *NF1* genotype. We also collected a Cell Painting dataset of *NF1*^{+/+} cells perturbed with siRNAs targeting *NF1*.
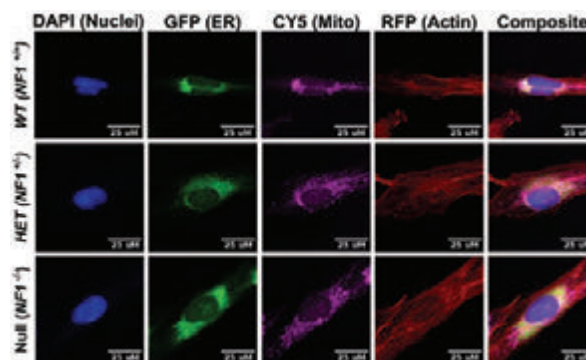
Figure 1. Example Cell Painting images of single Schwann cells for three *NF1* genotypes of an isogenic Schwann cell line (*ipn2.3 2 gamma*).
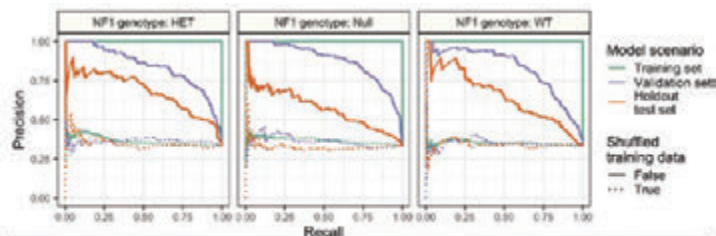
Figure 2. Precision-recall curves for predicting *NF1* genotype in Schwann cells. Each data split represents different cells. The dotted lines are a negative control, indicating performance from models trained on randomized data.

Results Summary: Our machine learning model predicted *NF1* genotype in a holdout test set (Schwann cells the model has never seen before) over two times better than random, but our models were over-fit to the training data indicating room for improvement (Figure 2). We identified that nuclei and endoplasmic reticulum morphologies were the most differential across *NF1* genotypes. Replicates of the *NF1* siRNA treatments were strongly correlated, and different siRNA constructs were more correlated than non-targeting controls (Figure 3). All machine learning code and data are publicly available on GitHub to accelerate NF1 patient benefit.

Conclusions: Machine learning can predict *NF1* genotype from high-content microscopy images of Schwann cells. The human eye cannot determine these differences; highperformance computing and machine learning are required. Our machine learning models can be improved to reduce overfitting, which will increase the chances that our models can generalize to new microscopy data. We identified offtarget effects with siRNA constructs, which we may overcome with CRISPRbased approaches. Overall, our study reveals promising indicators that can use this phenotype-based approach to identify/screen and prioritize new drugs that upregulate neurofibromin function in Schwann cells (by any mechanism) and improve health of NF1 patient Schwann cells while avoiding drug toxicity pitfalls[8].

Figure 3. siRNA treatment of *NF1+/+* Schwann cells by two constructs targeting *NF1* and several controls reveals high replicate correlation.

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Platform: Leveraging State-Of-The-Art Model Systems To Enable AI Drug Response Prediction Algorithms in NF1 Tumors

Tuesday, 25 June, 11:30 – 11:45

Sara JC Gosline, PhD, *Pacific Northwest National Laboratory, Seattle, WA, US*

Purpose: Despite the rising popularity of artificial intelligence (AI) algorithms that can predict tumor drug sensitivity from omics-level measurements[1], these algorithms are not currently generalizable to NF1 tumors. This is partially due to the overall scarcity of properly formatted data in NF1 as well as the lack of algorithms that generalize to new datasets. The purpose of this study is to build a harmonized dataset and access platform to support the broad adoption and development of AI-based drug sensitivity prediction that can be used in NF1 tumors.

Methods: We developed the Cancer Omics and Drug Experiment Response Data (coderdata) Python package that collates the most up-to-date cancer omics and drug response data. This package comprises an automated pipeline that collects and formats data from four public pan-cancer repositories[2,3] and the NF Data Portal[4]. We also collected drug structure information from PubChem and calculated diverse computational representations of chemical properties. The dataset is rebuilt with up-to-date information monthly and stored on FigShare for convenient access within the coderdata package.

Results: To date we have curated molecular measurements (genomics, transcriptomics, proteomics, copy number) from approximately 5000 samples across multiple model systems such as two-dimensional cell lines (2160), organoid models (309), patient-derived xenografts (20), and tumor data (2513) including data from patient-derived NF1 tumors – malignant peripheral nerve sheath tumors and cutaneous neurofibromas. We also have single and combination drug sensitivity data for ~300 of these samples for up to ~50,000 different drugs. The data package can be found at https://pnnl-compbio.github.io/coderdata/.

Conclusions: With harmonized data, AI algorithms can leverage cancer model system and drug sensitivity data to predict drug sensitivity in rare tumors such as those from NF1 patients and be benchmarked against other algorithms. Coderdata provides this dataset in an easily accessible python package to facilitate training and evaluation of AI algorithms in NF1. To date it is the only benchmark dataset that combines existing cell line data with ex vivo model system data, the most recent developments in NF1[5,6]. The platform is open source and available for additional contributions from other datasets.

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References: 1. Partin et al., *Frontiers in Medicine* 2023. 2. Tsherniak, et al. *Cell* 2017 3. Goncalves et al., *Cancer Cell* 2022 4. Allaway et al., *Scientific Data* 2019 5. Larsson et al., *Neuro-oncology* 2023 6. Nguyen et al. *Cell Reports Methods* 2024.

Platform: Localized Magnetic Resonance Imaging Features of the Anterior Visual Pathway are Associated with Visual Acuity Loss in Children with NF1-OPG

Tuesday, 25 June, 11:45 – 12:00

Zhifan Jiang, PhD, *Children's National Hospital, Washington DC, US*

Purpose: Optic pathway gliomas associated with neurofibromatosis type 1 (NF1-OPG) occur throughout the anterior visual pathway (optic nerve, chiasm, tract; AVP). The overall shape and volume of AVP measured from magnetic resonance imaging (MRI) have been recently associated with visual acuity (VA) loss. To explore the added value of shape, volume, and detailed image intensity patterns within the optic nerves (ONs) and chiasm, referred to as localized MRI radiomic features, we propose an automatic deep-learning framework for VA loss prediction from volumetric and radiomic analysis of ONs, chiasm, and AVP.

Methods: MRIs of 60 children with NF1-OPG from Children's National Hospital (CNH, GE platform) and 75 from Children's Hospital of Philadelphia (CHOP, Siemens platform) with 3 MRI sequences (high-resolution T1-weighted, low-resolution anisotropic T2-weighted and T2-FLAIR) were included. The average resolutions of the three sequences were $0.9 \times 0.78 \times 0.78$ mm^3, $0.49 \times 0.55 \times 2$ mm^3, and $0.43 \times 0.43 \times 4.8$ mm^3, respectively. Volumetric ground truth was established by expert annotation of the AVP. Neuro-ophthalmic evaluation identified VA loss ($>= 0.2$ LogMAR decline) in 28 children from CNH and 24 from CHOP. Automatic AVP segmentation was performed using a knowledge-transferred Swin transformer network. The ONs and chiasm were split through template-based registration. The brain volume, tumor location, child age, and 1,172 radiomic features were used to predict VA loss using support vector machines. Sequential feature selection was performed using sensitivity and univariate statistical tests (ANOVA) to identify VA loss risk factors. We first assessed predictions based on AVP alone and then explored whether refined analysis on ONs and chiasm was an improvement.

Results: The average Dice volumetric overlap for automatic AVP segmentation was 0.791 ± 0.075, consistent with the reported inter-observer variability for manual AVP segmentation (0.75 ± 0.06). Cross-validated assessment of VA loss prediction based on AVP alone resulted in accuracy, sensitivity, specificity, and AUROC of 0.865, 0.647, 0.971, 0.728, respectively. Analyzing ONs and chiasm led to balance and improvements in these metrics, resulting in 0.865, 0.882, 0.857, 0.899, respectively. Significant risk factors were maximal image intensity and gray level run length entropy in T2-FLAIR for ONs, and image intensity range in T2-FLAIR for chiasm. The increase in AUROC was statistically significant (DeLong's test, $p = 0.028$).

Conclusion: Deep learning-based analysis indicates an association of new localized MRI radiomic features in the ONs and chiasm with VA loss, which show enhanced capability in predicting VA loss. This automated framework has the potential to guide the treatment decisions for children with NF1-OPGs.

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Exploration of Exosomes as Blood-Derived Biomarkers and Therapy Indicators for NF2-SWN

Tuesday, 25 June, 13:00 – 13:30

Lars Björn Riecken, PhD, *Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany*

NF2-related schwannomatosis (NF-SWN) is a disease caused by loss of *NF2* gene function and associated with the development of multiple nervous system tumors. While benign, these tumors severely impact life quality, e.g. due to compression of affected nerves and nearby structures such as brain stem and spinal cord. Currently, no effective treatment is available; systemic pharmacological interventions are only partially responsive, afflicted by progressive side effects, and tumors becoming resistant over time. Often, surgery remains the last treatment option but is associated with a high risk of nerve damage and potentially injury-induced tumor relapse, as previous work indicates:

In our multifactorial model of NF2-SWN induction, schwannoma arise from cellular and molecular changes induced by loss of *NF2* expression that result in a disturbed cell-cell communication during nerve regeneration. As a key component of this disturbed cell-cell communication, we identified the neuronal surface molecule Neuregulin 1 (Nrg1). Nrg1 constitutes an important differentiation factor for Schwann cells to recognize (regenerated) axons, (re-)attach to them and support normal nerve function. In *NF2*-deficient neurons Nrg1 is significantly downregulated, and in this environment NF2-deficient Schwann cells appear unable to re-differentiate and support normal nerve regeneration, instead giving rise to Schwannomas.

In a proof-of-concept pilot study, we investigated the therapeutic potential of injecting soluble recombinant Nrg1 to replace the missing differentiation cue and demonstrated a significant reduction of Schwannoma formation as well as improvement of nerve regeneration and functional recovery. Further seizing the opportunity, we also utilized this paradigm to explore exosomes as potential biomarkers for NF2-SWN.

Exosomes are extracellular nano-sized vesicles that can be found in most bodily fluids, are secreted by virtually all cell types and potentially distributed throughout the entire body. Carrying a molecular signature derived from their producing cell, they are not only important microenvironmental modulators but also carry valuable information about the cellular biology of their cell of origin. In turn, studying exosome composition may reveal potential NF2-SWN schwannoma biomarkers.

To this end, we isolated blood-derived exosomes from both a schwannoma-bearing NF2-SWN mouse model as well as from human NF2-SWN patients. Indeed, we found the abundance of several proteins to be significantly changed when compared to healthy controls. In mice, we found 122 significantly changed proteins – 43 of which could be reversed by Nrg1 protein replacement therapy. Cross-species comparison between mouse and human ultimately identified 13 potential NF2-SWN biomarkers with translational validity – 4 of which (Ppia, Apoc3, Apom, F11) responded to Nrg1 protein replacement therapy.

Platform: Multi-omics Analysis of Multiple Meningiomas in *NF2*-Related Schwannomatosis

Tuesday, 25 June, 13:30 – 13:45

Yu Teranishi, MD, PhD, *Paris Brain Institute, Paris, France*

Purpose: Patients with *NF2*-related schwannomatosis (*NF2*-SWN) typically develop multiple meningiomas based on a single *NF2* germline mutation. Meningiomas in one *NF2*-SWN patient may present different histological grades, patterns of growth and diverse clinical aggressivity, thus questioning the presence of additional genetic and/or epigenetic events in addition to *NF2* gene inactivation to explain this intra-patient heterogeneity. The aim of this study is to further characterize the epigenetic, mutational and transcriptional landscape of *NF2* mutant meningiomas in order to decipher the mechanisms of different aggressiveness among *NF2* mutant meningiomas.

Methods: A long-term retrospective follow-up (81.2 ± 48.7 months) study involving a total 23 meningiomas in 11 patients with *NF2*-WN was performed. Their paired tumors' clinical / molecular characteristics were assessed using whole-exome sequencing, bulk-RNA sequencing, DNA Methylation analysis, and immunohistochemistry (H3K27me3, FOXM1, CD163). All *NF2*-WN patients had a severe phenotype. They harbored a median number of 6.1 ± 3.5 meningiomas. The mean annual growth rate (cm^3/year) was 7.9 ± 14.4 in resected meningiomas. Defining 4.0 cm^3/year as the cutoff of growth rate, 39.1 % of tumors were defined as fast growing (FG) and 60.9 % as slow growing (SG). Six tumors (26.1%) were WHO grade I, 16 cases (69.6%) were WHO grade II and 1 case (0.4 %) was Grade III. The overall recurrence rate was 21.7 % over the follow-up period.

Results: After multi-omics analysis, we didn't find any statistical difference in terms of copy number variation (CNV), mRNA expression profile, DNA methylation class, macrophage infiltration, or any established molecular prognostic marker (h*TERT, CDKN2AB,* H3K27me3) when comparing FG and SF meningiomas in *NF2*-SWN patients. There was also no molecular factor associated with recurrence. When investigating the molecular factors influencing the clinical aggressivity of *NF2*-SWN meningiomas at an individual level and found that paired tumors with identical mutation profile and CNVs might still present with different clinical behavior and on the opposite, that tumors from the same patient with different CNV profiles might present with similar histological grades and clinical behavior. More surprisingly, independent meningiomas arising from the same *NF2*-SWN patient were epigenetically independent, although germline *NF2* alteration was shared between tumors.

Conclusion: In conclusion, our study suggests that robust molecular prognostic factors validated in sporadic meningiomas might not be relevant to explain various tumor aggressivity in *NF2*-SWN.

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Platform: The PNF-ANF-MPNST Progression at Single Cell Level: Delving Deep into NF1-Related Tumors

Tuesday, 25 June, 13:45 – 14:00

Bernat Gel, PhD, *Hereditary Cancer Group, Germans Trias i Pujol Research Institute (IGTP), Spain*

Figure 1:
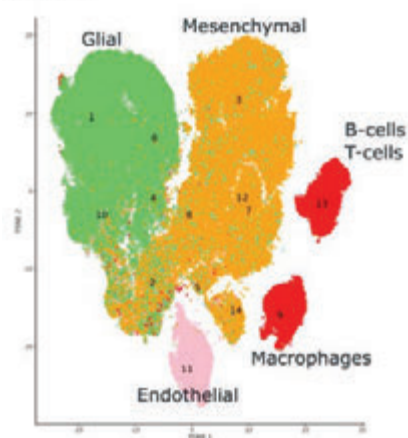
Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft-tissue sarcomas and the leading cause of NF1-related mortality. Over the years several efforts have been made to genomically characterize these tumors using different bulk technologies. However, some questions cannot be addressed without information at the single-cell level.

For this, we characterized 16 samples from the PNF-ANF-MPNST progression (4 control nerves, 4 PNF, 4 ANF and 4 MPNST) using five complementary technologies (scRNA-seq, scATAC-seq, SMARTseq2, scDNA-seq with cell surface markers and spatial transcriptomics) to obtain a complete picture of the genomics, epigenomics and transcriptomics of the whole progression at single-cell resolution. This is a unique dataset since it can be used to integrate multiple technologies in each single tumor type, multiple tumor types with a single technology or perform a full integration of multiple technologies in multiple tumor types. We performed different controls on sample preparation. Basic scRNA-seq analysis revealed that the main cellular composition of the tumors matched the expected proportions determined by histology. A custom bioinformatic quality control analysis identified and corrected cellular stress signals in some samples.

By performing an integrated analysis of all technologies across all tumor types, we were able to completely determine the cellular composition of each tumor type, including minor cell populations and different differentiation states along the neural crest - Schwann cell axis. We also characterized the systematic changes in cellular composition along the PNF-ANF-MPNST progression. The combination of this information with SMART-seq data allowed us to determine the exact cell populations affected by the inactivation of *NF1, CDKN2A* and PRC2 and thus identify the cells most probably originating the different tumor types.

Figure 2:
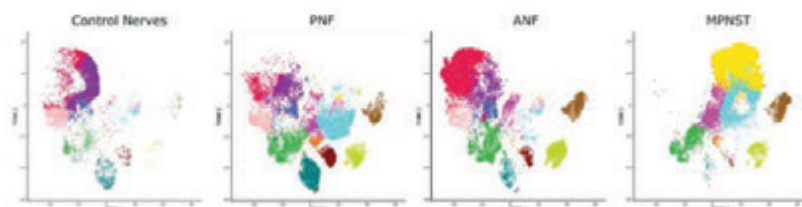
Figure 3:
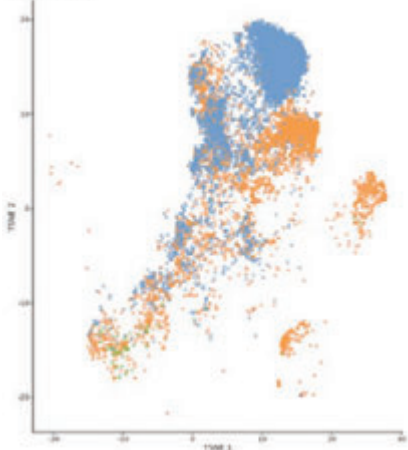
We also detected large cellular identity changes on the transformation from ANF to MPNST and explored the epigenetic changes determining them by combining transcriptomics and scATAC-seq data. Estimation of copy-number profiles from scRNA-seq data revealed different subclones within MPNSTs and their different transcriptomic patterns. Finally, we were able to map the different cell populations on tumor tissue using spatial transcriptomics, revealing the spatial organization of these tumors.

In summary, we performed an analysis of the whole PNF-ANF-MPNST progression at single-cell resolution using multiple techniques. The integration over different tumors and different data types revealed new biological insights into the mechanisms of MPNST progression with potential translation to the clinics. This whole dataset will be open and a valuable asset for the whole NF1 research community.

Figure Legends:
Figure 1: Broad cellular composition of the whole dataset
Figure 2: Changes in cellular composition over the PNF-ANF-MPNST progression
Figure 3: Example of broad subclone analysis in one MPNST

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<u>Platform</u>: A Comprehensive Algorithm to Predict Malignant Transformation of NF1 Nerve Sheath Tumors from Single-Cell Transcriptomic Profiling

Tuesday, 25 June, 14:15 – 14:30

Xiyuan Zhang, PhD, *Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, US*

Objective: A life-threatening complication of having NF1 is the development of an aggressive and highly metastatic malignant peripheral nerve sheath tumor (MPNST) at an earlier age compared to the general population. Currently there are no effective treatments for MPNST other than complete surgical resection with wide negative margins. To gain more insights of MPNST tumorigenesis, this study aims to decipher the alterations in the tumor microenvironment (TME) along the trajectory of malignant transformation from benign plexiform neurofibroma (PN) through precancerous atypical neurofibroma (AN) to MPNST.

Methods: To dissect the oncogenic mechanisms of NF1-deficient Schwann cells during the malignant transformation and describe the concurrent changes in the TME, we utilized single-cell RNA sequencing (scRNAseq) to profile the intra-tumoral heterogeneity of clinically annotated NF1 tumors collected from patients enrolled in the NF1 Nature History study. We built a NF1 tumor single-cell atlas (reference) by performing integrative analysis to correct for batch effects and to compare the transcriptomic profiles of 55 NF1 nerve tumors. Furthermore, we developed an algorithm to predict malignant transformation of NF1 nerve sheath tumors by comparing scRNAseq data from an independent cohort of 39 tumors to this reference.

Results: After quality control and filtering, we analyzed 421,377 cells from 25 PN, 25 AN, and 5 MPNST. A total of 34 transcriptionally distinct clusters were discovered to belong to seven major cellular compartments: fibroblasts, pericytes, myeloid and lymphoid immune cells, endothelial, Schwann, and malignant cells. These cellular compartments changed composition between benign PN, AN, and MPNST, with notable decreases of the fibroblast, myeloid immune cell, and Schwann cell populations over the course of malignant transformation. Conversely, MPNST exhibited increases in the lymphoid immune cell and tumor cell compartments. To further investigate the changes of immune components accompanying the malignant transformation, we performed subclustering analysis using the 199,921 immune cells and further grouped them into 32 transcriptionally distinct clusters, including functionally distinct cytotoxic T cells, regulatory T cells (Tregs), B cells, NK cells, NKT cells, mast cells, dendritic cells, monocytes, and macrophages. The immune-cellular composition was unchanged in the comparison of PN and AN. Notably, there was emergence of CTLA4+ Tregs and loss of activated macrophages in MPNST. Finally, we discovered a unique "transitioning" cell population in some PN and AN that express high levels of imprinted long non-coding RNAs. These benign NF1 nerve sheath tumors may possess high risk of malignant transformation. Using the signatures of these "transitioning" cells and the transcriptional signatures of malignant MPNST cells, we developed an algorithm for the early identification of MPNST.

Conclusions: In summary, we describe the cellular intra-tumoral heterogeneity of NF1 nerve sheath tumors using scRNAseq data from patients. We show that MPNST exhibits an immunosuppressive TME characteristic of diminished activated macrophages and the presence of Tregs, which may play a role in malignant transformation. Importantly, the discovery of a malignant cell signature presents a unique opportunity for early detection of high-risk NF1 nerve sheath tumors that may undergo malignant transformation. Once validated, our approach of using scRNAseq as a diagnostic tool will help identify tumors at risk in clinical practice.

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<u>Platform</u>: Epigenetic Profiling Improves Diagnostic Accuracy of Malignant Peripheral Nerve Sheath Tumors (MPNST)

Tuesday, 25 June, 14:30 – 14:45

Catena Kresbach, MD, *Institute of Neuropathology, University Hospital Hamburg-Eppendorf, Hamburg, Germany and Forschungsinstitut Kinderkrebs-Zentrum Hamburg, Germany*

Purpose: Patients with NF1 frequently develop plexiform neurofibromas (PNF) that harbor the risk of progression to premalignant lesions termed atypical neurofibromatous lesions of unknown biological potential (ANNUBP) and further to highly aggressive malignant peripheral nerve sheath tumors (MPNST) with poor response to radio- or chemotherapy. Therefore, it is highly relevant to detect MPNST as early as possible. However, MPNST show pronounced intratumoral histological heterogeneity, comprising areas histologically corresponding to PNF, ANNUBP or MPNST (Figure 1 and 2). Thus CT-guided stereotactic biopsies are not always representative of the entire tumor and harbor the risk of misdiagnosis.

We aim to delineate the intratumoral heterogeneity of MPNST to improve diagnostic certainty and to better understand the mechanisms of malignant progression from premalignant to malignant stages.

Methods: Histological workup including analysis of tissue morphology on H&E-stained sections and immunohistochemistryfollowed by array-based global methylation profiling (Illumina EPIC arrays) of suitable tissue areas.

Results: Epigenetic signatures have demonstrated high accuracy in diagnosing both central and peripheral nervous system tumors (Capper et al, 2018, Koelsche et al., 2021). We recently found that ANNUBPs show a rather distinct epigenetic profile, providing a helpful diagnostic tool for this histologically challenging premalignant entity (Kresbach et al., 2023). We proceeded to investigate whether the epigenetic landscape of MPNST with intratumoral histological heterogeneity might reflect the presence of premalignant tumor stages. So far, we have selected 7 MPNST and after careful histological examination, chose one area with histological PNF or ANNUBP morphology and one area with high-grade MPNST morphology in each tumor for methylation analysis. Clustering analysis revealed that in 5/7 cases, both areas from one tumor showed highly similar epigenetic characteristics and clustered with MPNST (Figure 3). Copy number profiles inferred from the methylation data showed marked alterations not only in the high-grade areas but also in the histologically benign areas in 5/7 cases.

Conclusions: Our findings are of high relevance when interpreting stereotactic biopsies of suspected MPNST when the histology remains doubtful. Further, they indicate that epigenetic changes might occur early during malignant progression. We are currently working on extending this cohort and correlating the findings with radiological features on MRI and PET-CTs. In addition, we are complementing the data with analyses of transcriptomic profiles and genome sequencing.
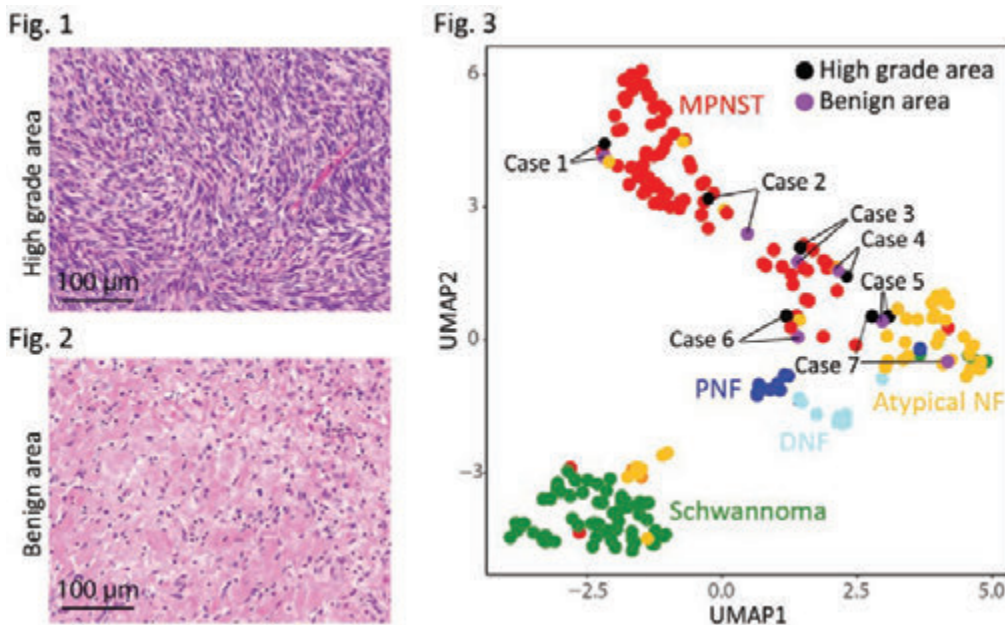
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Prevalence of NF1 in the United States: A Claims-Based Analysis

Rajeev K. Amar, MSc, PhD, *Alexion, AstraZeneca Rare Disease (Alexion Pharma GmbH), Baar, Switzerland*

Purpose: This purpose of this research is to address an important gap in the literature by providing recent and representative prevalence estimates of neurofibromatosis type 1 (NF1) as well as the frequency of plexiform neurofibroma (PN) in patients with NF1 in the United States (U.S.) population.

Methods: The analysis was performed using U.S. medical insurance claims data from the Clarivate™ Real World Database, which holds individual-level open claims data for >300 million unique patients since 2011 across various commercial and public payers/plans. The prevalent NF1 cohort included patients having ≥2 International Classification of Disease-10 codes (ICD-10) for NF1, at least 30 days apart during the study period; patients having ≥1 ICD-10 code for PN were flagged. Although this analysis reports annual prevalence from 2018-2022, the study period began in 2016 to capture prevalent patients diagnosed prior to 2018 who may not have had claims for NF1 during the period 2018-2022. This means that patients with NF1 identified in any year during the study period were prevalent patients in subsequent years until the last year they had a claim of any kind until end of 2022. The denominator was defined as all patients in the database utilizing the healthcare system during the same period. National prevalence was extrapolated using stratum-specific prevalence from the selected sample, based on matched age, gender, and state cohorts.

Results: The number of patients diagnosed with NF1 in the database during the study period was 44,613. The number of people in the U.S. diagnosed with NF1 in 2022 was estimated at ~79,500 or 23.9 per 100,000 population (95% CI: 22.3 – 25.6). Prevalence estimates ranged from 21.1/100,000 in 2018 to 23.9/100,000 in 2022. Prevalence was highest in those <18 years of age (49.2/100,000 in 2022), with lower estimates observed in older age groups (18-39: 26.4/100,000; 40-64: 12.8/100,000; 65+: 6.6/100,000). Prevalence in males was slightly higher than females (25.4 vs. 22.5/100,000, respectively). Overall, the proportion of patients with NF1 diagnosed with PN was 25.6%, while proportions in adults (ie, those ≥18 years) ranged from 30.4%-39.4% depending on age group.

Conclusions: The annual prevalence of NF1 in the U.S. in 2022 was ~24 per 100,000 population. This is the first U.S. prevalence estimate per 100,000 population to be reported, and it is similar to estimates in Northern European countries[1-5]. The proportion of U.S. patients with NF1 diagnosed with PN was ~26%.

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Targeting RNA-Binding Proteins: Role in Growth and Metastasis of Malignant Peripheral Nerve Sheath Tumors

Adrián Aparicio Rey, MSc, *University of Santiago de Compostela*

Background and study purpose: Malignant peripheral nerve sheath tumours (MPNSTs) represent highly aggressive soft tissue sarcomas characterized by high metastatic potential, primarily to the lungs. The challenging prognosis of MPNST patients underscores the pressing need for enhanced and efficacious treatment options. A promising therapeutic approach in cancer involves targeting dysregulated transcriptomic programs, with a focus on identifying the pivotal molecular regulators steering these programs. RNA-binding proteins (RBPs) are gaining increasing recognition as appealing targets due to their ability to govern the type and abundance of numerous transcripts. The primary aim of this research is to explore the functional and mechanistic roles of RBPs in driving the growth and metastasis of MPNSTs.

Methods: A bioinformatics analysis was performed on microarray datasets encompassing MPNST tumours, neurofibromas (NF), and nerves, to identify RNA-binding proteins (RBPs) characterized by overexpression in malignant tumours (adjusted p-value < 0.05 and fold change > 1.5). Other relevant target information was obtained with the use of Gepia2 and EURBP databases. After this identification, the expression of Gene A was validated in Schwann cells, NF and MPNST cell lines through Western blot. The effect of Gene A knockdown in different normal Schwann cells and MPNST cell lines was studied using two shRNAs vectors. The silenced cells were employed in a broad spectrum of functional assays, such as colony formation, ATP levels measurement, anchorage independent growth, BrdU incorporation and cell count.

Results: Data from datasets GSE14038, GSE41747 and GSE178989 support that Gene A is overexpressed in MPNSTs compared to NFs and normal nerves (Figure 1). Western Blot confirms the same trend in our cell lines (Figure 3). The overall survival of sarcoma patients is poorer in the group with higher gene expression (Figure 2). We noted a decline in the clonogenic and proliferative capabilities of tumour cells following silencing (Figure 4), accompanied by a decrease in total ATP levels and proliferation markers. Importantly, the impact of silencing was less conspicuous in immortalized human Schwann cells when compared to the notable effect observed in cancer cell lines.
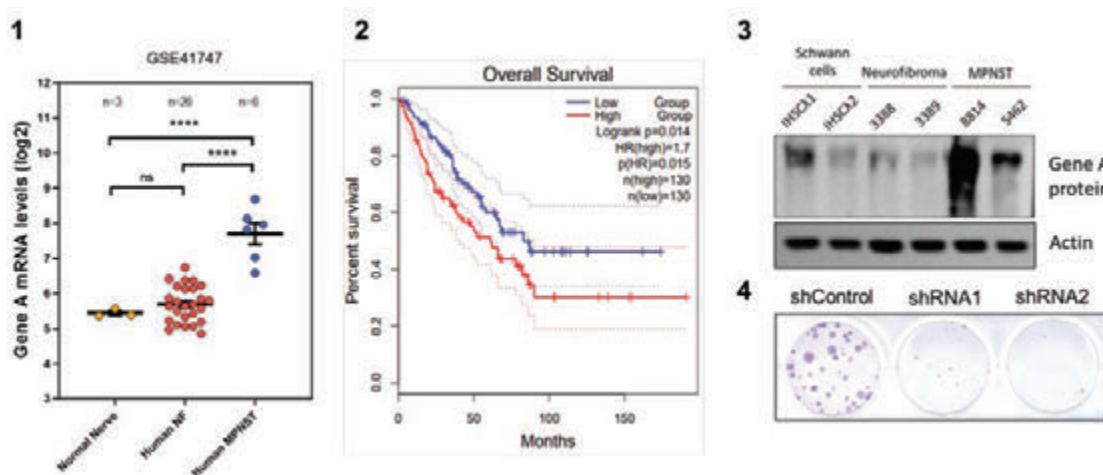
Conclusions: High Gene A expression is correlated with malignant transformation of Schwann cells. The inhibition of Gene A diminishes the tumorigenic properties of MPNST cell lines.

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Probing Disease Mechanisms in *HNF1B*-Associated Dysplastic Kidney Malformations with hPSC-Derived Kidney Organoids

Ioannis Bantounas, *University of Manchester*

Hepatocyte nuclear factor 1B (HNF1B) encodes a transcription factor expressed in developing human kidney epithelia. Heterozygous *HNF1B* mutations are the commonest monogenic cause of dysplastic kidney malformations (DKMs). To understand their pathobiology, we generated heterozygous *HNF1B* mutant kidney organoids from CRISPR-Cas9 gene-edited human ESCs and iPSCs reprogrammed from a family with *HNF1B*-associated DKMs.

Mutant organoids had abnormal anatomical features, such as enlarged malformed tubules displaying deregulated cell turnover. These mutant tubules also exhibited abnormal physiology, as they resisted cAMP-induced dilatation seen in controls. Bulk and single-cell RNAseq experiments showed downregulation of numerous genes implicated in Mendelian kidney tubulopathies. Further bioinformatic analyses of these results, indicated abnormal WNT, calcium, and glutamatergic pathways, the latter hitherto unstudied in developing kidneys. ScRNAseq further revealed abnormal populations of tubular epithelial and glomerular cells in the mutant without an equivalent in the controls. Conversely, normal proximal tubule epithelial cells were almost completely absent in the mutants.

Intriguingly, *Glutamate ionotropic receptor kainate type subunit 3 (GRIK3)* was one of several glutamate receptor subunits upregulated in malformed nephron tubules of mutant organoids and was also detected in *HNF1B* mutant fetal human dysplastic kidney epithelia. Moreover, scRNAseq analyses showed that it was expressed largely in the same cell populations as the mutant version of *HNF1B.* These results reveal morphological, molecular, and physiological roles for HNF1B in human kidney tubule morphogenesis and functional differentiation and illuminate the developmental origin of mutant-*HNF1B*-causing kidney disease.

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Using Human Pluripotent Stem Cell-Based Models to Study Neurofibromatosis Type-1

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Purpose of study: RASopathies are a family of genetic disorders, in which mutations in members of the RAS-signalling pathway lead to increased signalling. Neurofibromatosis type 1 (NF1) is a common RASopathy affecting 1 in 2500 people. Around 80% of children with NF1 have learning disabilities and behavioural impairments including autism and ADHD. Practical and ethical reasons have until recently prevented study of the mechanisms of this disease in human.

Methods: We generated induced pluripotent stem cell (iPSC) lines from families carrying mutations in NF1. We differentiated the iPSCs using protocols that produce cultures enriched in either glutamatergic or GABAergic neurons via intermediate stages representing the formation of neuroepithelium and then neural tube before final maturation.

Results: RNAseq analysis of transcriptomes during early differentiation identified several dysregulated genes. We also detected increased phosphorylation of signalling proteins downstream of RAS and observed an increased mobility of NF1-mutant neuroepithelial cells, which was reversible by pharmacological inhibition of downstream effectors of RAS. In the neural tube stage, we detected aberrations in epithelial cell-to-cell contact. We further characterised our model at later time points, where mature neurons were predicted to form. We report the formation of neuron subtypes representing all cortical layers, as well as glial cells in our mature cultures.

Conclusions: We have thus provided a human platform in which to mechanistically study NF1 and similar genetic diseases and test potential therapies.

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Preclinical Modeling of CDK4/6 Inhibitors for Late-Stage NF1 Tumors

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Purpose: The leading cause of premature death for patients with Neurofibromatosis type 1 (NF1) is the development of malignant peripheral nerve sheath tumors (MPNSTs) which render current medical treatment strategies largely ineffective, prompting an investigation of rational targeted therapies opposing the chronic cell cycle deregulation that promotes this malignant transformation.

Methods: Molecular and phenotypic responses to the targeted CDK4/6 inhibitors abemaciclib and palbociclib were evaluated in three human MPNST cell line models (*NF1* and *CDKN2A* null). Multiplexed kinase inhibitor bead (MIB) affinity chromatography coupled with mass spectrometry (MIB/MS) was used to analyze changes in kinase activity and expression following CDK4/6 inhibitor treatment, compared to DMSO control. *RB1* knockout clones were generated using CRISPR/Cas9 to evaluate the dependence of CDK4/6 inhibition on RB, on account of clinical studies identifying *RB1* loss as a potential mechanism for acquired resistance.

Results: MPNST cell lines demonstrated sensitivity to single agent treatment by CDK4/6 inhibition, with abemaciclib providing a greater reduction in cell viability than palbociclib. Both inhibitors elicited a diverse change in kinome profile compared to control, primarily exhibiting a reduced MIB binding of kinases that reflect a cell cycle arrest phenotype. Previously reported off-targets exclusive to abemaciclib were also identified as having reduced binding during MIB/MS. *RB1* knockout MPNST clones exhibited resistance to palbociclib but remained sensitive to abemaciclib in long-term exposure studies. Abemaciclib treatment also outperformed single agent inhibition of its non-CDK targets in both *RB1* proficient and knockout cells.

Conclusions: CDK4/6 inhibition shows promise as a targeted therapy against late-stage NF1 tumors with dysregulated cell cycle signaling. Single agent treatment with abemaciclib and palbociclib suppressed growth of MPNST cell lines, but only abemaciclib remained effective upon knockout of *RB1*. Lack of resistance in these clones, along with a unique kinase signature revealed by MIB/MS analysis, suggests the importance of off-target binding of abemaciclib in its effect on cell proliferation. Further characterization of abemaciclib treatment is therefore warranted, including the potential for synergistic combination therapies through other kinase targets.

Deciphering the Molecular Mechanism of Pathogenicity in Destabilized Variants of Neurofibromin Mutated in its SecPH Domain

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Pathogenic mutations in Neurofibromatosis type 1 gene *(NF1)* are numerous, almost 3000, and distributed throughout this gene. They correspond to deletions, nonsense or missense mutations, these latter representing 17% of the total pathogenic variants. Addressing the molecular basis responsible for the pathogenicity of *NF1* missense variants is crucial to establish a molecular diagnosis of the disease and develop more appropriate therapies. We focused on twelve NF1 missense and one small in frame deletion variants whose mutations are localized in SecPH, a domain adjacent to the GRD (GAP Related Domain) which harbours the Ras-GTPase activity of neurofibromin (Nf1), the protein encoded by NF1. We evaluated multiple biochemical and molecular features of full length Nf1 mutant proteins comprising steady state level, half-life and Ras-GTPase activity analysis. Furthermore, we also tested the pattern of SecPH SUMOylation (Small Ubiquitin-related Modifier), a structure-based Nf1 Post Translational Modification, we recently discovered (the unique mechanistic details of SecPH SUMOylation are depicted in B.Vallée poster). These features allowed us to delineate four groups of mutants. One of them is particularly interesting with profound defects in all the parameters we tested. The five variants of this group harbour mutations inducing a misfolding of the SecPH domain, which then triggers the ubiquitylation and proteasomal degradation of the corresponding full length Nf1 mutant protein thereby explaining the defects in all the parameters tested. These results deepen our understanding of the molecular mechanisms responsible for these variants pathogenicity. The instability of other *NF1* missense pathogenic variants in codons 844 to 848 has already been described (Li et al., 2016; Young et al., 2023) and it is likely that many other missense variants in *NF1* acquire their pathogenic character by a similar mechanism of misfolding and subsequent instability. New therapeutic approaches aimed at stabilizing Nf1 by preventing its ubiquitylation might constitute valued therapeutic options for this class of variants.

Targeting the MAPK-Pathway in Neurofibromatosis Type 1

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Purpose: Up to 50% of individuals with neurofibromatosis type 1 (NF1) develop plexiform neurofibromas and up to 20% might develop gliomas of the optic pathway upon homozygous loss of the neurofibromin 1 gene *(NF1)* in specific precursor cells. Up to now, the only drug approved in NF1 (for symptomatic neurofibroma in children) is a MEK inhibitor, with MEK being a key kinase of the MAPK-signaling pathway, hyperactivated in *NF1$^{-/-}$* cells. Therefore, we aim to evaluate novel drug candidates targeting different parts of the MAPK-pathway using cell models for plexiform neurofibroma and malignant peripheral nerve sheath tumors as well as NF1-associated low- and high-grade glioma.

Methods: Drug candidates (i.e. a SOS::KRAS inhibitor (BI-3406), a pan-KRAS inhibitor (BI-2493), a SHP2 inhibitor (batoprotafib), a BET-protac (MZ1), a FAK inhibitor (defactinib) and a MEK-inhibitor (trametinib)) were evaluated using patient-derived cells (in 2D primary cell cultures and 3D tumor spheroids) as well as commercially available, immortalized Schwann cell lines *(NF1$^{+/+}$, NF1$^{+/-}$, NF1$^{-/-}$)*. Cell viability and confluence assays were performed using a luminescence-based assay and an Incucyte live cell imaging system, respectively. In addition, drug combinations were evaluated for potential synergies.

Results: All evaluated drugs demonstrated single compound activity at low micromolar concentrations. However, different sensitivities were identified dependent on the *NF1* status. For instance, BI-2493 and batoprotafib were more potent at reducing metabolic activities of *NF1$^{-/-}$* Schwann cells compared to *NF1*-positive Schwann cells. In comparison, Trametinib, BI-3406, MZ-1 and defactinib affected both Schwann cell lines equally. With respect to 3D growth, defactinib, MZ1 and trametinib blocked 3D growth of all Schwann cell lines irrespective of *NF1* status. In contrast, BI-2493 and batoprotafib more potently blocked growth of Schwann cells lacking *NF1*.

Regarding NF1 patient-derived cells, BI-2493 and MZ1 were most effective at inhibiting proliferation. Interestingly, batoprotafib most potently blocked growth of NF1 patient-derived cells with a co-occurring SHP2 mutation. In addition, treatment with the individual drugs resulted in differences in morphology, pointing at different cellular responses, despite targeting of the same cellular signaling pathway.

Conclusions: Our findings support the potential of blocking key oncogenic pathways in *NF1*-negative tumors with small molecule inhibitors of the MAPK pathway and endorse further evaluations by performing combinatorial screens and the use of *in vivo* models.

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Impact of the Type II RAF Inhibitor Tovorafenib on Tumor Development in a Genetically Engineered Mouse Model of Plexiform Neurofibroma (PN)

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The purpose of this study is to interrogate the impact of Tovorafenib on PN leveraging a genetically modified mouse model (GEMM).

Genomic alterations and dysregulation of the MAPK pathway have been described in many different types of cancers. BRAF V600 point mutations and *BRAF* fusions, which are found in both pediatric and adult cancers, are oncogenic drivers that drive constitutive activation of the RAF pathway (Jones et al. 2009, Yaeger and Corcoran 2019). *NF1* loss-of-function (LOF) mutations, which occur in many cancer types, result in decreased neurofibromin GAP function, thus activating RAS (Basu et al. 1992). Tovorafenib is an investigational, selective, CNS-penetrant, small molecule type II RAF inhibitor which inhibits both RAF monomers and dimers and has been shown preclinically to impact the *KIAA1549::BRAF* fusion (Sun et al. 2017). In this study, the impact of tovorafenib was explored in a *Nf1[flox/flox]; Postn-cre* genetically modified mouse model (GEMM) harboring *NF1* LOF mutations specifically in Schwann cells. *Nf1[flox/flox]; Postn-cre* mice develop PN with 100% penetrance by 4 months of age. These PN recapitulate the development and progression of PN in human NF1 patients (Rhodes et al. 2019).

Tovorafenib 25 mg/kg or vehicle was orally administered daily to *Nf1[flox/flox]; Postn-cre* mice for 12 weeks and was well tolerated. After two weeks of treatment, a subset of mice were microdissected and pERK levels in the proximal nerve tissue were measured by western blot. Tovorafenib attenuated NF1-related ERK hyperphosphorylation in the proximal nerve tissues. Tumor burden was then assessed after 12 weeks of treatment by measuring peripheral proximal nerve volume and PN quantitation. Tovorafenib treatment did not reduce the tumor burden by either metric. Subsequent kinomic and transcriptomic studies are underway to characterize the mechanism(s) involved.

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Multiomic Analyses Reveal New Targets of Polycomb Repressor Complex 2 in Schwann Lineage Cells and Malignant Peripheral Nerve Sheath Tumors

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Neurofibromatosis Type 1 syndrome (NF1) patients are strongly predisposed to malignant peripheral nerve sheath tumor (MPNST) development. In NF1 syndrome, MPNSTs develop from benign precursors called atypical neurofibromas (ANF) which themselves form after somatic loss of the wild-type *NF1* allele, resulting in increased Ras-GTP activated signaling, and *CDKN2A* and *CDKN2B*[1]. This transformation is still not completely understood, but loss of the polycomb repressor complex 2 (PRC2) is a common event during the transition from ANF to MPNST[2]. SUZ12, EED and EZH2 are core components of PRC2, which is responsible for trimethylation of Histone H3 at lysine 27 (H3K27me3), a repressive epigenetic mark that silences genes through formation of heterochromatin. We hypothesized that loss of PRC2 has direct and indirect effects on gene expression, promoting MPNST development by affecting cell differentiation, survival, and proliferation. **The purpose of this study was to identify potential drivers of MPNST and therapeutic targets using multiomics. We engineered NF1-deficient human Schwann cells (iHSCs) with or without concomitant loss of function *SUZ12* or *EED* mutations.** We found major epigenomic changes in the histone code of *SUZ12* mutants including complete loss of H3K27me3 with concomitant gain in H3K27ac. **RNA sequencing revealed 1,210 genes are derepressed by PRC2 loss in *NF1*-deficient iHSCs and are potential mediators of the MPNST phenotype. Of these genes, 145 are differentially expressed when comparing ANF to MPSNT. Also, 172 of these genes are repressed by re-expression of *SUZ12* in *SUZ12*-negative MPNST cell lines which we coined as direct and indirect targets of PRC2. Chromatin immunoprecipitation (ChIP) sequencing of H3K27me3 and H3K27ac was used to identify direct versus indirect targets of PRC2. We found 81% of genes derepressed by loss of PRC2 are direct targets.** Pathway enrichment analysis on differentially expressed genes indicates many upregulated cancer related pathways when PRC2 is lost. **We found cell adhesion molecules, steroid hormone biosynthesis, Hedgehog signaling, NOTCH signaling, and various immune regulatory pathways enriched in PRC2-deficient iHSCs. NOTCH signaling has been implicated in Schwann cell development. NOTCH pathway members may be worthy candidates for drug targeting.** Strikingly, the downstream regulator of NOTCH signaling, HES1 was highly expressed in PRC2-deficient MPNSTs and patient-derived xenograft models. We confirmed these findings with Western blot, various transient cell line models, and drug assays.

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MEK Inhibition as a Therapeutic Strategy for the Neurobehavioral Manifestations of NF1

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Neurofibromatosis type 1 (NF1) is a neurodevelopmental disorder mainly characterized by benign tumors of the nervous system. It was recently found that pharmacologically inhibiting mitogen-activated protein kinase kinase (MEK) successfully shrinks NF1-associated tumors, and MEK inhibitors have now been approved for this purpose. However, individuals with NF1 also experience sleep disturbances, hypersensitivity to touch, learning difficulties, growth defects, and increased rates of ADHD and autism. Although these non-tumor manifestations affect up to 80% of the NF1 population, the underlying mechanisms remain largely unknown, and no treatments are currently available. Interestingly, these traits are highly conserved across species, having been well documented in *Drosophila* models of NF1. Here, we leverage this powerful animal model to test whether MEK inhibition is a viable strategy to improve non-tumor manifestations of NF1.

Using the genetic accessibility of Drosophila, we first used RNAi to knockdown MEK expression specifically in neurons of *Nf1* mutant flies and assessed effects on social behavior, repetitive grooming behavior, circadian rhythms, cognition, and sensitivity to touch, all of which have been shown to be impaired in fly models of the disorder. Our results show that decreasing MEK expression in *Nf1*-/- flies reduces atypical social behavior and repetitive grooming, and improves defects in circadian rhythm, olfactory associative learning, and sensory responsivity.

Next, we began to explore whether the MEK pathway can be targeted pharmacologically to treat non-tumor phenotypes. We administered MEK inhibitor cobimetinib to *Nf1*-/- flies throughout development and observed rescue of the pupal growth defect of *Nf1*-/- flies. This initial finding raises the possibility that behavioral deficits described above may be amenable to pharmacological MEK inhibition.

Together, these results provide molecular insights into neurobehavioral phenotypes in NF1, suggesting that MEK activity plays a role. MEK-inhibitors have already shown success in treating NF1-associated tumors, and our work demonstrates their potential for treating a wide variety of other symptoms.

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Synthetic Lethal Screens Identify Selective and Non-Selective Autophagy as a Therapeutic Option in *NF1*-Deficient Tumors

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Neurofibromatosis type 1 (NF1) is a diverse and systemic genetic condition resulting in multiple symptoms, including the formation of benign tumors within the peripheral nervous system. There are few therapeutic options for treating these tumors, resulting in a clinical unmet need for identifying novel treatment strategies. To address this, we have performed synthetic lethal screens in two model organisms to identify novel therapeutic targets. Synthetic lethality occurs when two mutated genes are independently viable, but when co-expressed, result in cell death. These synthetic lethal screens both identified inhibition of either selective or non-selective autophagy as a potential therapeutic strategy in NF1.

In a high-throughput synthetic lethal screen carried out in *S. cerevisiae,* we screened for compounds that were synthetic lethal with loss of an ortholog of *NF1 (IRA2)* and identified several lead compounds, including the small molecule Y102. Treatment of cells with Y102 perturbed autophagy, mitophagy, and lysosome positioning in NF1-deficient cells. Utilizing two different proteomics approaches, click chemistry and a cellular thermal shift assay, we identified the BORC complex, which is required for lysosome positioning and trafficking, as a potential target of Y102. siRNA knockdown of a BORC complex subunit recapitulated the phenotypes observed with Y102 treatment, and colocalization was observed between azide-tagged Y102 and a subunit of the BORC complex.

In a separate synthetic lethal screen, we made use of a novel *NF1*-deficient *Drosophila* cell line generated using CRISPR gene editing. A genomic RNAi library was used to screen for perturbation of pathways resulting in specific vulnerability for NF1-deficient cells compared to wild type controls. This revealed five candidate genes which could be targeted with existing drugs, including autophagy inhibitors. Inhibition of autophagy using chloroquine (CQ) resulted in reduced cell viability in a panel of human *NF1*-deficient cell lines and a *Drosophila in vivo* model at concentrations lower than those currently used for treatment of malaria, while combination treatment with CQ and the MEK inhibitor, selumetinib, resulted in further reduction of *NF1*-deficient cell viability. Additionally, we have validated CQ in a mouse xenograft model and found that it outperforms selumetinib by about 2-fold in this system.

Together, these studies provide evidence that inhibiting selective and non-selective autophagy may provide therapeutic strategies for NF1-deficient tumors.

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Disclosures: SJB has two patents on the small molecule Y102.

Unlocking Neurobiological Insights of NF1 Using Patient-Derived Stem Cells

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Study Purpose: To identify the molecular mechanisms driving the neurodevelopmental challenges in NF1 using patient stem cell derived neuronal models

Methods: As part of a cross-sectional study, we have recruited 200 children with NF1 and collected extensive cognitive and behavioural data to classify their neurodevelopment profile. From this study we have selected 12 individuals, 6 that have NF1 and 6 that have NF1 and co-occurring neurodevelopment issues to assess the neurobiological mechanisms that may be contributing to NF1-related neurodevelopmental issues. Whole exome sequencing was used to determine the pathogenic NF1 variants. We used CRISPR Cas9 gene editing technology to simultaneously reprogram and edit peripheral blood mononuclear cells into induced pluripotent stem cells (iPSCs). The gene edited iPSCs serve as an isogenic control for each patient line. Flow cytometry and immunofluorescence were used to validate pluripotency of the iPSCs and differentiation potential was validated using an embryoid body protocol. iPSC derived neurons were generated using a lentivirus based NGN2 differentiation protocol. Protocols for immunofluorescence assays and multielectrode array protocols have been established to model structural and functional deficits.

Results: We have generated 12 NF1 patient iPSC lines and their gene corrected isogenic controls. The pluripotency and their potential to differentiate into the three germ layers, ectoderm, endoderm and mesoderm was validated. The differentiation of iPSC into cortical neurons have been established and we have developed an extensive pipeline of molecular techniques that will be used to model key aspects of neuronal development, morphology and function in our NF1 patient lines and their isogenic control iPSC lines. These include immunofluorescence assays to visualise structural deficits particularly focusing on number of branching, branch length as well as puncta. Multielectrode arrays which enable the measurement of electrical activity of neuronal networks in real time to assess functional deficits.

Conclusions: We have established and validated human iPSC-derived neuronal models to understand key aspects of neuronal development, morphology, and function in NF1. These models and study outcomes will enable future high throughput drug screening analyses to identify targeted therapies to treat the causes of the neurodevelopmental issues in NF1.

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Obesogenic Diet Exposure Modulates Risk of Nf1-OPG Formation Induced by Specific Germline Mutation

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Neurofibromatosis Type 1 (NF1) is a cancer predisposition syndrome caused by germline mutations in the *NF1* gene. Children with NF1 have ~15-20% risk of developing low-grade gliomas of the optic pathway (NF1-OPG). However, it is currently unclear which children will develop tumors. Recent studies have demonstrated that an individual's specific *NF1* germline mutation may contribute to their risk, with some mutations associated with higher rates of brain tumor development than others. However, germline mutation alone is insufficient to explain the degree of phenotypic heterogeneity in Nf1-OPG formation and outcomes, suggesting that other factors may also contribute to an individual child's risk. One such factor may be maternal obesity; in the general population, children of obese mothers develop several types of tumors at increased rates, including brain tumors. Importantly, obesity in the U.S. is driven in large part by diet, given the abundance and accessibility of high-fat, high-sugar food choices. Based on these data, we hypothesized that maternal obesogenic exposure would modulate the risk of Nf1-OPG formation for a given germline mutation.

Methods: Utilizing a series of Nf1-OPG mouse models, we exposed dams and offspring to an obesogenic high-fat, high-sucrose diet (Ob) to mimic dietary conditions prevalent in the U.S., with control diet-exposed (CD) animals as a comparison. Offspring were continued on their respective maternal diets and optic nerves analyzed at 6w-3mo.

Results: We demonstrated that progeny from Ob dams displayed increased glioma formation compared to CD in two low-penetrance models of *Nf1*-OPG (C383X[KO] and G848R[KO]). The latter model does not typically form tumors, but did so at a low rate with Ob-exposure. This exposure also resulted in earlier tumor onset in a high-penetrance *Nf1*-OPG model (R681X[KO]). We then analyzed optic nerves to determine if the increased risk of tumor formation in Ob mice occurred through further upregulation of paracrine signaling circuits previously shown to contribute to Nf1-OPG growth. Increased T-cells and microglia were observed in the Ob-exposed optic nerves, consistent with this hypothesis. However, Ob exposure did not result in increased expression of the cytokine genes *CCL2, CCL4, CCL5,* or *Mdk,* key mediators of the abnormal paracrine circuit created by *Nf1* germline mutation.

Conclusion: These findings demonstrate that Ob exposure increases the risk of Nf1-OPG formation for a given germline mutation. However, it does not so through further upregulation of the abnormal paracrine circuits created by *Nf1* mutation, suggesting that an alternative mechanism underlies this effect.

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Importance of *SPRED1* in Cutaneous Melanoma

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Background: Inactivating germline *SPRED1* mutations cause Legius syndrome, characterized by pigmentation abnormalities reminiscent of neurofibromatosis type 1 (NF1) [1]. *Spred1-/-* mice show hyperpigmentation, similar to conditional *Nf1* knockout mice [2], suggesting *Spred1* loss affects normal melanocyte function. *NF1,* classified as one of the most frequently mutated genes in cutaneous melanoma (CM) [3], encodes neurofibromin which interacts with SPRED1, thereby negatively regulating RAS-MAPK pathway [4,5]. *SPRED1* alterations were found in 3-4% of CM and low *SPRED1* expression correlates with poor prognosis as well as resistance to MAPK-targeted therapies [6,7]. However, the role of *SPRED1* in CM remains understudied.

Purpose/aim: To investigate the role of *SPRED1* in melanogenesis and melanomagenesis.

Methods: To assess the role of *Spred1* in melanogenesis, the amount of melanin and melanin-producing melanocytes in the interfollicular epidermis of *Spred1-/-* and WT mice were quantified and compared. Additionally, *Spred1* was inactivated at postnatal day 23, 25 and 28 in *TyrCre;Spred1$^{flox/flox}$* mice to induce and investigate hyperpigmentation. CM was induced in *Braf$^{CA/+}$;Pten$^{flox/flox}$* and *Braf$^{CA/+}$;Ink4a$^{-/-}$* mice crossed with *Spred1$^{flox/flox}$* mice (*Braf;Pten;Spred1* and *Braf;Ink4a;Spred1* respectively). The effect of *Spred1* loss on melanoma initiation and dermal invasion was assessed through lineage tracing of fluorescently-labelled melanoma cells in the tail interfollicular epidermis of *Braf;Pten* and *Braf;Pten;Spred1* mice. *Braf;Ink4a(;Spred1)* mice were monitored weekly to assess CM initiation and progression. Differential gene expression and enrichment analyses were performed on RNAseq data from *Braf;Ink4a;Spred1* and *Braf;Ink4a* back skin tumours. CM metastasis was assessed by immunohistochemistry and fluorescent microscopy.

Results: Tail skin hyperpigmentation in *Spred1-/-* mice was caused by a significant increase in melanin and melanin-producing melanocytes in the interfollicular epidermis. In *TyrCre;Spred1$^{flox/flox}$* mice hyperpigmentation was absent. Inactivating *Spred1* in *Braf;Pten* mice did not accelerate CM initiation and dermal invasion. However, Spred1 loss in *Braf;Ink4a* mice significantly increased the risk for CM development to 87% with a latency of 3,3 months compared to 21% and 7 months in *Braf;Ink4a* mice, but without metastasis. Enrichment analysis showed significant upregulation of immune related Gene Ontology (GO) terms and significant downregulation of melanogenesis and skin development GO terms.

Conclusions: *Spred1* loss increases melanogenesis, however results indicate importance for *Spred1* in melanogenesis in early murine development. Effect of *Spred1* inactivation on melanomagenesis was redundant in *Braf;Pten* mice, due to aggressive and early melanoma onset. Loss of *Spred1* in *Braf;Ink4a* mice significantly increased CM penetrance and reduced latency. Further analysis will focus on understanding the molecular and functional behaviour of these melanoma cells.

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Role of CDKN2a Co-Deletion in Driving Tumor Invasiveness in NF1 Mutant Glioma

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Background: Neurofibromatosis type 1, caused by mutations in the NF1 gene, is a common autosomal dominant genetic disorder associated with an increased risk of glioma tumor development. A high occurrence of CDKN2a codeletion in NF1-mutant high-grade gliomas (HGG) compared to low-grade gliomas (LGG) implies a potential role of CDKN2a loss in the transformation of NF1-mutant LGG to HGG. In our study, we aimed to elucidate the role of NF1 and CDKN2a in tumor transformation using a panel of glioma cell line models with varying NF1 and CDKN2a expression.

Method: A diverse panel of glioma cell lines, encompassing LGG (1667-ON, RES259, RES186, JHH-NF1-PA1) and HGG (GBM511, GBM110, SF8628, U87-MG, TM-31, 7316-2189, 7316-3058, 7316-4917), was cultured. Their growth rates and invasiveness were further evaluated using cell proliferation and Boyden chamber invasiveness assays.

Results: LGG cell lines exhibited relatively lower growth rates and invasiveness compared to their high-grade counterparts. Intriguingly, RES259, a LGG cell line harboring both NF1 and CDKN2a variants, demonstrated the highest growth rate and invasiveness among LGG cell lines, surpassing even those with intact NF1 and CDKN2a, such as JHH-NF1-PA1 and RES186. To further elucidate the roles of NF1 and CDKN2a, CRISPR-Cas9 technology was employed to knockout these genes individually and in various combinations in RES186. Our findings unveiled that sole NF1 knockout had negligible impact on RES186. Conversely, CDKN2a knockout increased both growth rate and invasiveness. Strikingly, the codeletion of NF1 and CDKN2a in RES186 significantly exacerbated its growth rate and invasiveness. These results underscore the intricate interplay of NF1 and CDKN2a in governing the growth and invasiveness dynamics of glioma cells.

Conclusion: In conclusion, our preliminary study suggests that the concurrent loss of NF1 and CDKN2a promotes enhanced growth and invasiveness in gliomas, shedding light on the collaborative role of these genetic alterations in tumor transformation.

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The Refinement of Hexokinase 2-Targeting Peptides as an Anti-Neoplastic Approach for Malignant Peripheral Nerve Sheath Tumor Treatment

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Objective: In several neoplasms Hexokinase 2 (HK2), the first glycolytic enzyme, resides in Mitochondrial Associated Membranes (MAMs) where it shields tumor cells from death. We have previously proven that HK2 dislodgment from MAMs with a specific peptide represent a novel antineoplastic approach that efficiently triggers massive apoptotic death of human MPNST-derived cells. Here, we have further developed this technology by designing an activatable pro-peptide (HK2pep) suitable for *in vivo* application. HK2pep carries a protease cleavage sequence to specifically release the active form in tumors. Modulating this cleavable site, it is possible to adjust the HK2pep delivery to the MPNST microenvironment. Since this technology is flexible and adaptable and it is known that MPNSTs express specific Matrix Metallo-Proteinases (MMPs), the aim of this project is to tailor the anti-HK2 antineoplastic approach to MPNSTs to set the stage for future clinical applications.

Methods: We analyzed HK2 and MMPs expression in human and murine samples mining MPNST public dataset and performing immunohistochemistry and western blot analysis. We treated several MPNST cell lines with the active portion of HK2pep and measured MPNST cell viability. We designed and synthetized HK2pep variants suitable for MPNST delivery *in vivo.* First, we tested the efficacy of these new HK2peps in *in vitro* tumorigenic assays (*foci* and spheroid formation). Then, we selected the most effective peptide variant for subsequent administration in MPNST-bearing mice.

Results: Our study shows that HK2 is expressed by 80% of human MPNSTs, it is present in MPNST xenografts and in MPNST-derived cells, which are efficiently killed by the active form of the anti-HK2 dislodging peptide. According to the MMPs expression revealed in human and xenograft MPNST samples, we designed new HK2peps variants carrying different MMPs cleavable sites. All variants reduced *foci* formation but only two variants decreased spheroid size. The most promising HK2pep variant was administered to mice bearing xenograft MPNST finding a relevant impairment in MPNSTs growth.

Conclusions: This project identifies HK2 as a good target to be employed against MPNST cells and highlights that it is possible to tackle MPNSTs *in vivo* with a wise tailoring of HK2peps. HK2pep and its adaptive variants represent a promising and innovative antineoplastic platform to target MPNST according to its microenvironment. Furthermore, if required, this technology allows the combination of two or more HK2pep variants for an optimal delivery of the active drug.

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Identification of Novel Biomarkers and Therapeutic Candidates in Neurofibromatosis Type 1-Associated Malignant Peripheral Nerve Sheath Tumor

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Neurofibromatosis type 1 (NF1) exhibits diverse neurological, skeletal, and neoplastic manifestations, especially with an increased risk of tumors in the central and peripheral nervous systems. Malignant peripheral nerve sheath tumor (MPNST) is an aggressive soft tissue sarcoma with high invasiveness, posing a significant mortality risk for patients with NF1. MPNST affects approximately 8-13% of NF1 patients, with a 5-year survival rate below 50%. Complete resection is not possible in many cases, and despite surgical resection, MPNST recurrence remains a concern. Current treatment strategies lack specific targeting for MPNST, emphasizing the need for novel therapeutic approaches and biomarkers to help its early detection. In this study, we aimed to find the new biomarkers and therapeutic targets for MPNST through proteomic analysis in the paired samples of benign NF and MPNST in NF1 patients. Utilizing high-resolution liquid chromatography–mass spectrometry, we identified 141 proteins specific to MPNST samples, contrasting with 23 proteins detected only in benign NF tissues. Among them, our study focused on elevated Matrix metallopeptidase 8 (MMP-8), a neutrophil collagenase, in MPNST by immunohistochemical (IHC) analysis, along with the increased MMP-8 levels in plasmas of the MPNST patients. Additionally, several therapeutic candidates were found to be overexpressed in MPNST compared to benign NF, validated by IHC analysis. Together, our results indicate the novel biomarkers and therapeutic candidates for MPNST treatment.

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Human Induced Pluripotent Stem Cell (iPSC) and Murine Immune-Proficient Preclinical Models of Atypical Neurofibroma for Evaluation of Small Molecule and Immune Therapies

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There are currently a small number of NF1-associated atypical neurofibroma (ANF) models despite their importance as the likely premalignant precursor to life-threatening malignant peripheral nerve sheath tumors (MPNST). Many ANFs are thought to arise from benign *NF1*^{-/-} plexiform neurofibromas (PNFs) through loss of *CDKN2A/CDKN2B* which causes defects in cell cycle regulation and p53 response pathways in the face of RAS pathway hyperactivation. To identify potential vulnerabilities following loss of these genes, we used CRISPR/Cas9 to generate gene knockout combinations of *NF1, CDKN2A,* and *CDKN2B* in human induced pluripotent stem cell derived Schwann lineage cells (iSC). The resulting mutant iSCs and their precursors were treated with drugs we have previously found to be selectively lethal to *NF1* deficient immortalized Schwann cells or are mechanistically rational based on ANF genetics. Additionally, we sought to develop a novel immune-competent murine model of ANF to test small molecule inhibitors or immune therapies *in vivo* in an *Nf1*^{+/-} microenvironment. To this end, we created the following mice: *Dhh-Cre; Nf1*[flox/-]; *Rosa26-Lox-STOP-Lox-Cas9-IRES-eGFP*. These mice harbor heterozygous *Nf1*^{+/-} somatic cells and *Nf1*^{-/-} Cas9-expressing GFP+ Schwann cells. Fluorescence activated cell sorting (FACS) of GFP+ primary Schwann cells from adult quad peripheral nerves yielded pure *Nf1*^{-/-} Schwann cells that expressed Cas9 and S100b. These *NF1*^{-/-} Schwann cells could be cultured upwards of 30 passages and seemed to spontaneously transform into a MPNST-like state after extended passaging. Targeted *in vitro* knockout of *Cdkn2a/b* achieved 87-95% editing efficiency at each locus. Implantation of *Nf1*^{-/-} or *Nf1*^{-/-} + *Cdkn2a/b*[-/-] Schwann cells into the sciatic nerve pocket of *immunocompromised* mice led to 100% tumor penetrance with no significant difference in survival. Both *Nf1*^{-/-} and *Nf1*^{-/-} + *Cdkn2a/b*[-/-] Schwann cells were also implanted in the sciatic nerve pocket of *Nf1*^{+/-} *immunocompetent* recipient animals. Recipients of *Nf1*^{-/-} + *Cdkn2a/b*[-/-] Schwann cells demonstrated 100% tumor penetrance and a significant decrease in latency compared to recipients of *Nf1*^{-/-} Schwann cells, suggesting an increased ability of ANF-like tumors to avoid detection by the immune system. Additional efforts are ongoing to characterize the expression profiles of primary mouse Schwann cells, primary tumors, and secondary tumor derived cell lines harvested from both immunodeficient and immunocompetent recipient animals. Studies utilizing this model to interrogate drug sensitivities in ANF-like tumors are also ongoing. Future studies using this model include characterizing the immune landscape and identifying potential targets for immunotherapeutic intervention, all in the context of an immune proficient *Nf1*^{+/-} microenvironment and immune system.
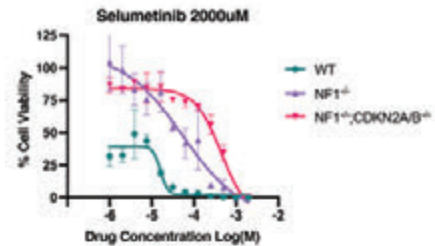
Figure 1: A 12-point two-fold dose response curve for the MEK-inhibitor Selumetinib performed on iPSC-derived Schwann cell precursors demonstrating increased resistance of mutant cells to treatment. Cells were treated with compound for 48h at the noted concentrations in triplicate and viability was assessed via Alamar blue staining. The highest compound dose is noted in graph title.
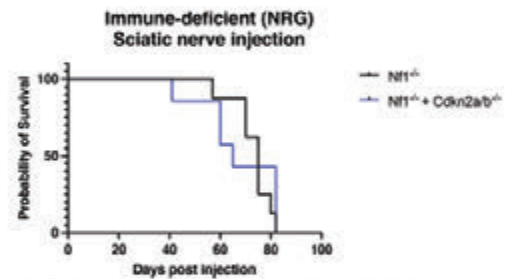
Figure 2: Kaplan-Meier survival plot of immune *deficient* NRG mice receiving sciatic nerve pocket injections of murine Schwann cells of the indicated genotype. (n > 5 per genotype).
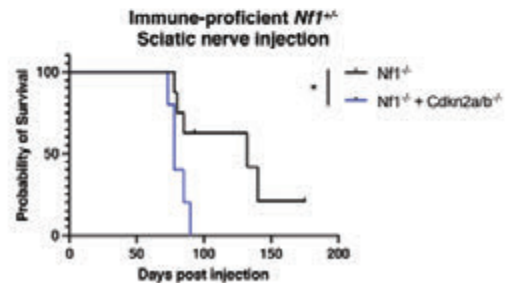
Figure 3: Kaplan-Meier survival plot of immune *proficient Nf1*^{+/-}; *Dhh-Cre; R26-LSL-CAG-Cas9-IRES-eGFP* mice receiving sciatic nerve pocket injections of murine Schwann cells of the indicated genotype. (n > 5 per genotype, * = p < 0.05).

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Fate Mapping of the Early Steps of Malignant Transformation in NF1

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Neurofibromatosis type 1 (NF1) is a genetic disease caused by mutations in the NF1 tumor suppressor gene. A common symptom of NF1 is the development of benign nerve sheath tumors, neurofibromas, due to the loss of NF1 in the Schwann cell lineage. Approximately half of NF1 patients will develop plexiform neurofibromas (pNFs), and 10% of cases will progress to malignant peripheral nerve sheath tumors (MPNST), with no effective treatment to date. Malignant progression of pNFs passes through a transient and poorly characterized pre-malignant state (pmNFs).

We designed an NF1 mouse model (NF1-KO) developing a full spectrum of nerve tumors, including pNFs and their stepwise progression to MPNSTs. We explored this model extensively and discovered that: (i) the glial-mesenchymal transition (GMT) in tumor cells is the earliest molecular event and signature of malignant progression. GMT is characterized by the extinction of glial (Sox10, S100ß) cells and the activation of mesenchymal markers, notably the transcription factor Sox9, making this factor a biomarker of GMT and a potential therapeutic target (ii) GMT is followed sequentially by the acquisition of a bi-allelic loss of Cdkn2a (Cdkn2a -), then of pathogenic variants in other tumor suppressor genes such as p53.

The aim of this study is to decipher the mechanisms governing GMT in tumor cells, our hypothesis being that targeting this switch in pmNFs could prevent/delay malignant transformation.

To this end, we subcutaneously transplanted tumor cells from tumors at different stages of malignancy into nude recipients and showed that: (i) Sox9+ tumor cells isolated from MPNSTs give rise to rapidly growing MPNSTs, (ii) Sox9+ tumor cells isolated from "late stage" pmNFs progressively evolve into MPNSTs, (iii) Sox10+, S100ß- tumor cells isolated from "early stage" pmNFs also evolve, through GMT, into MPNSTs and finally (iv) Sox10+, S100ß+ tumor cells isolated from pNFs give rise to slow-growing tumors without transition to MPNSTs at 8 weeks. We conclude that extinction of S100ß in tumor cells might play an important role in their malignant progression.

To further explore the mechanisms associated with S100ß extinction and GMT we cultured tumor cells isolated from "early stage" pmNFs, assessing their proliferation potential and the impact of passaging on their GMT. Subsequently, these cells will be grafted and characterized, providing a potential timely controlled model to explore new insights into the molecular dynamics and mechanisms underlying the malignant transition.

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Mechanistic Insights into Neurofibromin Function from Pathogenic NF1 Missense Mutations

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Neurofibromatosis type 1 (NF1) is a genetic disorder that affects 1 in ~3,500 newborns. NF1 symptoms occur in multiple body tissues, and among the most serious are the Schwann cell-derived plexiform neurofibromas that can transform into malignant peripheral nerve sheath tumors (MPNSTs). Over 3,000 distinct pathogenic mutations have been identified within the *NF1* gene. Missense mutations (MMs) constitute the most significant subset of genetic abnormalities, comprising approximately 38% of cases. Unlike nonsense mutations, the pathogenic mechanisms of NF1 MMs remain unclear. Recent research has proposed that one potential consequence of NF1 MMs might be related to the decreased stability of neurofibromin dimers. We focus on elucidating novel mechanisms of how MMs within specific neurofibromin domains affect their function. To investigate this, we have engineered immortalized human Schwann cells (SCs) using CRISPR-based gene editing strategies to introduce epitope tag–encoding DNA into the N- or C-termini of the *NF1* gene. Molecular analysis and western blotting have confirmed that the levels of endogenous neurofibromin tagged with HiBiT or FLAG remain unchanged in these cell lines. Furthermore, since the 11 amino acid HiBit epitope tag can produce a bioluminescent signal upon binding to its complementation partner, LgBiT, we have used luciferase assays of SC NF1-HiBIT lines to quantify neurofibromin levels, thus establishing a platform to investigate the effects of MMs on neurofibromin stability. Additionally, we have employed prime editing to introduce specific NF1 pathogenic MMs into NF1-HiBiT SC lines. Immunoprecipitation using the tagged neurofibromin from engineered cell lines and subsequent proteomic analysis is underway to identify novel potential interactors of neurofibromin that may be disrupted by pathogenic mutations. This approach may reveal new functions and potential pathways that could be targeted for therapeutic intervention.

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Assessment of the Impact of NF1 on Quality of Life in People of 65 Years or More

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Investigating individuals with Neurofibromatosis type 1 (NF1) aged over 65, our study aims to address the under-characterization of this specific demographic. Our objective is to assess and characterize the quality of life in this population segment, highlighting the need for a more comprehensive understanding of their health and well-being.

Conducted as a cohort analysis, our study involved the identification and characterization of all individuals aged 65 or older diagnosed with NF1 in our database. Demographic information and quality of life parameters were examined. The statistical analysis was descriptive due to constraints in sample size, offering an understanding of the current impact of NF1 on the quality of life. Inclusion criteria encompassed individuals aged 65 or older with a diagnosis of NF1, who had been admitted to the clinic until December 31, 2023, and could provide consent to respond to the questionnaire. The data collection instrument was the translated and validated Portuguese version of the questionnaire on the impact of NF1 on Quality of Life (INF1-QOL).

Out of the 32 individuals aged 65 or older diagnosed with NF1 and admitted to the clinic until December 31, 2023, 10 were excluded due to death, 6 due to incapacity to provide consent for the questionnaire, and 3 due to refusal. Consequently, a sample of 13 participants, averaging 71 years of age, was considered. Anxiety and depression (85%), bone-related problems (77%), pain (69%) and problems with mobility/walking (69%) were the items that were scored most frequently by the participants. Pain was considered the symptom with the highest impact on quality of life. There was moderate or severe pain intensity in 6 of the participants (31%) and 4 (31%) reported experience of pain quality that interferes moderately with daily activities or prevents those activities. Only one participant reported moderate or severe issues regarding the impact of NF1 on their role and outlook on life, and two participants expressed concerns about the cosmetic appearance of neurofibromas. Study limitations include a small sample size, a single administration of the questionnaire, telephone-based application, and challenges in distinguishing whether the impact on quality of life (QOL) is attributed to NF1 or concurrent comorbidities.

Assessing the QOL in older patients is important, emphasizing the significance of considering the impact of NF1 in this specific population. NF1 may have unique implications in advanced age, underscoring the need for a comprehensive approach in evaluating QOL.

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Standardization of the Preclinical Trials Targeting Cutaneous Neurofibromas in the *Nf1-KO* Mouse Model

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Cutaneous neurofibromas (cNF), present in 95% of individuals with neurofibromatosis 1 (NF1), can significantly alter their quality of life. Promising drugs are being tested. Our laboratory has generated and characterised a *Nf1-KO* mouse model which faithfully recapitulates these tumors and allows to test different candidate compounds. However, robust methods are essential in preclinical assays to ensure reliable results that can be extrapolated to clinical trials.

The aim: to develop and validate a standardized method for the evaluation of cNF in the *Nf1-KO* mouse model.

Two therapeutic strategies have been implemented to test the capacity of drugs on shrinking cNF (curative strategy) or preventing their development (preventive strategy). In both, the evaluation of cNF consist in 3 steps. First, 2D macroscopic pictures taken under epifluorescence microscopy for precise visualization of the back skin of anesthetized mice, where cNF develop. Indeed, in this model, the NF1 mutant Schwann cells (SC), at the origin of cNF, express Tomato reporter. Second, immunofluorescent (IF) profiling of sections of cNF after animal sacrifice with a panel of markers (SC and microenvironment). According to these two steps, a third of FACS analysis is eventually carried out. These 3 steps need to be standardized by: defining outcomes and endpoints, and automating the methods of data acquisition and analysis. In parallel, defining homogenous cohorts of animals and a rigorous monitoring of treatment tolerance and animal well-being are necessary.

Table 1: Definition of the macroscopic, pathological and cellular endpoints for preclinical trials targeting cutaneous neurofibromas in the Nf1-KO mouse model. *Imaging endpoints should be defined as primary endpoint whereas **pathological and cellular as secondary endpoints. Abbreviations: complete response (CR), cutaneous neurofibroma (cNF), partial responses (PR), progressive disease (PD), stable disease (SD), total fluorescent surface area (TFSA).

We defined endpoints (Table 1) and the corresponding outcomes: fluorescence intensity, total fluorescent surface area, and the number of cNF (macroscopic pictures), and fluorescence intensity, total Tom+ area, and the number of Tom+ SC (IF pictures). We developed an automated analysis script to assess the previously defined outcomes. We compared the results of the scripts to those generated manually (two blinded investigators). We confirmed the perfect reproducibility of the automated method (intraclass correlation coefficient), that the results were strongly correlated with those generated manually (Spearman's coefficient), with a good level of agreement (Bland-Altman diagram). In addition, we defined a minimum set of antibodies to be used for FACS analysis: Tomato (SCs), Epcam and CD90 (fibroblasts), CD45 (immune cells).

This study offers a standardized procedure that allows reproducible and robust results by minimizing the variability of preclinical trials in *Nf1-KO* mice. This process is paramount if we seek to compare the efficacy of different treatments. The next step will be to apply this method to the upcoming preclinical trials in our laboratory.

Examining the Role of Conserved Neuronal Activity Pathways in the Progression of Neurofibromatosis Type 1-Associated Plexiform Neurofibroma

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PN, in themselves, are a leading cause of morbidity in Neurofibromatosis Type 1 (NF1), often disfiguring or threatening vital structures. During formation of plexiform neurofibroma (PN), a complex tumor microenvironment (TME) develops, with recruitment of multiple neoplastic and non-neoplastic cell types being critical for growth and progression. We applied single cell transcriptomic analysis paired with spatial transcriptomics to PN to provide a clearer understanding of the complex TME harbored by PN.

Single-nuclei RNA-sequencing (snRNA-seq) was applied retrospectively to 9 frozen PN to provide a large enough sample cohort required to adequately describe the disease TME. Additionally, 4 frozen PN samples were OCT embedded and spatial transcriptomics (ST) was run, adding morphological context to the transcriptomic data generated. SnRNA-seq analysis definitively charted the heterogeneous cellular subpopulations in the PN TME, including predominant stromal fibroblast populations and a smaller population of neoplastic non-myelinating Schwann cells (NMSC), the putative PN cell of origin. PN have a remarkable amount of inter-sample homogeneity regarding cellular subpopulation proportions despite being resected from a variety of anatomical locations. ST analysis showed mapping of novel fibroblast and PN subpopulations to classic histological features in aberrant nerve fascicles and surrounding by areas of prototypical neurofibromatomous growth. Interestingly, a close interaction between NMSC and fibroblasts was observed based on ST spot deconvolution in multiple regions, including endo-, peri- and epineurial zones. Schwann cell/fibroblast interactions were further characterized by ligand/receptor interaction analysis (CellChat) that was applied to snRNA-seq data. A high probability of Neurexin 1/Neuroligin 1 (NRXN1/NLGN1) ligand-receptor cross-talk was predicted between NMSC and fibroblast subpopulations, respectively. Elevated NRXN1 expression is seen in PN associated NMSC compared to normal NMSC. Preliminary data using novel *in-vitro* functional validation illustrated an increased rate of growth of PN cells in the presence of fibroblasts alone, supporting a role for fibroblasts in propagating PN-NMSC tumor growth, potentially through NRXN1/NLGN1 signaling. The NRXN1/NLGN1 pathway has never been described in PN, but has been observed in other NF1 associated tumors, and may indicate a clear and direct communication pathway between putative PN NMSC cells of origin and surrounding cancer associated fibroblasts, potentially driving disease progression. This finding could provide translational therapy options for patients with these devastating tumors of childhood and early adulthood.

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Development of an Adeno-Associated Virus (AAV) Toolkit to Modulate Signaling Pathways Altered in Neurofibromatosis Type 1 (NF1)

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Neurofibromatosis type I (NF1) is an autosomal dominant disorder caused by loss of function mutations in the *NF1* gene that encodes the neurofibromin protein. Neurofibromin is a GTPase activating protein that acts as a negative regulator of the Ras-mitogen activated protein kinase (MAPK; Ras-Raf-MEK-ERK) signaling cascade. The clinical phenotype of NF1 is characterized by a predisposition to peripheral nerve sheath tumors that can be locally destructive and have potential for malignant transformation. Targeted gene therapy that rescues neurofibromin function could provide a method for tumor prevention and treatment. Adeno-associated virus (AAV) represents an appealing vector for gene therapy; however, its small packaging capacity prevents delivery of the entire neurofibromin gene. Here, we describe the generation and *in vitro* validation of a viral toolkit featuring AAV-compatible transgenes that modulates MAPK signaling up- or downstream of Ras or affects other signaling pathways perturbed in NF1. This includes the design of a bicistronic expression vector that allows for easy identification of transduced cells with a nuclear localized YFP that is independent of the therapeutic transgene. The transgenes chosen have previously been shown to downregulate MAPK signaling or affect other pathways dysregulated in NF1. These include three functional variants of the neurofibromin GAP-related domain (GRD); four dominant negative mutant G-proteins (HRas, NRas, KRas, and Rac1); Sprouty-related, EVH1 domain-containing proteins (SPRED1, 2, and 3); dual specificity protein phosphatase 6 (DUSP6); dominant negative immediate early genes; the GsDREADD for orthogonal control of cAMP production; etc. We found that all versions of the GRD AAV vectors reduced growth factor stimulated ERK phosphorylation, proliferation of *NF1*-null immortalized human Schwann cells and *Nf1*-null primary mouse Schwann cells, and Schwann cell precursor (SCP) sphere formation relative to YFP control viruses. These effects were dose-dependent and acted primarily by reducing proliferation rather than inducing cell death. Dominant negative KRas and HRas were also efficacious in ERK phosphorylation, proliferation, and SCP sphere formation assays. We will present additional data regarding the validation and application of these vectors *in vitro* and in Rasopathy mouse models. We will also describe progress in the screening of AAV capsids for Schwann tropism in a mouse model of plexiform neurofibroma. Overall, these studies represent a necessary first step in the development of a diverse set of targeted AAV gene therapies for NF1.

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Targeting Valosin-Containing Protein Shrinks Plexiform Neurofibroma

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Neurofibromatosis type 1 (NF1) patients are predisposed to develop plexiform neurofibromas (PNFs). By cross comparison of RNA sequencing and RUNX1-CHIP sequencing data on mouse plexiform neurofibroma (PNF), we found that transcript encoding the NF1 interacting p97/valosin-containing protein (VCP) gene is overexpressed in PNF. Co-immunoprecipitation confounded that VCP bounded to NF1. Immunostaining confirmed VCP protein overexpression in both mouse and human PNFs. Treatment of primary mouse PNF Schwann cells and immortalized human PNF Schwann cells with CB-5083, a p97/VCP inhibitor, led to accumulation of poly-ubiquitinated proteins and generation of irresolvable proteotoxic stress. In vivo treatment with CB-5083 on the *DhhCre;Nf1^{fl/fl}* mouse model of PNF significantly inhibited cell proliferation, increased cell apoptosis and reduced neurofibroma volume. However, combination with MEK inhibitor did not increase the efficacy, supporting the idea that the proteotoxic stress in NF1 Schwann cells occurs downstream of Ras-MAPK signaling. The significant effects of VCP inhibition in this pre-clinical study suggest a potential novel therapy for patients with PNFs.

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Targeting Inflammatory Signaling in Cutaneous Neurofibromas

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The purpose of this study is to determine the role of epigenetic reinforcement of inflammatory RAS signaling in cutaneous neurofibroma (CNF) symptom evolution and tumor growth. CNFs are highly heterogeneous tumors comprised of multiple cell types including tumorigenic Schwann cells, as well as fibroblasts and immune cells. We recently demonstrated that CNFs support inflammatory pain signaling through epigenetically reinforced RAS/MKK3/p38 pathway activation that drives increased COX2 expression via chromatin remodeling, however the key cell types that promote these pain and inflammation pathways remain unknown. Using publicly available scRNA-sequencing data of CNFs we have identified a unique subpopulation of inflammatory and transcriptionally active fibroblasts that express high levels of COX2. To assess the therapeutic relevance of this finding, we developed a patient derived explant (PDE) *ex vivo* culture model of CNF tumors and normal skin from individuals with NF1. This model preserves the structural and cellular architecture of CNFs and shows robust maintenance of viability and molecular phenotype beyond one week. It also recapitulates the heterogeneity of immune cell infiltration and MAPK signaling observed in CNFs. Using CNF PDEs as a model system, we found that proliferation was associated with increased T cell infiltration. Further, we identified a pattern of reciprocal inflammatory signaling in CNF PDEs in which tumors rely on prostaglandin or leukotriene mediated signaling pathways. As proof of principle, we show that *ex vivo* glucocorticoid treatment reduced expression of pro-inflammatory genes, confirming CNF PDEs are a useful model for both mechanistic studies and preclinical drug testing. Collectively, these data suggest that targeting epigenetic reinforcement of inflammatory RAS signaling could reduce CNF symptoms. Future work will determine how corticosteroid treatment in combination with MEK inhibition alters genome wide DNA methylation and chromatin accessibility, as well as expression of inflammation and hormone regulated genes at the single cell level.

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The Potential of PacBio Long-Read Sequencing for NF1 Diagnostics: Identification of a Reciprocal Translocation Affecting *NF1* Transcripts is a First Promising Result

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Background: Comprehensive mutation analysis combining genomic DNA sequencing (gDNA) and transcript analysis has been shown to reach *NF1* pathogenic variant detection rates of 94% in familial cases (Messiaen et al. 2009; PMID: 19920235). In our laboratory, gDNA short-read next generation sequencing (NGS) is performed for the detection of point mutation and copy number variants. For transcript analysis we use direct cDNA sequencing, which is based on reversed-transcriptase (RT-)PCR amplification of the entire *NF1* coding region starting form mRNA transcripts extracted from short-term lymphocyte cultures. In addition to identifying and delineating splice mutations, direct cDNA sequencing may indicate unequal allelic expression, which in turn may indicate presence of gross genomic alterations that escape short-read NGS or alterations in regulatory regions of the gene.

With the aim to improve the mutation detection rates, identify novel mutation mechanisms, and provide a genetic diagnosis to patients, we started to explore the potential of long-read sequencing for *NF1* diagnostics.

Methods: From our cohort of >1000 individuals comprehensively analysed for *NF1* variants two individuals showing unequal allele expression by analysis of two linked polymorphic variants in the 5´-region of the *NF1* gene. gDNA of the two individuals were fragmented using Femto Puls. Subsequently a SMARTbell library was created following the protocols of the provider and both samples underwent sequencing on a PacBio Revio machine utilizing two Revio SMRT cells. For data analysis, the generated HIFI bam files were aligned to the GRCH38/HG38 reference genome (GCA_000001405.15_GRCh38_no_alt_analysis_set) using pbmm2 v.1.13.1, followed by detecting the structural variants and deep variants using pbsv v.2.9.0 and Deepvariant v.1.6.0, in turn. The whole pipeline was generated under SMRT Link v.13.0 with pbcromwell v.1.3.1 as workflow manager.

Result: In one of the individuals a heterozygous reciprocal translocation t(16;17)(p12.3;q11.2) was identified with one breakpoint in *NF1* intron 29 and the other in an intergenic region on the short arm of chromosome 16. This translocation likely leads to non-functional transcripts causing nonsense-mediated decay, detectable by allelic imbalance. Analysis of the long read sequencing data of the second individual is still ongoing.

Conclusion: The identified translocation could have be identified also by cytogenetic analysis. Since long-read sequencing identified no gross structural variants in the second individual, we expect to identify in this individual a different type of alteration that would also escape cytogenetics. Our results also show that testing for allelic imbalance is a suitable approach to select patients for long-read sequencing in routine diagnostics.

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Contribution of NF1 Exon 23a Alternative Splicing Isoform to Schwann Cell Biology

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The *NF1* gene undergo alternative splicing to generate multiple splicing variants. The most characterized NF1 isoforms are the one excluding exon 23a (isoform 1) and the one including exon 23a (isoform 2). NF1 isoform 2 has a much weaker Ras inhibitory activity compared to isoform 2. Since Ras inhibition appear to be key to the biology of *NF1*^{-/-} Schwann cells, we decide to study the contribution of NF1 exon 23a alternative splicing to the phenotype of *NF1*^{-/-} Schwann cells.

To do so, we designed Targeted Oligonucleotide Silencer of Splicing (TOSS) and antisense Phosphoroamidate Morpholino Oligomers (PMOs) to shift NF1 exon 23a favoring isoform 1. We successfully validate the splicing shift of TOSS and PMO targeting NF1 exon 23a in several *NF1*^{-/-} Schwann cells (ipNF95.6, ipNF05.5, ipNF95.11b). We are now determining the impact of these antisense strategies on cell proliferation and Ras signaling as well as performing a direct comparison with a MEK inhibitor.

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The Combination of the Retinoid X Receptor Agonist MSU-42011 and the MEK Inhibitor Selumetinib Dereases pERK Levels in NF1-Deficient Cells, Inhibits Cytokine Production in Macrophages, and Reduces Tumor Burden In Vivo

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Neurofibromatosis type 1 (NF1) is a cancer predisposition syndrome that increases the risk of developing plexiform neurofibromas (PNFs) in 30-50% of patients with NF1. Approximately 10% of PNFs can progress to highly aggressive malignant peripheral nerve sheath tumors (MPNSTs). Selumetinib is the only FDA-approved drug for NF1-associated PNFs, however, the anti-tumor effects are limited in MPNSTs and have dose-limiting side effects. During the formation of neurofibromatosis, a complex tumor microenvironment (TME) develops, with the infiltration of macrophages critical for growth and progression. Targeting tumor-promoting immune cells could be an alternative approach for treating or preventing the progression of NF1. The novel retinoid X receptor (RXR) agonist MSU-42011 reduces tumor growth in experimental Kras-driven lung cancers by decreasing pERK levels, reducing tumor-promoting immune cells like CD206+ macrophages and regulatory T cells, and increasing activated cytotoxic T cells.

Purpose: Given the similarities in RAS activation and immune cell infiltration in NF1 and Kras-driven lung cancer, we hypothesized that MSU-42011 can be used as an alternative approach to selumetinib and reverse macrophage numbers and phenotypes within the NF1 TME.

Methods: We treated NF1-deficient cells and macrophages with MSU-42011 and selumetinib, either alone or in combination, using monoculture and conditioned media (CM) conditions.

Results: In human PNF cells and mouse MPNSTs, combination treatment with MSU-42011 and selumetinib reduced pERK levels. CM from human and mouse PNF cells increased the mRNA expression of monocyte chemoattractant *CCL2* (C-C motif chemokine ligand 2) and the secretion of IL-6, TNF alpha, and CCL2 in human THP1 monocytes/macrophages and bone marrow derived macrophages (BMDM). Notably, combination treatment with MSU-42011 and selumetinib inhibited *CCL2* and *TNF alpha* mRNA expression in macrophages stimulated with CM from PNF cells and MPNSTs. The combination of MSU42011 and selumetinib also significantly reduced tumor burden, pERK levels, and tumor-promoting CD206+ macrophages in a LL2 model of lung cancer driven by an activating *Kras* mutation.

Conclusions: As NF1 is a complex multisystem disorder, a combination of drugs with different mechanisms will most likely be more effective than single agents. Our initial results form the justification for further preclinical evaluation *in vivo.* Currently, we are assessing the immunomodulatory and anti-tumor effects of MSU-42011 and selumetinib in syngeneic models of PNF and MPNST, with the eventual goal of translating these findings into clinical applications.

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Patent applications covering the novel compounds described in this work have been applied for on behalf of Michigan State University; Karen T. Liby is a named inventor on the patent applications and a founding scientist of Akeila Bio.

Low Variant Allele Fraction in Germline Genetic Testing Predicts Pathogenicity of *NF1* Variants

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Background: Patients with a clinical diagnosis or suspicion of neurofibromatosis type 1 (NF1) undergo germline genetic testing for the *NF1* gene. *NF1* is also included in multigene panel testing for cancer predisposition and neurodevelopmental disorders. Low variant allele fraction (VAF) in *NF1* variants is frequently observed in germline genetic testing (next-generation sequencing) using blood or saliva and may result from mosaic/segmental NF1, circulating tumor DNA, or clonal hematopoiesis of indeterminate potential. *NF1* may provide a selective advantage to hematopoietic stem cells when mutated, thereby driving clonal hematopoiesis. In this study, we sought to determine whether low VAF is associated with pathogenicity of *NF1* variants.

Methods: Rare *NF1* variants (total allele frequency $<0.1\%$ in gnomAD v2) were identified in patients who submitted blood or saliva for germline genetic testing from 2016 to 2023. To ensure sufficient coverage and minimize technical interference in variant calling, only single nucleotide substitutions in coding sequence and within $+/-$ five nucleotides from exons were included in analyses. VAF $<10\%$ was filtered out to exclude potential sequencing artifacts. VAF in blood or saliva was available for 4,145 *NF1* variants in 43,457 patients – 387 likely pathogenic/pathogenic (LP/P), 2,263 variant of unknown significance (VUS), and 1,495 likely benign/benign (LB/B). *NF1* VAF was compared between blood and saliva, as well as between LP/P and LB/B variants. Low VAF was defined as three standard deviations below the mean in patients with LB/B variants (35.1%).

Results: There was no statistical difference in *NF1* VAF between blood and saliva, and thus, both specimen types were combined for the subsequent analyses. Mean VAF of rare *NF1* variants was significantly different among patients with LP/P, VUS, and LB/B – 41.1%, 46.8%, and 47.4%, respectively. VAF of both LP/P and LB/B variants peaked near 50% as expected for heterozygotes. However, a significantly higher proportion of the patients with LP/P variants had low VAF – 23.1% with LP/P and 0.84% with LB/B. Low VAF was observed with 8.4% (189/2,263) of the VUSs.

Conclusions: Rare *NF1* LP/P variants were significantly associated with low VAF in blood and saliva. While the exact cause of low VAF may be unknown, *NF1* pathogenic variants may be driving clonal hematopoiesis in patients undergoing germline genetic testing. Our data indicates that low VAF can predict pathogenicity of *NF1* variants and provide *in vivo* functional evidence to aid classification of *NF1* variants. *NF1* VUS observed with low VAF may benefit from reassessment.
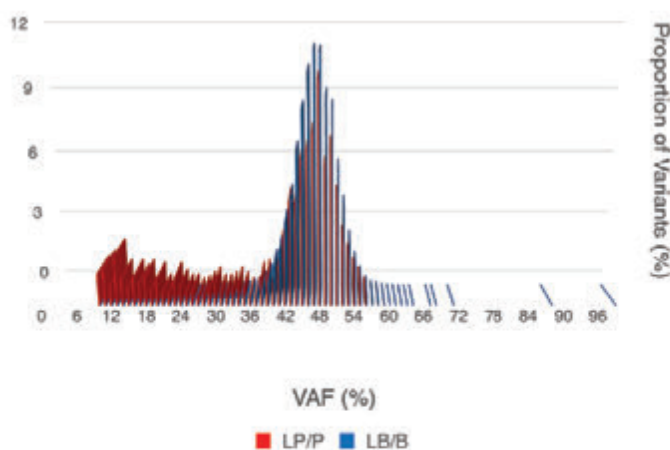
Figure 1. VAF distributions of rare *NF1* variants in blood and saliva (880 patients with LP/P and 36,033 with LB/B). A significantly higher proportion of the patients with LP/P variants had low VAF, compared to those with LB/B variants (Fisher's exact test p-value < 0.0001).

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Identifying Cancer Stem Cells Mechanisms Driving Therapeutic Resistance in NF1-Associated MPNST

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Considering the poor prognosis and high rate of drug resistance in patients with NF1-associated MPNST, a better understanding of the processes underlying the development of this malignancy is urgently needed to enable the development of effective therapies.

Previously described by Sun *et al.,* our laboratory has highlighted a specific population of cells that underlies the intra-tumoral heterogeneity of NF1-associated MPNST by harboring tumor-initiating capabilities, quiescent properties leading us to characterize these cells as cancer stem cells (CSCs). Ultimately, the presence of these CSCs in the Nf1 -/-; Trp53 -/- (NP) mouse model provided a partial explanation for the limited success of anti-mitotic therapies in effectively blocking tumor progression as their elimination leads to the tumor shrinkage. These findings emphasized the need for a complete molecular characterization of cancer stem cells during tumor initiation, progression, and relapse in NF1-associated MPNST with a view to targeting them as a potential new treatment.

To this end, we have used a similar approach as described in Sun *et al.,* using an additional genetically engineered mouse model of MPNST harboring the pilot mutations Nf1/Cdkn2a (NC) coupled with a transgene called CGD, that uses rat Nestin regulatory elements, CreERT2, enhanced nuclear GFP, and human diphtheria toxin receptor (hDTR).

After investigating and confirming the presence of the stem-like cell population in the tumor landscape of the NC mouse model and its role in tumor initiation by serial transplantation, we have analyzed the transcriptional and epigenetic properties of the NP and NC tumors by single-nucleus multi-omics analysis. Strikingly, at the transcriptional level, the cancer stem cells exhibit a similar signature in both mouse models, arising from the neural crest-derived Schwann cells lineage, cell of origin of this malignancy. However, the tumor cells that constitute NI tumors differ from those in NP tumors, as a subset of tumor cells in NI tumors contain important matrix constituents, as well as genes expressed in less differentiated states of Schwann cells than cells in NP tumors. These results lead us to hypothesize that, depending on the mutated gene, the Schwann cell differentiation may be differentially affected. We are currently validating the genes enriched in the CSC population and determining their role in the initiation and relapse of MPNST after antimitotic therapy. This work will provide a better understanding of the mechanisms underlying tumor relapse, which will lead to the development of novel strategies to curb malignancy.

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Preoperative Classification of Peripheral Nerve Sheath Tumors on MRI Using Radiomics

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Introduction: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas occurring in neurofibromatosis type 1 (NF1) patients. High-grade MPNSTs carry a high risk of metastasis and recurrence, making early recognition and surgical resection crucial for improved survival. The resection of high-grade MPNSTs often leads to postoperative complications, while neurofibromas can be adequately resected with minimal nerve damage. Therefore, preoperative differentiation between benign peripheral nerve sheath tumors (BPNSTs) and MPNSTs is important. However, current imaging tools may not always provide sufficient diagnostic accuracy, leading to the need for biopsies and associated burdens. Clear distinguishing features in magnetic resonance imaging (MRI) are not yet known. Radiomics provides a potential new tool in the diagnostic armamentarium, which may reduce the need for biopsies. This study aims to develop a radiomics model that utilizes quantitative imaging features and machine learning to differentiate BPNSTs from MPNSTs based on T1- and T2-weighted MRI sequences.

Materials and Methods: We collected T1- and T2-weighted MRI sequences from BPNST and MPNST patients at our tertiary referral center for sarcoma. Lesions were manually and semi-automatically segmented on the MRI sequence where the tumor was best visible. Segmentations were warped to the other sequences using image registration. For each lesion, on each sequence, 564 radiomics features were extracted. For classification, the WORC algorithm was used, which includes a large set of commonly used radiomics methods and uses automated machine learning to determine their optimal combination based on the training set. Evaluation was performed using a 100x random-split cross-validation with 20% of the data for testing. Performance was compared to manual scoring by two radiologists who had access to the scans of the complete MRI sessions.

Results: A total of 35 MPNSTs and 74 BPNSTs were included. The radiomics models had a mean test area under the curve (AUC) of 0.71 on T1-weighted MRI, 0.68 on T1-weighted MRI with interactive segmentations. The two radiologists had AUCs of 0.75 and 0.60.

Conclusions: Radiomics based machine learning using T1- and T2 weighted MRI sequences can provide a valid tool for improved clinical decision-making in the management of these tumors. Further validation and refinement of the radiomics model are warranted to enhance its diagnostic accuracy and clinical utility.

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The Role of Radiotherapy in Malignant Peripheral Nerve Sheath Tumors: A Multicenter Cohort Study

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Introduction: Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are rare and aggressive malignant soft-tissue sarcomas (STS), with 40% associated with neurofibromatosis type 1 (NF1). Surgical excision is the primary treatment for localized disease, but MPNSTs have a high likelihood of local recurrence (LR). Radiotherapy (RT) is increasingly used to improve local control in STS without affecting survival. However, its use is controversial due to the potential for higher major wound complications and increased risk of secondary malignancies in NF1 patients, as MPNSTs commonly arise from plexiform neurofibromas. This study evaluates the use of RT in these rare tumors and tries to understand its impact in local control, especially in the NF1 setting.

Material & Methods: Surgically treated primary MPNSTs from 1988 to 2019 in the MONACO multicenter cohort were included. Demographic and treatment differences, specifically the use of RT, between NF1 and non-NF1 cases were analyzed. Univariate and multivariable logistic regression analyses identified factors associated with RT utilization. Multivariate Cox regression analyses identified factors associated with LR in NF1 patients.

Results: A total of 516 patients (32.6% NF1) were included, with 149 (28.9%) developing LRs. RT was administered to 54.5% of patients (56.0% in NF1). Among them, 26.0% received neoadjuvant RT, and 79.2% adjuvant RT. Treatment modalities were similar between patients with and without NF1. Multivariable regression analysis showed high-grade tumor as the only independent factor associated with RT utilization. RT use in NF1 did not alter after correcting for tumor-, patient- and surgical factors. RT did not impact overall survival in sporadic and NF1-associated MPNST. In NF1 patients, a microscopically positive margin (R1) (HR 2.2; 95% CI, 1.21-3.91) was the only independent risk factor for LR development. After adjusting for tumor-, patient-, and surgical-related factors, the use of RT was not associated with lower rates of LR, in contrast to the sporadic population.

Conclusions: RT is commonly used in MPNST treatment regardless of its origin in NF1. While it may affect LR rate in sporadic patients, its impact in NF1 patients is less clear. Further studies are needed to evaluate its role and indications in NF1 associated MPNST.

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Contribution of Fibroblasts to Tumor Growth and Invasiveness in 3D NF1 Plexiform Neurofibroma Cultures

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Purpose: Neurofibromatosis Type 1 (NF1) plexiform neurofibroma (PN) is a complex tumor composed of abnormal Schwann cells and cells of the surrounding tumor microenvironment (TME), i.e., cellular microenvironment. Local invasion and enormous growth by NF1 PN are significant problems that lead to morbidity and often prevent complete surgical resection. However, it remains less understood how the cellular microenvironment affects growth and invasion of NF1 PNs. Delineating molecular mechanisms by which fibroblasts, major cell types in the cellular microenvironment, contribute to tumor growth and invasion is crucial to designing new therapies to prevent NF1 PN progression.

Methods: To study roles of fibroblasts in the growth and invasion of NF1 PN, we are using a three-dimensional (3D) heterotypic co-culture model of human NF1 PN Schwann cells *(nf1$^{-/-}$)*, ipNF95.11bC and ipNF05.5, grown with human primary fibroblasts *(nf1$^{+/-}$; PN-fibroblasts)* in our novel microfluidic culture devices (Patent: US 10,227,556 B2) that we designed and fabricated. These culture devices support growth of the 3D co-cultures, live-cell confocal imaging in real-time and non-invasive, high-content analysis and drug testing over extended long-term culture periods. We use live-cell assays and 3D quantitative analysis of temporal and dynamic changes in NF1 PN tumor cell:PN-fibroblast interactions in correspondence with changes in their growth and invasiveness.

Results: We cultured NF1 PN tumor cells in the presence and absence of human PN-fibroblasts in 3D cultures for 6 days. We observed that the cell numbers of NF1 PN tumor cells were significantly greater in NF1 PN tumor cell: PN-fibroblast cocultures than in NF1 PN tumor cell monocultures. We detected a slight increase in growth of PN-fibroblasts in the cocultures than in PN-fibroblast monocultures. In addition, NF1 PN tumor cells grew faster in media conditioned by PN-fibroblasts, implicating that the secretome from PN-fibroblasts increases the growth of NF1 PN tumor cells. The increased growth of NF1 PN tumor cells by PN-fibroblast conditioned media (CM) is abolished by exosome-depleted PN-fibroblasts CM, suggesting that PN-fibroblasts increase NF1 PN tumor growth via exosome-mediated paracrine pathways.

Conclusions: Our 3D NF1 PN culture model combined with the microfluidic devices will allow provide mechanistic insights into how the cellular microenvironment facilitates growth of NF1 PN and the technology required to screen therapeutic candidates for translation to the clinic. Our results suggest that fibroblasts secrete exosomes that may be therapeutic targets for reducing NF1 PN growth.

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Dissecting the Mechanistic and Functional Importance of an RNA-Binding Protein for Malignant Peripheral Nerve Sheath Tumour Growth and Metastasis

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Introduction: Malignant peripheral nerve sheath tumours (MPNSTs) are highly aggressive soft tissue sarcomas with strong metastatic proclivity for which there is no effective therapy. It can affect both children and adults. The dismal prognosis of MPNST patients poses an urgent need for improved effective therapeutic modalities for this challenging disease.

Genetic alterations in cancer cells invariably lead to a global remodelling of their transcriptome allowing them to acquire advanced functional capabilities for survival, proliferation and dissemination. Targeting dysregulated transcriptomic programs in cancers has emerged as a promising therapeutical strategy, and there is an intense focus on identifying the key molecular regulators that drive these programs. In particular, RNA-binding proteins (RBPs) are increasingly recognised as attractive targets because they can regulate type and abundance of hundreds of transcripts.

The main was to investigate the functional and mechanistic role of RBPs in MPNST growth and metastasis.

Methods: A bioinformatics analysis of microarray databases of MPNST tumors, neurofibromas, and nerves was conducted, and we selected the RBPs that were overexpressed in malignant tumours (adjusted p-value < 0.05 and fold change > 1.5). The expression of RBPx.1 was validated in MPNST cell lines and human samples by Western blot and RT-qPCR. The effect of silencing RBP.1 in MPNST cell lines (S462 and T265) and immortalized normal Schwann cells (iHSCλ2) was assessed through a series of functional assays. We performed a xenograft transplant assay, and we are trying to understand the mechanism of action of RBPx.1.

Results: We have found that there are 106 RBPs that are differentially expressed in MPNSTs with respect to neurofibromatosis, which might potentially play key roles in MPNST growth and metastasis. We confirmed the elevated expression of RBPx.1 at the RNA and protein levels in MPNST cell lines and in patient samples. Using functional assays, we observed a decrease in clonogenic and proliferative capacity of tumour cells, as well as a reduction in total ATP levels and proliferation markers after genetic silencing, an effect which was more pronounced in cancer cell lines compared to on immortalized human Schwann cells We observed that RBPx.1 silencing prevents MPNST tumour growth in vivo.

Conclusions: RBPx.1 could be a promising marker for tumour progression in MPNST, and could be regulating the oncogenic properties of MPNST cells.

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Big Data Analysis Expedites Drug Discovery Targeting Plexiform Neurofibroma Heterogeneity

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Background: The paucity of patients limits research in rare diseases like plexiform neurofibroma (PN). With the access to the biomedical big data pool reflecting different aspects of PN, such as NF data portal, the integrated data mining significantly expedites the drug discovery in PN. This study combines single cell RNA (scRNA) sequencing, PN bulk sequencing, cell modeling, drug screening, gene regulation network analysis and drug combination prediction, which can be applied to elaborate the nature of PN tumors and identify novel treatment. This strategy prioritizes HSP90 inhibitors (HSP90i) to target the stem-like tumor cells in PN. The combination between HSP90i and Selumetinib can be therapeutic alternatives to Selumetinib monotherapy that has limited responses and long-term adverse effects.

Purpose: This effort aims at targeting the vulnerability of stem-like cell population within PN tumor revealed by the big data analysis with HSP90i. Additionally, the potential of combining HSP90i with Selumetinib to reduce the dose and toxicity of Selumetinib monotherapy is explored

Methods: scRNA sequencing analysis is employed to identify PN tumor heterogeneity and stem cell-like signatures. Gene regulation network analysis of bulk sequencing characterize the conserved signaling among PN cell lines and primary tumors. Integrated drug mining is used to reveal candidates from a drug screening study to affirm the differential activity related to the PN heterogeneity. Drugs combination prediction premises the HSP90i combination with Selumetinib. We choose four different HSP90i and five different PN cell lines to evaluate the single HSP90i and its combination with Selumetinib by cell viability assay. The synergistic analysis is used to determine the effects of drug combination.

Results: Four HSP90i are shortlisted by integrated analysis, including SNX2112, SNX5422, Retaspimycin, and Geldanamycin. We tested the candidates to target the stem-like properties exhibited by the PN cell lines and the differential responses corresponding to tumor heterogeneity. Moreover, HSP90i show synergistic effect with Selumetinib and significantly reduce Selumetinib dosage.

Conclusion: Integrated data analysis with different data types is a powerful tool to discover and prioritize novel research initiatives for fast-track medical interventions. HSP90i is effective in targeting stem-like tumor cells and can reduce Selumetinib dose and toxicity in combination for better therapeutic outcomes.

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Functional Restoration of Neurofibromin in Fibroblasts from Neurofibromatosis Type 1 Patients with Nonsense Mutations

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Neurofibromatosis Type 1 (NF1) is one of the most common genetic disorders, caused by the tumor suppressor *NF1* gene, affecting 1 in 3000 individuals worldwide. Neurofibromin (encoded by *NF1*) tightly regulates cell growth and survival by suppressing activated RAS, GTP bound RAS, which primarily stimulates the MEK/ERK signalling pathway. In Korean NF1 patients, approximately 30% of the patients harbor nonsense mutations that generate premature termination codon (PTC). Ataluren is a nonsense suppressor drug that selectively induces ribosomal readthrough of PTCs, but not that of normal termination codon. Recently, Ataluren efficacy of PTC possessed *NF1* was tested by using a mouse or minipig model, but in human fibroblasts yet. Herein, we isolated fibroblasts from 22 Korean *NF1[NS/+]* patients and evaluated the efficiency of Ataluren treatment. The results exhibit that Ataluren induced restored Neurofib romin significantly alleviates the hyperactive GTP bound RAS in 23% of *NF1[NS/+]* fibroblasts. Furthermore, the cellular levels of phosphorylated ERK decreased after Ataluren treatment. Because of the differential readthrough efficacy of Ataluren, we divided two groups according to drug responsiveness. Based on our transcriptome wide profiling, we analyzed a subset of genes in Ataluren treated *NF1[NS/+]* fibroblasts whose expression was reduced in Ataluren responsive cells, but not in non responsive cells. Collectively, our study suggests that Ataluren might be considered for therapeutic purpose in some *NF1[NS/+]* patients.

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Spine Deformity in Mice Lacking *Nf1* Gene in Boundary Cap-Derived Osteoblasts

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Background and purpose of the study: Neurofibromatosis type 1 (NF1), a genetic disorder due to mutations in the *NF1* gene, affect patients with a variety of symptoms including spine deformities, cutaneous and plexiform neurofibromas (cNFBs and pNFBs). Neurofibromas result from a second *NF1* hit mutation in Schwann cells. Although a second *NF1* hit mutation has been reported in NF1 patients with spine deformity, the affected cell type(s) and the mechanisms remain unknown. *Prss56[Cre]; R26[tdTom]; Nf1[fl] (Prss56-Nf1 KO)* mice lack *Nf1* gene in Prss56-expressing boundary cap (BC) cells, a neural crest-derived population giving rise to multiple derivatives such as Schwann cells. *Prss56-Nf1 KO* mice faithfully recapitulate cNFBs, pNFBs, and pseudoarthrosis, another bone manifestation of NF1. To elucidate the molecular and cellular bases in NF1 spine deformities, we explored the *Prss56-Nf1 KO* mouse model.

Methods: We used in vivo micro-CT and lineage tracing analyses from 3 to 16 months of age to characterize the spine phenotype in *Prss56-Nf1 KO* mice and the affected cell types.

Results: Micro-CT analyses showed that *Prss56-Nf1 KO* mice recapitulate scoliosis (front-lateral deformity equal to or greater than 10 degrees) in approximately 20% of mice at 12 and 16 months of age. *Prss56-Nf1 KO* mice showed significantly increased spine curvature in the sagittal plane compared to control at 12 and 16 months of age, recapitulating kyphosis. Spine deformities in *Prss56-Nf1 KO* mice were associated with vertebral anomalies (fusion, scalloping, wedging), as observed in NF1 patients.

In vivo, fate mapping of BC-derived (tdTom+) cells revealed a significant increase of tdTom+ cells in the vertebrae of *Prss56-Nf1 KO* mice compared to control starting from embryonal stages until 16 months of age. TdTom+ cells were increased specifically in trabecular bone, corresponding to a significant increase of tdTom+ osteoblast number. In addition, the osteoblast/osteoclast ratio was increased. Moreover, the increase of tdtom+ signal correlated with increased bone volume and with kyphosis spine curvature, indicating that an increase in *Nf1*-deficient BC-derived osteoblasts in mutant vertebrae can lead to changes in bone parameters and subsequently to kyphosis spine deformity.

Conclusion: Overall, our results establish a new mouse model of NF1 spine deformity, suggesting the role of Prss56+ BC cells as the cell of origin in NF1 spine deformity.

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Digital Eye: Single-Cell Multiomics Simulation to Model and Study Cell-Cell-Interactions During Development, Regeneration and in the Pathogenesis of Retinal and Optic Nerve Diseases

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We propose a new method of analyzing transcriptomics data by creating a "digital eye" that allows to computationally introduce diseased cells into healthy tissue and vice versa to simulate stages of disease progression and study and model the way cells interact at the ligand-receptor level with the true single-cell resolution.

To showcase the method in the absence of NF1 human optic nerve and retina data, we start with the scRNA-seq data for human adult healthy and age macular degenerated (AMD) retinas. Our pipeline includes the methods of data processing (Harmony, SCTransform, ForceAtlas2) and cell-cell interactions analysis (CellChat, Scriabin). We focus on combining the datasets of healthy and AMD retinas to generate the following conditions: 1) healthy, reference 2) healthy with AMD microglia 3) healthy with AMD endothelia 4) healthy with AMD RPE 5) healthy with AMD glia 6) AMD 7) AMD with healthy RGC, Control.

We identify cell-cell interactions that profile the disease staging. For healthy condition: ApoE, TAFA, GALECTIN, GDNF, ANNEXIN, IL16, SELPG. For healthy with AMD microglia: IFN-II, GH, VWF, 2-AG, MMP, LHB, SEMA5, ADGRG, CRH, TIGIT, CD200, PACAP, CX3C, ESAM, PVR, ENHO. For AMD: IFN-I, Ach, MIF, IL16, CSF3, LHB, NMU. The upregulated interactions in healthy with AMD microglia condition are CD45, PCDH, SLITRK, NT, PSAP, IL2, L1CAM, THY1, IGFBP, Chemerin, NPVF, CD96, with their upregulation in addition to new interactions of BTLA, CLDN, LIGHT, SEMA4, CypA, PDGF, FLRT in the AMD condition. We demonstrate the gradual increase of PCDH interactions in the AMD progression, first affecting ON-bipolar and horizontal cells. We show 95% similarity of cell-cell interactions between the AMD and AMD Control conditions.
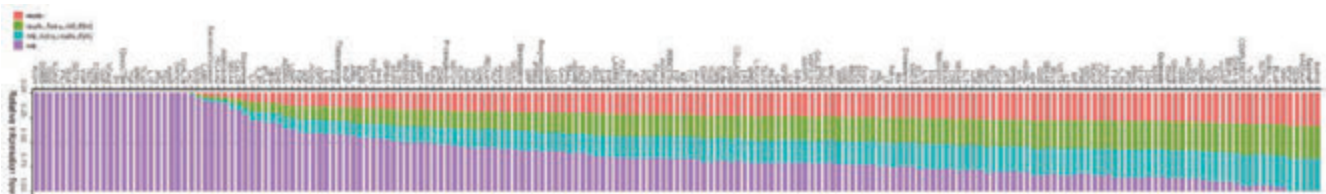
Fig. 1. CellChat panel demonstrating the cell-cell interactions differences between the conditions of human healthy PBMC and healthy retina, healthy PBMC and AMD retina, AMD PBMC and healthy retina, and AMD PBMC and retina.

We describe a new approach of single-cell data analysis for recapitulating the disease progression staging based on comparison of cell populations and microenvironment in retina in the mix of healthy and diseased cells. Our pipelines pinpoint multiple ligands and receptors that can be modulated at the early stage of AMD progression.

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A Multiomics Approach to Identifying Synergistic Drug Combinations Using an *Ex Vivo* Microtissue Model of Malignant Peripheral Nerve Sheath Tumors (MPNST)

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Purpose: We have been able to acquire a series of well characterized MPNST patient tumors and use them to create a framework by which we create patient derived xenografts (PDX) and assemble the PDX into microtissues *ex vivo.* The purpose of this study is to identify drug combinations that could inform human clinical trials via time-series multiomics measurements of drug treatments in this framework.

Methods: Leveraging the expertise of a multi-institutional collaboration, we derived *ex vivo* microtissues from a genomically diverse set of MPNST PDX. Using these PDX-microtissues (PDX-MT), we tested 26 drug compounds representing six different drug classes. These drugs were tested in an 8-point dose response manner and we assessed cellular viability after 48 hours. To determine the adaptive response to short term drug treatment, we tested PDX assembled in ECM and treated them with sublethal doses of 15 drugs at multiple early time points. We have measured the phospho- and global proteome and the transcriptome of these treated PDX to identify dynamic transcriptional, translational, and signaling changes that inform drug resistance mechanisms. These analyses have the potential to identify druggable targets to allow rationally designed combinations with the likelihood of antitumor activity in PDX.

Results: Of the 26 compounds tested at their human Cmax dose, we identified at least 10 to be promising as they inhibited cell viability more than 50% in at least one third of PDX-MT. While some drugs were not considered promising by us as single agents, they offered potential for effective combination therapy. As such, we show that the combination of a MEK inhibitor and digoxin was either additive or synergistic in four different PDX-MT. This combination was also effective at either reducing tumor burden or slowing tumor growth in mice, xenografted with MPNST cells, better than either single agent alone. Analysis of the omics data for 15 drugs, is ongoing, and demonstrates classic up- and downregulated ERK transcriptional output genes in the PDX-MT in response to a MEK inhibitor.

Conclusion: The current PDX-MT framework enables medium throughput drug sensitivity assays that, when paired with multiomic measurements, can inform drug combination experiments. This system may prove useful as a precision medicine tool, by allowing patient derived microtissues, which recapitulate PDX-derived *in vivo* findings with a higher throughput potential compared to traditional *in vivo* testing. A host of drug compounds could be screened using the PDX-MT in as little as a week's time and effective, personalized treatments may result.

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Targeting the Link Between Cancer Cell Metabolism and the ECM to Defeat Peripheral Nerve Neoplasms

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Purpose: The purpose of our study is the identification of metabolism-based anti-neoplastic treatments to hit the interaction between the extracellular matrix (ECM) and cancer cells in malignant peripheral nerve sheath tumors (MPNSTs), aggressive sarcomas arising in Neurofibromatosis type 1 (NF1) patients.

Methods: First, we analyzed the expression of variants of collagen, the main structural component in the ECM of MPNSTs, and tested whether glutamine (Gln) could affect their abundance, as Gln metabolism is required for collagen synthesis. To this aim, we performed qPCR, Western blot (WB) and immunofluorescence (IF) assays, as well as metabolic flux analyses by using an isotope-labelled $^{13}C_5$-$^{15}N_2$-Gln. Second, we set up spheroid assays to test the importance of Gln metabolism and collagen synthesis for MPNST cell tumorigenicity. Finally, we knocked-out by CRISPR-Cas9 technology the mitochondrial chaperone TRAP1 to assess its contribution in tuning Gln-related collagen synthesis and MPNST neoplastic growth.

Results: We observed that MPNST cells express collagen III, IV, and VI, but only IV and VI isoforms are modulated by Gln metabolism, as their expression is proportional to Gln availability. We found that MPNST cells are avid consumers of Gln, which is rapidly transformed into Pro, and their propensity to grow and invade in a 3D matrix increases in high Gln conditions. Together, these data suggest that Gln metabolism sustains the assembly of the ECM by regulating collagen content and that this mechanism may underpin the ability of MPNST cells to spread. We had previously demonstrated that the mitochondrial chaperone TRAP1 is a master regulator of bioenergetic circuits in NF1-related tumor models. Here, we find that TRAP1 controls the biosynthesis of collagens IV and VI in MPNST cells through the regulation of the Pro biosynthetic pathway.

Conclusions: Our study dug into MPNST metabolic pathways, bridging the Gln-to-Pro axis to the production of collagen molecules and to the assembly of a pro-tumorigenic ECM supporting cancer cell invasion. These new findings could pave the way to the identification of novel targets for developing effective therapeutic strategies.

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Exploring the Molecular Basis of Enhanced Anti-Tumor Activity: Cordycepin and FT895 Combination Therapy in MPNST

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Neurofibromatosis type 1 (NF1) is a common neurocutaneous disorder, with about a quarter of NF1 patients develop the plexiform neurofibromas (PNs). PNs can potentially progress into malignant peripheral nerve sheath tumors (MPNST) for which effective treatment is lacking. The purpose of this study was to evaluate the potential of cordycepin, an extract from cordyceps militaris known for its anti-inflammatory and anti-tumor properties, in inhibiting the proliferation of malignant peripheral nerve sheath tumors (MPNST) cells. Furthermore, FT895, a novel HDAC11 inhibitor, was found to jeopardize the mitochondrial biogenesis and function in MPNST cells. The efficacy and its associated molecular mechanisms were evaluated. In both in vitro and in vivo experiments, cordycepin exhibited notable anti-tumor properties. Treatment with cordycepin led to a decrease in the levels of ERK, survivin, pAKT, p53, and Sp1 proteins in MPNST cells. Additionally, tubulin levels, but not actin or GAPDH, decreased in a dose-dependent manner. ChIP-qPCR assays revealed a reduction in Sp1 binding to the tubulin promoter regions. Treatment with FT895 reduced basal, maximal, and ATP-production-coupled respiration in MPNST cells, accompanied by a decrease in mitochondria-related proteins XBP1, PINK1, and Parkin. CHIP-qPCR analysis demonstrated a significant reduction in the copy numbers of promoters of several genes involved in mitochondrial function. RNA-seq analysis highlighted the HIF-1α signaling pathway's prominent role post-FT895 treatment, aligning with the observed impairment in mitochondrial respiration. Combining treatment with cordycepin and FT895 exhibited a synergistic anti-tumor effect on MPNST cells both in vitro and in vivo. This combined treatment decreased the protein levels of α-tubulin and KIF18A, potentially disrupting microtubule organization and inducing aneuploidy. In conclusion, both cordycepin and FT895 showed promising anti-tumor effects on MPNST cells via distinct mechanisms, with their combination demonstrating enhanced efficacy in suppressing MPNST cell proliferation. These findings suggest the potential therapeutic utility of cordycepin and FT895 in the treatment of MPNST.

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Progressive Gender-Dependent Learning/Memory Issues in *Nf1* Mutant Mice: Potential Central Myelin Mechanisms

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Purpose: NF1 patients present with neurological issues throughout their life; while learning deficits and ADHD are common in children and presumably continue into adulthood, higher risk for depression and dementia develops with aging. Neuron-centered research identified mechanisms for disrupted learning in NF1 and therapy strategies were proposed; unfortunately, subsequent clinical trials could not establish treatments. Abnormalities of the brain white matter and myelin are common in NF1 patients and show variations throughout life. While evidence for the involvement of myelin plasticity in learning and disease rapidly grows, the myelin biology in NF1 remains poorly understood. Hence, our purpose is revealing myelin abnormalities in correlation with learning/memory issues in NF1 models.

Methods: We subjected mice with germline or cell-type specific mutations of *Nf1* to learning/memory tests and histological analyses.

Results: Mice with *Nf1* haploinsufficiency were subjected to a myelin-regulated learning/memory test. Young adult *Nf1* mutant females showed memory and putative motivational issues, while older and aging females presented broad progressive learning/memory defects, as compared with controls. No changes in oligodendrocyte (OL; myelin producing cells) numbers, but increased OL precursor cells (OPC) were observed in the corpus callosum (CC) of young *Nf1* mutants. With age, the overall OPC number progressively decreased to control levels mainly due to their lower proliferative capacity. Scattered CC regions retained higher OPC numbers in aging *Nf1* mutants; however, failure to increase OL numbers at normal rates upon the learning challenge, as well as localized decreased proliferation, were also observed. Interestingly, mutation of *Nf1* specifically in adult OPCs suggests increased proliferation and mild learning advantage in the short-term.

Conclusion: Together with previous data on learning issues following *Nf1* mutation in adult OLs, our current results support the idea that abnormal myelin biology contributes to neurological issues throughout the lifespan of NF1 patients.

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Optogenetic Rescue and Cortico-Striatal Neural Recordings of Social Deficits in a Preclinical Model of Neurofibromatosis Type 1

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The purpose of this study is to investigate autism spectrum disorders (ASDs) in Neurofibromatosis type 1 (NF1) using optogenetics and neural recordings in a preclinical model.

Introduction: NF1 is a common genetic disorder with varied symptoms including neurocutaneous lesions, tumors, and neurodevelopmental disorders. Cognitive symptoms affect many patients, with up to 90% having difficulty in school, 60% having social impairments, and around 40% being diagnosed with ASDs.

Methods: We tested male and female wildtype and *Nf1* haploinsufficient *(Nf1$^{+/-}$)* mice on behavioral tasks measuring social interaction and memory. Next, we measured neural firing using electrodes simultaneously implanted in the nucleus accumbens and prefrontal cortex, brain areas involved in decision making and motivated action. Electrodes recorded individual neuron activity called spikes and group neuron activity called local field potentials. Finally, we used optogenetics to modulate neural firing in these brain areas during social interaction to modify behavior.

Results: Male *Nf1$^{+/-}$* mice exhibit social deficits in the three chamber social recognition test. Neural recording studies demonstrate underlying neural differences due to the *Nf1* mutation. Increasing neural activity in either the nucleus accumbens or prefrontal cortex using optogenetics rescued social behavior. "Power" indicates strength of neural signal while "synchrony" measures how well two brain areas are communicating. *Nf1$^{+/-}$* mice exhibited altered power in the prefrontal cortex and nucleus accumbens, with decreased synchrony between the brain areas.

Conclusions: These studies are the first to use *in vivo* awake-behaving recordings during social behavior in preclinical NF1. Simultaneous recording of the nucleus accumbens and prefrontal cortex is highly valuable to understanding function of this circuit during social interaction. Local field potentials captured by electrodes are translationally relevant to electroencephalogram (EEG) in patients. Although each of the brain regions is making increased effort to communicate a signal (increased power), the signal does not go through (decreased synchrony), resulting in deficits when coordinating complex social behavior. An ideal treatment approach would increase synchrony of the corticostriatal circuit, decreasing noisy and inefficient oscillatory power. Correction of dysfunction in either brain area through optogenetics is sufficient to rescue social deficits in *Nf1$^{+/-}$* mice. Optogenetic stimulation during the familiarization phase is sufficient to restore social recall 24 hours later, a robust response. Overall, *Nf1$^{+/-}$* male mice recapitulate behavioral phenotypes of NF1 and are a useful model system to identify alterations in neural circuitry associated with ASD, a significant concern for patients and families.

Identification of Different MPNSTs Subtypes Through Precise Genomic Analysis

Miriam Magallón-Lorenz, PhD, *Germans Trias i Pujol Research Institute (IGTP)*

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft-tissue sarcomas appearing either in the Neurofibromatosis type 1 (NF1) context or sporadically. MPNSTs tend to metastasize and have a poor 5-year survival rate. The diagnosis of MPNSTs can be challenging, especially outside the NF1 context, since different tumor entities may share overlapping histological characteristics. Misdiagnosis of MPNST negatively impacts basic and preclinical research and can even hinder clinical trials by adding noise that leads to discarding potential new treatments. The main objective of this work is to obtain an accurate and genuine characterization of MPNSTs and translate this information for improving MPNST diagnosis, management and treatment.

The genomic and transcriptomic analyses of a set of 20 clinically diagnosed MPNSTs helped us to provide a genomic definition of MPNSTs with a classic histological presentation and differentiate them from other confounding entities. Classic MPNSTs are characterized by: bearing the complete inactivation of *NF1, CDKN2A* and *PRC2;* presenting copy neutral (CN) loss of heterozygosity (LOH) of 1p, 4, 9p, 10, 11 and 17p; highly gained chromosomes 7 and 8; absence of activating mutations either by point mutations or fusion-genes; a low mutational frequency. In addition, *CDKN2A* is commonly inactivated by inter-chromosomal translocations. This genomic definition was confirmed using an MPNST validation set from Cortes-Ciriano *et al.* (2023).

We built an MPNST classifier solely based on these genomic features and it correctly discriminated between classic MPNSTs, other newly defined MPNST subtypes and other entities, bearing distinctive histological and genomic features. The model was built on multiple genomic traits and provided a better classification than PRC2 status, transcription factor profile or any other single genomic feature of the tumors.

The correct separation between true MPNSTs and other histologically overlapping entities, and the identification of different MPNST subtypes will have a profound impact on MPNST diagnostics and management and can be key for the identification of new treatments for these deadly tumors.

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NF1(-/-) Schwann Cell Differentiation as a Therapeutic Strategy for Cutaneous Neurofibromas

Helena Mazuelas, PhD, *Hereditary Cancer Group, Germans Trias & Pujol Research Institute*

The development of multiple cutaneous neurofibromas (cNFs) constitutes one of the major concerns of Neurofibromatosis type 1 (NF1) affected persons. Complete loss of *NF1* in a cell of the Schwann cell (SC) lineage composing the subepidermal glia is necessary for cNF development, although other cell types present in cNFs also seem to play a key role.

The aim of this study is to find new therapeutic approaches for cNFs. We previously reported that the combined activation of the cAMP pathway by GPR68/Ogerin and inhibition of the Ras/MAPK pathway by Selumetinib treatment, resulted in the induction of SC myelinization, decreased cell viability, and increased proportion of cells in apoptosis in human cNF-derived SC cultures. Consistently, we also observed an increased sphere disaggregation and cell death in a human 3D iPSC-based neurofibromasphere model after Ogerin/Selumetinib co-treatment (Mazuelas et al. 2022 & Mazuelas et al. 2024). Furthermore, in co-treatment experiments we obtained similar results when replacing Ogerin by direct cAMP elevators (forskolin and 8CPT-cAMP) and other GPR68 activators (positive allosteric modulator 71) (Yu et al., 2019). We also showed that Selumetinb treatment alone elicits a complete stop of *NF1*(-/-) SC proliferation, but similarly to what may happen in patients, SCs resume proliferation after drug removal. Contrarily, Ogerin/Selumetinib co-treatment induces a permanent halt in proliferation through *NF1*(-/-) SC differentiation.

We are currently investigating other cAMP-pathway activating compounds, selected to target cAMP pathway component isoforms specifically expressed in cNF-derived *NF1*(-/-) SCs. Most promising compounds are being tested as potential co-treatment partners with Selumetinib in human cNF-derived SCs and in an iPSC-based 3D neurofibromasphere model. In addition, the readout possibilities of the iPSC-based 3D model are being expanded to easily measure pathway activation (Ras/MAPK; cAMP) and SC maturation status (SC precursor; mature SC) in different *NF1* genotypes.

The combination of MEK inhibitors with cAMP elevators is emerging as an interesting potential therapeutic option for cNFs.

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Characterization of the PRC2-Regulated Protein ZNF423 in NF1-Related Malignant Peripheral Nerve Sheath Tumors

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Purpose: To identify potential therapeutic targets for NF1-related MPNST by probing the molecular drivers and mechanisms underlying MPNST cellular lineage, identity, and proliferation.

Methods: Human MPNST cell lines (control and PRC2-restored) and murine tumor cells-of-origin (*Nf1*$^{-/-}$ and *Nf1;Arf*$^{-/-}$) models were employed for RNA sequencing, immunoblotting, RNA interference, proteomic analysis of the kinome, and cell-based assays.

Results: To investigate the transcriptional changes that occur as a result of PRC2 loss, we restored *SUZ12* in two PRC2-deficient MPNST lines. RNA sequencing revealed fourteen common transcription factors downregulated by PRC2 reconstitution. Amongst those downregulated was *ZNF423,* a transcription factor expressed in numerous immature cell populations, including neuronal and olfactory precursors, where it functions as a lineage-specific transcription factor by regulating differentiation-promoting functions. To validate these results, we utilized genetically engineered mouse models of benign plexiform neurofibroma and MPNST to isolate tumor cells-of-origin known as DNSCs (DRG/nerve root neurosphere cells). By RNA sequencing, we observed that 7 of 14 common transcription factors affected by PRC2 status were upregulated in murine cells. Upon *Nf1* inactivation, which occurs during PNF development, *Zfp423* (orthologous to human *ZNF423*) was repressed compared to wild type. However, when both *Nf1* and *Arf* were inactivated, expression was significantly higher than wild type. Ablation of *Suz12* by CRISPR/Cas9 to disrupt PRC2 function did not further elevate *Zfp423* transcription. We are currently testing whether reintroduction of ectopic SUZ12 will drive repression of *Zfp423.* Preliminary analysis of RNA sequencing following depletion by siRNA in human NF MPNST cell lines reveals that ZNF423 regulates key neuronal differentiation programs and may contribute to MPNST signatures of dedifferentiation.

Conclusions: ZNF423 depletion significantly reduces MPNST cell viability and proliferation suggesting that its disruption could interfere with tumor growth. Ongoing studies will further delineate ZNF423-dependent signalling pathways in human and murine MPNST models using omics approaches, cell-based phenotypic assays, and *in vivo* studies.

The Association of Body Image on Quality of Life, Skin Involvement, the Appearance of Their Neurofibromas, Psychological Counseling and Social Support in Neurofibromatosis Type 1: Cross-Sectional Study

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Purpose: This research explores the Body Image (BI) of Neurofibromatosis type 1 (NF1) patients and its association with quality of life (QoL), skin involvement and the appearance of their neurofibromas. Also, the study evaluates the extent to which social support and psychological counseling is necessary for patients.

Methods: Two hundred and five patients with NF1 participated in the study. They responded to the questionnaires about QoL, BI and other sociodemographic data. Correlations, simple and multiple regressions are used to assess the relations between variables.

Results: The results show that BI problems increase if NF1 patients have concerns about the aspect of their neurofibromas (.828; <.001) and if they have major skin severity involvement (.245; <.001). BI impairments diminish the QoL by .605 (<.001), while skin severity is not clearly related. If you have BI impairments, you are prone to do psychological counseling (.218; <.01). The results also show that when patients with NF1 inform to have social support (-.210, <.01) or to receive psychological counseling (-.238; <.001) they have decreased QoL.

Conclusion: Body image concerns are a key feature to detect NF1 patients' impairments in QoL rather than skin involvement. When healthcare professionals detect BI impairments, it is crucial for them to collaborate with the patients and either provide or refer them to psychological interventions. This approach helps improve social support, enabling patients to benefit from both their professional and personal environments.

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SWI/SNF ATPase Modulation of the DNA Damage Response in Malignant Peripheral Nerve Sheath Tumors Induces a Therapeutic Vulnerability in This Disease

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Background: Malignant peripheral nerve sheath tumors (MPNST) are rare and aggressive sarcomas of the peripheral nervous system. MPNST is typically therapeutically resistant; complete surgical resection of tumors with wide negative margins is currently the only treatment that statistically improves the overall survival of patients[1–3]. Therefore, there is an urgent need to identify novel vulnerabilities and treatment options for MPNST. The purpose of this study is to investigate core ATPase components of SWI/SNF epigenetic regulatory complexes as novel therapeutic targets in MPNST.

Methods: Short-Interfering RNA (siRNA) knockdown (KD) of core SWI/SNF components identified the loss of the SMARCA4 ATPase gene as decreasing MPNST cell growth and viability. RNA sequencing (RNAseq) of these KDs identified biological roles of SMARCA4 underpinning these phenotypic effects. Findings were validated through flow cytometry. SMARCA4 was therapeutically targeted using a small molecule inhibitor, BRM014, and chemotherapeutics were screened for synergistic combinations, analyzed using SynergyFinder in R.

Results: Western blotting of SMARCA4 validated protein expression across a panel of 9 MPNST cell lines, while immunohistochemistry staining of 3 MPNST patient-derived xenograft tumors found high SMARCA4 expression. SMARCA4 KD using siRNA reduced viability and proliferation across 3 MPNST cell lines from 20-40% (p-val<0.001) when compared to a non-targeting (NT) control. Additionally, small molecule inhibition of SMARCA4 via BRM014 reduced MPNST growth and proliferation in a dose-dependent manner.

To establish the biological mechanism underlying SMARCA4 contribution to MPNST cell viability, RNAseq of three MPNST cell lines was carried out comparing SMARCA4 KD to NT control. Cell cycle and DNA replication KEGG pathways, as well as the E2F target pathway, were significantly (p-adj<0.05) downregulated upon SMARCA4 KD across these three cell lines. EDU/DAPI staining confirmed these findings, where SMARCA4 KD arrested MPNST cells at the G1 phase. Further, these results could be mimicked using BRM014 inhibitor, as MPNST cells containing a fluorescent cell cycle marker arrested in the G1 phase under single-agent treatment.

Due to the identified role of SMARCA4 in MPNST cell cycle and DNA replication, DNA damaging agents were screened in combination with BRM014 to highlight synergistic compounds. Etoposide, a TOP2A poison, was found to synergize with BRM014 at low doses, increasing the induction of G1 arrest through the ATM DNA damage response pathway.

Conclusions: This study identifies SWI/SNF ATPases as novel therapeutic targets in MPNST. Further, it identifies a synergistic therapeutic regimen in combination of etoposide and BRM014 in MPNST treatment, which is currently being tested *in vivo.*

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Establishment of Schwann Cell Precursors from NF1-Derived iPS Cell Lines

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Questions/Purposes: Neurofibromas in Neurofibromatosis type 1 (NF1) frequently progress to plexiform neurofibromas (PN) and then become malignant peripheral nerve sheath tumor. To evaluate the mechanism of developing PN, we tried to establish disease-specific iPS cells from them.

Methods: Peripheral blood mononuclear cells (PBMCs) derived from NF1 patients were isolated and initialised using PBMCs and SRV-iPSC4-Vector, after which colonies were picked up and cultured as single colonies. iPS cells from healthy individuals without NF1 mutation were also established as controls.

Results: The established iPS cells were confirmed to be pluripotent by fluorescence immunostaining using pluripotency markers (NANOG, OCT4) to confirm marker expression. The flow cytometry showed that almost 100% of the cells were SSEA4 positive. Targeted sequence analysis of the *NF1* exon confirmed that the pathological *NF1* mutation of derived iPS cells was insistent with that of original PBMCs.

Next, healthy and patient-derived iPS cells were induced to differentiation into neural crest cells. Increased mRNA expression was observed by qRT-PCR using neural crest markers (SOX10, P75, TFAP2B) and protein-level expression was also confirmed by fluorescent immunostaining using neural crest markers (AP-2a, SOX10). The flow cytometry confirmed that almost all cells were P75 positive. Finally, these cells were induced to differentiation into schwann cell precursors. Increased mRNA expression was observed by qRT-PCR using schwann cell markers (P75, SOX10, GAP43, ITGA4) and protein-level expression was also confirmed by fluorescent immunostaining using schwann cell markers (P75, SOX10, GAP43, S100β). From these results, it was concluded that both healthy and patient-derived iPS cells were successfully induced to differentiate into schwann cell precursors.

Conclusions: This study enables the generation of the new NF1 model that closely resembles the actual pathology by using iPS cells with patient genomic information, and brings us one step closer to understanding the mechanism of developing PN in NF1 patients.

Glial-to-Mesenchymal Transition Drives Malignant Progression of Plexiform Neurofibromas in *Nf1-KO* Mice

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Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft-tissue sarcomas for which there is no effective treatment to date. In the context of NF1, MPNSTs arise from plexiform neurofibromas (pNFs), benign nerve sheath tumors. The aim of this study is to decipher the stepwise progression of pNF to pre-malignant and then to MPNST, which we consider crucial for designing therapeutics to prevent or regress these aggressive tumors.

This was achievable thanks to a new mouse model *(Nf1-KO)* in which *Nf1* loss and expression of fluorescence reporter Tomato (Tom+) were targeted in boundary capderived Schwann cells. *Nf1-KO* mice recapitulate several NF1-associated symptoms, including the development of pNF and their progression into *bona fide* MPNSTs. Exploring this model leads us to the following observations:

By performing whole exome sequencing on primary tumors, we showed that the biallelic loss of *Cdkn2a* is the earliest genetic event accompanied by low copy number variants and single nucleotide variants. Interestingly, successive transplantations of *Cdkn2a-* tumor cells into Nude mice leads to an increased number of genetic alterations, including pathogenic *p53* variant, suggesting that primary tumors in our model correspond to low grade (LG) MPNSTs.

Using single-cell RNA sequencing data, we showed that malignant transformation is characterized by extinction of Schwann cell markers and activation of mesenchymal markers, a process defined as glial-to-mesenchymal transition (GMT), and which has been described in NF1 patients. IHC of tumor cells revealed that the GMT is concordant with disruption of cellular adhesion, increased tumor cells proliferation, and acquisition of migratory and invasive properties. In some MPNSTs, GMT precedes the loss of *Cdkn2a,* suggesting that GMT might be driven by epigenetic events and highlighting a possible reversibility of the process.

Differential expression analyses during GMT provided a list of candidate genes, including *Sox9,* whose expression is activated and maintained in mesenchymal-like tumor cells. Partial *Sox9* inhibition in tumor cells reduce their proliferative activity in culture and tumor growth after transplantation into Nude mice. Consequently, *Sox9* was used as biomarker of GMT and a read out for screening a library of 3500 FDA-approved drugs. Very unexpectedly, all of the top 12 drugs, with high efficacy to reduce *Sox9* expression and tumor cell proliferation, correspond to inhibitors targeting distinct checkpoints of the RAS pathway, highlighting the role of this pathway in malignant transformation.

The ongoing study consists to test the efficacy of some of those drugs *in vivo* using our *Nf1-KO* model of MPNSTs.

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Generation of a Chromosome 8 Gain;*NF1*-/- hiPSC-Derived Schwann Cell Precursor Model

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Background: Neurofibromatosis type 1 (NF1) is the most common cancer predisposition syndrome and is caused by a germline mutation in the *NF1* gene. Somatic loss of the second copy of *NF1* within schwann cell precursors (SCPs) leads to the development of benign plexiform neurofibromas (PNs), which can potentially transform into malignant peripheral nerve sheath tumors (MPNSTs). Our lab previously demonstrated chromosome 8 (Chr8) gain as a frequent event in NF1-MPNST patient-derived xenografts (PDX) and paired patient tumors. To explore the role of Chr8 gain, we generated isogenic *NF1*-/- iPSC cell lines with and without Chr8 gain.

Methods: A mosaic population of fibroblasts with trisomy 8 was obtained from Coriell and subsequently single-cell cloned to establish both wildtype (WT) and trisomy 8 (Chr8 gain) populations. The Genome Engineering & Stem Cell Center (GESC@MGI) at Washington University assisted in reprogramming these cells into human induced pluripotent stem cells (hiPSCs). *NF1* knockout lines were also generated by GESC@MGI using synthetic gRNA, and CRISPR/Cas9 to introduce *NF1* mutations into these cells. WT, Chr8 gain, *NF1*-/-, and Chr8 gain; *NF1*-/- iPSC cell lines were differentiated into Schwann cell precursors (SCPs) using a differentiation protocol that included TGF-beta inhibitor (SB431542), GSK-3 inhibitor (CT099021), N2, and B27 supplements. NRG-1 was added at day 6 of the protocol. The iPSC and SCP states of the cell lines were verified with qPCR after the completion of the differentiation protocol. Cell survival and proliferation of SCPs were assessed by Incucyte cell survival and CellTiter-Glo® 2.0 Cell Viability Assay.

Results: First, we validated the iPSCs with FISH and karyotyping. High expression of iPSC markers OCT3/4 and SOX2 was observed in all four iPSC lines. Second, after completing the differentiation protocol, we observed high expression of SCP markers GAP43 and ITGA4 in WT, *NF1*-/-, and Chr8 gain;*NF1*-/- SCP lines compared to their iPSC state, consistent with their morphology. However, the Chr8 gain iPSC line was unable to survive the differentiation process to SCPs, consistent with detrimental effects of aneuploidy in the absence of other oncogenic changes. Third, we observed that Chr8 gain; *NF1*-/- SCPs proliferate faster compared to WT and *NF1*-/- SCPs, and have enhanced survival compared to WT. Taken together, these findings support that Chr8 gain improves proliferation and cell survival in the setting of *NF1* loss.

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Increased Extracellular-Matrix Deposition and Disorganization in Neurofibromin-Haplodeficient Dermal Fibroblasts Cultured in 3D

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Background: Cutaneous neurofibromas (cNF) are peripheral nerve benign tumors mainly composed of Schwann cells, fibroblasts, and immune cells embedded in an abundant extracellular matrix (ECM). Accumulating evidence indicate a major role of fibroblasts in the establishment of the tumoral microenvironment (TME). The purpose of this study is to characterize the ECM produced by neurofibromin-haplodeficient dermal fibroblasts (NF1^{+/-}) isolated from patients, to depict the role of dermal fibroblasts in the establishment of TME favouring NF1-associated skin tumor formation.

Methods: Dermal fibroblast sheets, made using a tissue-engineered approach and 3D cell culture, was generated using skin fibroblasts isolated from NF1 patients and healthy individuals. The organization and abundance of collagen fibers in the tissues were evaluated by polarized light microscopy. Genes and proteins expression were assessed by RNA sequencing and liquid chromatograph-coupled mass spectrometry, respectively. Subsequent bio-informatics analyses were conducted on our datasets.

Results: NF1^{+/-} dermis were significantly thicker, indicating an increase of ECM secretion and deposition. Polarized light microscopy displayed an overabundance and disorganization of collagen fibers. Transcriptomic and proteomic analyses revealed that multiple genes involved in the secretion and organization of the ECM, cell migration, and proliferation were significantly overexpressed in NF1^{+/-} fibroblasts cultured in 3D. Finally, bioinformatic analyses revealed that the NF1^{+/-} reconstructed dermis were enriched by cancer- and tumors-associated proteins.

Conclusions: Our study suggests that haploinsufficiency of the *NF1* gene strongly alters the expression of genes and proteins associated with the matrisome in dermal fibroblasts culture in 3D. Consequently, fibroblasts could potentially play a major role in modifying the TME and promoting the formation and growth of cNF.

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Characterization of Resected Peripheral Nerve Sheath Tumors Using Radiologic Imaging, Histopathologic and Genomic Features

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Background: NF1 is characterized by peripheral nerve sheath tumors (PNST) in the spectrum of neurofibroma (NF), plexiform NF (PN), NF with cytologic atypia (AN), atypical neurofibromatous neoplasia of unknown biologic potential (ANNUBP), and malignant peripheral nerve sheath tumors (MPNST). The clinical, imaging, and genomic features which predict for malignant transformation of PNST are incompletely understood. Here, we comprehensively assessed the relationship between histologic, imaging, and genomic features of PNST resected at the NIH because of clinical concern.

Methods: All PNST tumors resected at the NIH between 2005 and 2023 were classified by an independent pathologist based on the 2017 diagnostic criteria[1]. PNST growth rate was calculated using volumetric MRI analysis (% change per year within 3 years of resection). Where available prior to resection, the minimal apparent diffusion coefficient (ADC_{min}) from diffusion weighted imaging (DWI) on MRI and maximal standard uptake value (SUV_{max}) from 18-fluoro-deoxy-glucose positron emission tomography (FDG-PET) were also captured. TruSight Oncology 500 Gene Panel sequencing was performed on PNST resected after 2017 including *CDKN2A/B* status. Statistical analyses including descriptive statistics, simple linear regression, ANOVA, and receiver operating characteristics (ROC) area under the curve (AUC) analysis were performed on Rv4.3.1.

Results: Sixty-nine PNST with MRI and histopathology available were resected from 32 patients. Of these, 62 (90%) appeared as distinct nodular lesions on MRI. Median number of resected tumors per patient was 1.5 (range 1-7), and the median age at resection was 20 years (range 10.4-51.3). Histologically, the 69 lesions were: neurofibroma (NF) ($n=13$), PN ($n=13$), cellular NF ($n=1$), AN ($n=16$), ANNUBP ($n=19$), or MPNST ($n=7$). NF and PN were grouped for comparisons. Among the 52 PNST with ≥ 2 MRI, annualized growth rates were similar between NF, AN, and ANNUBP, while MPNST ($n=4$) had higher growth rates ($p<0.001$). Of the 45 PNST with PET imaging, ANNUBP ($n=16$) and MPNST ($n=5$) had higher SUV_{max} (median 6.8, 12.3 respectively) than NF ($n=14$, median 3.3) (NF vs ANNUBP: $p=0.03$; NF vs MPNST: $p<0.001$). ROC analysis of SUV_{max} showed moderate discriminative ability between NF and ANNUBP (AUC 0.79). Among the 27 PNST with DWI, there was substantial variability in ADC_{min} and no significant difference between NF, ANNUBP, and MPNST. No correlation was found between growth rate and SUV_{max} ($p=0.14$), or between ADC_{min} and SUV_{max} ($p=0.11$). Eleven out of 12 cases with *CDKN2A/B* loss were histopathologically AN, ANNUBP, or MPNST.

Conclusions: In this cohort, ANNUBP and MPNST had significantly higher SUV_{max} on FDG-PET compared to NF and AN. In the PNST with ADC_{min}, it alone did not distinguish between NF, ANNUBP and MPNST. Contrary to our expectation, only MPNST had higher growth rates when compared to NF, AN, and ANNUBP. *CDKN2A/B* loss was almost exclusively identified in AN, ANNUBP and MPNST, consistent with prior reports. While SUV_{max}, growth rate, and ADC_{min} are crucial in tumor characterization, a multimodal approach integrating imaging, genomic, and molecular features may be needed for risk stratification. Additional analyses are ongoing, including correlation of histopathology (nuclear density/atypia) with imaging and genomic findings.

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A NF1 Genotype-Phenotype Strategy Based on Transcriptomic and Xenograft Mouse Models Suggests Reveal the Plasticity of *NF1*-/- Schwann Cells and May Unveil Potential Tumorigenicity Markers

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Background: With over 3,000 NF1 pedisposing mutations in the NF1 gene, existing genotype-phenotype relationships cover less than 10% of NF1 patients. To improve genotype-phenotype relationship and focus on the tumor manifestations, we hypothesized that transcriptome investigations of *NF1*-/- Schwann cells harboring various *NF1* mutations and tumorigenic potential may yield markers/signature correlating with tumorigenicity.

Methods: The tumorigenicity of the commercially available *NF1*-/- Schwann cells (ipNF95.11b, ipNF05.5, ipNF95.6) was assessed by implanting them into the vicinity of the injury-induced sciatic nerve of an immunocompromised (SCID) mouse. Published RNAseq datasets from Ferrer et al. (SciData, 2018) were reanalyzed, and results were confirmed using RT-qPCR.

Results: Histological evaluation (H&E) and immunostaining [S100 (Schwann-cell specific), Ku80 (human-specific)] indicate that xenograft of ipNF95.11b show robust tumor formation (4/4, 100%) whereas the ipNF05.5 (1/4, 25%) shows moderate and the ipNF95.6 (0/4) shows no tumors. Unexpectedly, looking closely at the expression of development-specific neural crest stem cell (EDNRB, ITGA4); Schwann cell precursor (CDH19), immature Schwann cell (GAP43, GFAP, MPZ, PLP1, PMP22, S100B) and general Schwann cell markers (ERBB3, SOX10) did not strongly correlate with of the tested *NF1*-/- Schwann cells. However, our results suggest a plasticity of *NF1*-/- Schwann cells from neural crest stem cell markers (EDNRB, ITGA4) to non-myelinating schwann cell markers (GalC, ITGA1, NGFR). Additionally, we tested different tumorigenic markers, unrelated to Schwann cell development-specific markers. Surprisingly, only ipNF95.11b exhibited downregulation of the MPNST driver CDKN2A, whereas all *NF1*-/- Schwann cells exhibited upregulation of the cancer-associated fibroblast marker SPP1.

Conclusion: To derive novel genotype-phenotype correlation, we developed a strategy where genome-wide transcriptomic data are correlated with tumorigenicity of *NF1*-/- Schwann cells harboring various NF1 mutations. So far, our results suggest that the expression of development-specific Schwann cell markers is not indicative of tumorigenicity in a xenograft model. However, our results suggest a plasticity of NF1 -/- Schwann cells from neural crest cell to non-myelinating Schwann cells. Additional markers, such as SPP1 and CDKN2A, as well as xenograft models suggest a general potential tumorigenicity of *NF1*-/- Schwann cells, especially ipNF95.11b Schwann cells.

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Decipher the Mechanisms Governing Cutaneous Neurofibromas Development in a Mouse Model of Neurofibromatosis Type 1

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Cutaneous neurofibromas (cNFs) are a major burden for NF1 patients and yet their pathogenesis is poorly understood, resulting in a lack of treatment to prevent or reverse their development. This study aims to identify the cells of origin among the SC lineage and decode the cellular and molecular cues driving cNFs pathogenesis to identify therapeutic targets.

This study was conducted using an *Nf1*-KO mouse model (*Prss56[Cre/+], R26[Tom/+], Nf1^{fl/fl}*), carrying bi-allelic *Nf1* loss and expression of Tomato fluorescent reporter into boundary caps-derived SCs. *Nf1-KO* mice develop bona fide cNFs from 1 year[3] and skin trauma induced by bites in grouped-house males accelerates this process. This cNF-inducible model (*Nf1*-KOi) was used to explore their natural history by collecting and analysing tumours at successive stages of development using immunohistochemistry. Three stages: initiation, progression, and stabilization were identified in *Nf1*-KOi mice and validated in patients[4]. They are defined by changes in cellular composition and activity. Initiation and progression periods are characterized by increased proportions of Tom+ mutant SCs, fibroblasts, immune cells accompanied by abnormally dense innervation. At the stabilization phase, the proportions of immune cells and potentially fibroblasts decrease before stabilizing. Mutant SCs proliferation is transient and restricted to initiation and progression phases while virtually all SCs from stabilized tumours appear quiescent with undetectable MAPK activity (readout of the RAS activity). Finally, cNFs are characterized by a dense innervation of PGP9.5+ axons.

Next, to define the transcriptomic signature of tumour SCs and their microenvironment at successive stages of cNFs development, scRNAseq analyses of cNFs, adjacent "healthy" (HLS) and control skin (CS) have been performed at the progression and stabilization phases. Since proportion of Tom+ SCs in the skin is low, scRNAseq analysis for each condition was conducted separately on purified Tom+ SCs and the skin microenvironment. Bioinformatics analyses are focused on horizontal (mutant vs. control cells at the same stage) and vertical (mutant cells between progression and stabilization phases) comparisons. Results reveal: (i) a profibrotic and proinflammatory activity of a cutaneous *Aqp1*+ non-myelinating SCs (nmSCs) population in cNFs, (ii) those cNFs' *Aqp1*+ SCs express SCs-axon crosstalk-related genes not found in HLS or CS during the progression, (iii) dermal and nerve-associated fibroblasts are responsible for cNFs important fibrotic activity, (iv) *Postn* and *Tnc* are potential therapeutic targets expressed by tumor and microenvironment cells during the progression and stabilization phases or progression phase, respectively.

In summary, this study demonstrates that the development of cNFs is transient and follows three successive stages: initiation, progression, and stabilization. scRNAseq experiments have allowed us to build ctrl and *Nf1*-KO transcriptomic skin atlases at the single cell resolution in both young and older mice. The scRNAseq analyses emphasize the significance of a specific population of Aqp1+ nmSCs in the formation and maintenance of cNFs. Additionally, they suggest that these nmSCs, along with dermal and nerve-associated fibroblasts, contribute to the fibrotic activity of cNFs. Finally, scRNAseq analyses point to *Postn* and *Tnc* as novel therapeutic targets for the treatment of growing and/or mature cNFs.

Full List of Authors: Fanny Coulpier, PhD, Audrey Onfroy, MSc, Katarzyna Radomska, PhD, and Piotr Topilko, PhD

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Spatial Nanomechanical and Transcriptomic Profiling of NF1 Tumors

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Purpose: We are undertaking a systematic investigation of the altered biomechanics and mechanobiology that accompany the development of plexiform neurofibromas (PNFs) and malignant peripheral nerve sheath tumors (MPNSTs). We gather this data for the purpose of further understanding the role of the microenvironment in tumor development and malignant transformation as well as identifying new targets for PNF and MPNST treatment.

Methods: PNFs were generated spontaneously in the dorsal root ganglion and trigeminal nerves of *Nf1[Fl/-]; Krox20[Cre/+]* mice.[1] MPNSTs were generated via CRISPR-Cas9 injection in the sciatic nerves of NF1 and wildtype mice.[2] Tumor progression was monitored, and mice were euthanized at humane or tumor endpoints. All mouse work was approved by the RPI IACUC and performed under the supervision of a resident veterinarian. Tumors and control tissues were excised and cross-sectioned for mechanical testing, staining, and spatial transcriptomics. Nano-indentations—force versus indentation depth—were collected over the cross-section with an atomic force microscope and the relative resulting stiffness values—soft, intermediate, and stiff—were mapped to histological sections. Spatial transcriptomics was optimized and performed following 10x Genomics and Visium protocols for fresh frozen sections.

Results: We find NF1 tumors are on average stiffer than healthy tissues or sporadic tumors, with PNFs having the largest mean stiffness. Combining our stiffness maps with histological and spatial transcriptomic mapping, we are identifying how tissue structure, cell types, extracellular matrix components, and differentially expressed proteins contribute to the mechanical micro-environment. For example, mapping sporadic MPNSTs revealed that, in the intermediate stiffness regions, the matrix remodeling protein MMP9 was upregulated, whereas the mast cell gene CMA was down regulated. Full spatial analysis of these tumors and tissues highlights differentially expressed genes correlated to increased stiffness in the NF1 tumor microenvironment.

Conclusions: Tumors with an NF1 background demonstrate altered mechanical properties compared to healthy peripheral nerve tissue and sporadic MPNSTs. Using spatial transcriptomics, tumor stiffness can be correlated to gene expression revealing the molecular link to the mechanical environment of these tumors. Armed with this information we will be able to design targeted studies into the functionality of these differentially expressed genes, elucidating their effects on the NF1 tumor mechanical micro-environment.
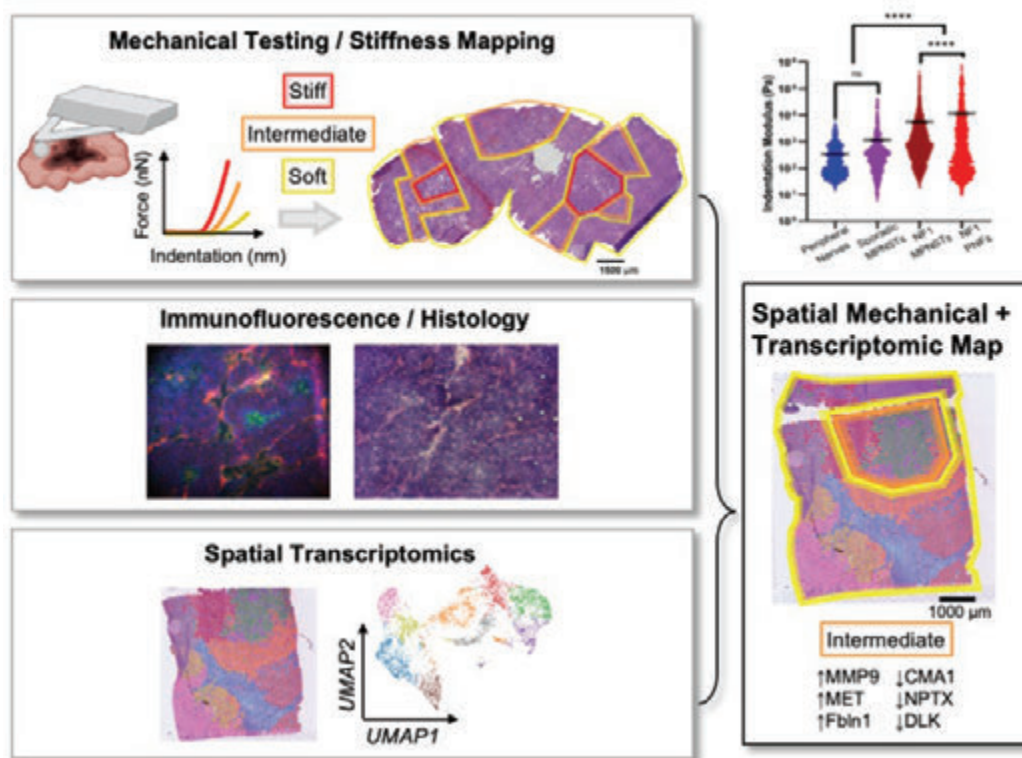
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A Systematic Review of Diagnostic Modalities and Strategies for the Assessment of Complications in Adult Patients with Neurofibromatosis Type 1

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Background: Neurofibromatosis Type 1 is an autosomal dominant tumour-predisposition condition commonly diagnosed in childhood and fully penetrant by adulthood. Long-term monitoring through imaging is inconsistent and varies between high and low-income countries. Implementation of a clinical practice guideline through a multidisciplinary clinic is instrumental to the care of adult Neurofibromatosis Type 1 patients. We aim to systematically review international diagnostic modalities and strategies to evaluate any association between a country's socioeconomic status and diagnostic modalities or strategies used for Neurofibromatosis Type 1 patients.

Methods: We searched PubMed, Embase, Web of Science and Cochrane. Relevant clinical information on the surveillance of adult Neurofibromatosis Type 1 patients worldwide was reviewed, extracted, and synthesised.

Results: We identified 51 papers reporting on 7724 individuals. Multiple imaging modalities are actively employed in high-income and upper-middle-income countries for surveying adult Neurofibromatosis Type 1 patients. We did not find any relevant papers from low- and middle-income countries.

Conclusion: This systematic review suggests that there is robust data on diagnostic modalities for adult Neurofibromatosis Type 1 patients in high-income countries, but not for low- and middle-income countries. There is a lack of data on consolidated diagnostic strategies from both high and low-income countries. Efforts should be made to publish usual clinical practice in low- and middle-income countries to develop clinical practice guidelines describing best medical practice to fit a local context.

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Combining SHP2 Inhibition with CX3CR1 Inhibition Shrinks Tumors and Normalizes the Plexiform Neurofibroma Microenvironment

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Purpose: Initially developed to target oncogenic signaling, targeting RAS–MAPK signaling is increasingly recognized for immunomodulatory effects that contribute to anti-tumor effects and sensitize the tumor microenvironment to further immunomodulatory therapies. We used a genetic model of benign neurofibroma formation in neurofibromatosis type 1 to compare the effects of inhibiting two distant nodes in the RAS-MAPK pathway, SHP2 and MEK, on tumor growth and tumor immune cells, with the goal of identifying combination therapies for improved pre-clinical efficacy.

Methods: Tumors were assessed by volumetric imaging, multi-spectral flow cytometry, and single-cell RNA-Seq.

Results: SHP2 and MEK inhibitors each significantly shrank plexiform neurofibromas, as assessed by repeated volumetric measurements. MEK and SHP2 inhibitors also each significantly shrank the immune compartment in neurofibromas, as assessed by single-cell RNA-Sequencing and by flow cytometric analyses. However, the two also had different effects. SHP2 inhibition affected the frequency of circulating and tumor monocytes, tumor CD163- macrophages, and altered T cell phenotypes, effects that were not observed after MEK inhibition. In contrast, MEK inhibition but not SHP2 inhibition targeted CD163+ parenchymal macrophages. We identified the chemokine receptor CX3CR1 as a marker of PNF monocytes, macrophages, and T cells. Anti-PD1 driven T cell activation increased CX3CR1 on T cells, and reversed effects of SHP2 inhibition on tumor shrinkage, implicating T cells in resistance to therapy in these benign tumors. Finally, combining SHP2i and CX3CR1 inhibition significantly improved tumor shrinkage and normalized immune cell phenotypes.

Conclusions: In a preclinical model of plexiform neurofibroma, combining CX3CR1 and SHP2 inhibition prevents T cell accumulation, restores immune populations to those resembling normal tissue, and shrinks the benign plexiform which are characteristic of Neurofibromatosis type 1. These findings underscore the unique immune microenvironment present in PNF and suggest a new direction for neurofibroma therapy.

GDNF Signaling to Neurons Modulates Pain in a Preclinical Model of NF1

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Purpose: In the current study, we wanted to determine the role of glial cell-derived neurotrophic factor (GDNF) in modulating pain in preclinical model of neurofibromatosis 1 (NF1), and whether it acted through binding its co-receptor, GDNF family receptor α1 (GFRα1), expressed on mechanical nociceptors.

Methods: We performed operant behavioral assessments of mechanical sensitivity, and single unit electrophysiological recordings of sensory neurons using a novel *ex vivo* skin/nerve/dorsal root ganglion (DRG)/spinal cord preparation from mice with SC-specific knockout of *Nf1* (DhhCre;Nf1$^{f/f}$) that were injected with an AAV9 containing a shRNA against GFRα1 into the both sciatic nerves compared to controls (AAV9 with GFP) to analyze the role of GDNF signaling to neurons in NF1-related pain development. The knock down of the GFRα1 was validated with immunohistochemistry in the DRGs for GFRα1. As GDNF is also released by macrophages, we depleted macrophages by systemic delivery of clodronate containing liposomes (i.v.) prior to behavioral analyses to determine the role of other cells in onset of pain in NF1.

Results: *Nf1* deletion in SCs prior to tumor formation at 4-5 months of age produced mechanical hypersensitivity using choice-based behavioral assessments (mechanical conflict avoidance). This correlated with sensitization of myelinated A-fiber nociceptors and unmyelinated polymodal C-fibers (CPM) to mechanical stimuli compared to controls using *ex vivo* recording as shown in our recent work. The depletion of macrophages present in the nerves and DRGs of DhhCre;Nf1$^{f/f}$ mice by i.v. delivery of clodronate containing liposomes; however, did not alter the mechanical hypersensitivity observed in the NF1 mouse model when compared to controls (*$p < 0.0475$, vs control two-way ANOVA with Tukey post-hoc; Mean ± SEM, Control w Clod, n= 9,; DhhCre;Nf1f/f with PBS, n= 7,;DhhCre;Nf1f/f w Clod, n= 11) (Fig 1). The depletion of macrophages using this strategy was verified by IHC (*$p < 0.0001$vs control (PBS injected), unpaired t-test; Mean ± SEM) (Fig 2). Conversely, the mechanical hypersensitivity in the DhhCreNf1$^{f/f}$ mice was reduced to control levels after injection with AAV9-m-GFRα1-shRNA when compared to the mice injected with control AAV9 (GFP) (*$p = 0.0036$ vs control two wayANOVA with Tukey post-hoc; Mean ± SEM Control w AAV(GFRα1), n=11, DhhCre;Nf1f/f w AAV (GFRα1), n=11, (GFP), n=8, DhhCre;Nf1f/f w AAV (GFRα1), n=7) (Fig 3).

Conclusions: The current study shows that GDNF signaling to GFRα1 containing nociceptors plays an important role in the onset of pain in a mouse model of NF1. Results provide insight into potential therapeutic treatment approaches for pain NF1 patients.

Fig1: Depletion of macrophages does not change the mechanical hypersensitivity observed in the DhhCreNf1$^{f/f}$ mice.
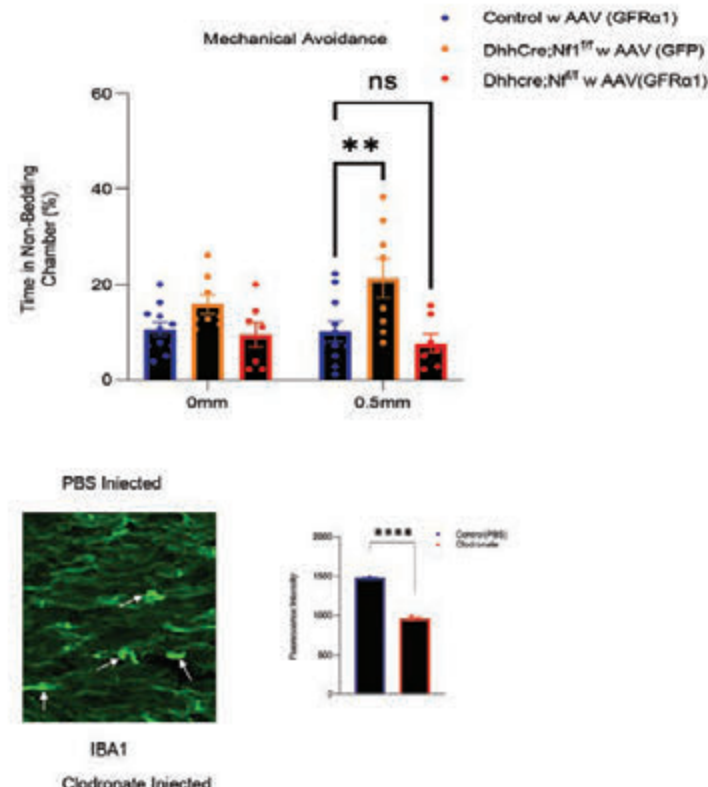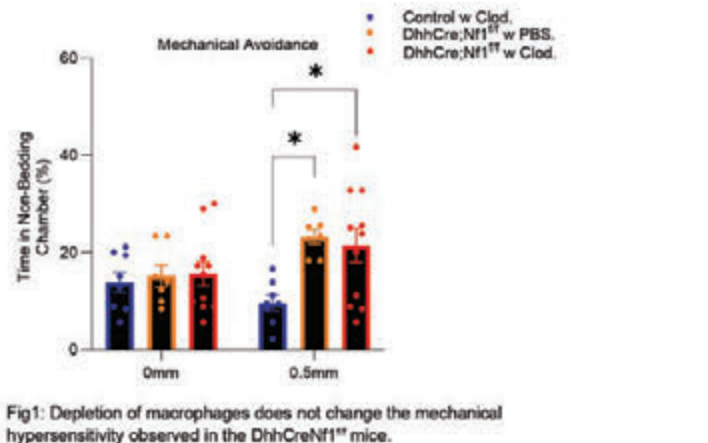
Fig 2: Depletion of macrophages by injecting clodronate containing liposomes (top left), compared to PBS injected animals (bottom left) with NF1 (DhhCre;Nf1$^{f/f}$), and quantification (right)

Molecular and Circuit Mechanisms of Visual Hypersensitivity in Neurofibromatosis Type 1 Model Mice

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The neurocognitive symptoms of neurofibromatosis type 1 (NF1) include impaired executive functioning, autistic features, speech and language delays, and sensory processing abnormalities. NF1 patients also have a high incidence of attention deficit/hyperactivity disorder, which is associated with diminished ability to suppress distractive stimuli, such that irrelevant environmental cues are assigned exaggerated stimulus salience. Using a heterozygous knockout *(Nf1^{+/-})* mouse model of NF1, we've found that *Nf1* haploinsufficiency causes hypersensitivity to salient visual stimuli, including distracting light stimuli or threatening looming discs that promote escape by simulating predator approach from above. However, the molecular pathways and brain circuits that contribute to these phenotypes are unknown. At the 2024 Global NF Conference, we will present evidence that sensory hypersensitivity in NF1 model mice is driven by enhanced mitogen-activated protein kinase signaling (MAPK; Ras-Raf-MEK-ERK), as novel transgenic mice with an activating *Mapk1* (ERK2) mutation recapitulate several phenotypes observed in *Nf1^{+/-}* mice, including increased reactivity to looming visual threats. Additionally, we have performed electrophysiological, histological, and physiological characterization of retinal circuits in *Nf1^{+/-}* mice and *Nf1^{-/-}* conditional knockout mice, which suggest that neurofibromin loss does not significantly alter retinal ganglion cell function, as has been previously hypothesized. Rather, visual response phenotypes originate in downstream visual processing circuits. Presently, we are employing circuit- and cell-type specific genetic MAPK manipulations, as well as calcium imaging techniques, to better understand the etiology of sensory processing abnormalities in NF1 model mice. If successful, these studies will significantly improve our understanding about how *NF1* haploinsufficiency and MAPK pathway activation contribute to attentional deficits and impacts sensory function.

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Proteomic Analysis of Small Extracellular Vesicles Secreted by NF1 Patients-Derived Skin Fibroblasts

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Background: Increasing evidence points to a pivotal role of fibroblasts in shaping the tumor microenvironment (TME) within the context of NF1. Small extracellular vesicles (sEVs) are multifunctional entities known to participate in the establishment of TME. This study aims to assess the protein composition of sEVs derived from 3D-cultured skin fibroblasts isolated from both NF1 patients and healthy controls.

Methods: sEVs were purified from the conditioned media of tissue-engineered 3D dermis, made of skin fibroblasts and self-secreted extracellular martrix (ECM), using a commercial reagent and centrifugation. Particle shape and size were assessed through transmitted electron microscopy, while size distribution and concentration were determined using semi-automated nanoparticle tracking-based analysis. Identification and quantification of sEV proteins were carried out with liquid chromatograph-coupled mass spectrometry (LC-MS/MS). Bioinformatics analyses were subsequently performed on the obtained datasets.

Results: sEVs with a close-to-spherical shape and a diameter of approximately 100 nm or less were successfully isolated. The size distribution of particles was nearly identical between both groups. Among the 772 proteins identified through mass spectrometry, 561 proteins met the right conditions to be properly quantifiable. A total of 105 differentially expressed proteins were detected, with 48 up-regulated in NF1 sEVs and 57 down-regulated, compared to the controls. Enrichment analyses unveiled an overrepresentation of proteins involved in ECM organization and collagen formation in NF1-derived sEVs. Notably, 66.5% of the quantified proteins were associated with the ECM, with a total of 62 significantly modulated proteins that were annotated as matrisomal proteins. We also identified, through in-depth bioinformatic pathway analyses based on the functional annotation of the dysregulated proteins, numerous predicted pathological functions associated with both the immune response and the angiogenic process.

Conclusions: This study provides strong evidence that sEVs, isolated from NF1 skin fibroblasts-conditioned media, display specific disease signature associated with ECM and collagen formation. These findings suggest that fibroblasts could participate in the establishment of the cellular microenvironment to promote tumor growth in NF1 patients. Our model could become a unique tool to better characterize the pathogenic mechanisms associated with skin tumor genesis. Ultimately, it could provide better tools to develop new therapies for patients through the development of personalized medicine strategies.

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Hydroxychloroquine Prevents Resistance and Potentiates Antitumor Effect of SHP2 Inhibition in NF1-Associated Malignant Peripheral Nerve Sheath Tumors

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Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas and the primary cause of mortality in patients with neurofibromatosis type 1 (NF1). These malignancies develop within pre-existing benign lesions called plexiform neurofibromas (PNs). PNs are driven solely by biallelic *NF1* loss eliciting RAS pathway activation and respond favorably to MEK inhibitor (MEKi) therapy. MPNSTs harbor additional mutations and respond poorly to MEKi. Effective therapies are urgently required. Given the well-established phenomenon of compensatory feedback upstream activation following MEKi, we reasoned that use of inhibitors upstream of RAS in lieu of MEKi could perhaps mitigate such secondary activation in MPNST. The protein-tyrosine phosphatase SHP2 (encoded by *PTPN11*) functions as a positive signal transducer upstream of SOS1/2 and NF1. Therefore, we re-examined MEKi in comparison with SHP2 inhibition (SHP2i) in multiple NF1 MPNST mouse models.

Methods: Our preclinical screening platform includes tumor-derived primary cultures from *Nf1-/-;Trp53-/- (NP) or Nf1-/-;Ink4a/Arf-/- (NI)* mutant mouse models that were used to triage promising mono- or combination therapies followed by progression through orthotopic mouse tumors for rapid *in vitro* and *in vivo* screening, culminating in validation in orthotopic patient-derived xenograft (PDX) models of NF1-MPNST.

Results: Our analysis of genetically engineered and orthotopic PDX MPNST tumor models indicates that MEKi has poor anti-tumor efficacy. By contrast, upstream inhibition of RAS through the protein-tyrosine phosphatase SHP2 reduced downstream ERK signaling and suppressed *NF1* MPNST growth, although resistance eventually emerged. To investigate possible mechanisms of acquired resistance, kinomic analyses of resistant tumors was performed, and data analysis identified enrichment of activated autophagy pathway protein kinases. Combining SHP2i with hydroxychloroquine, an autophagy/lysosomal inhibitor resulted in durable responses in *NF1* MPNSTs in both genetic and orthotopic xenograft mouse models.

Conclusions: We demonstrate intrinsic MPNST resistance to MEKi monotherapy and identify SHP2 inhibition as an actionable vulnerability upstream of RAS. Furthermore, anti-tumor effects are extended and enhanced by dual exposure to autophagy pathway inhibition. Validation of these results as the most effective therapy to date in multiple genetically engineered models and in orthotopic PDXs justify a clinical trial to evaluate SHP2i in conjunction with hydroxychloroquine as a novel treatment approach for *NF1* MPNSTs.

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HLX-1502: a novel potential treatment for neurofibromatosis type 1 plexiform neurofibromas

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Identified using Healx's AI-informed drug discovery approach, HLX-1502 has a novel mechanism of action, and is supported by data that suggest a favourable safety profile. Healx plans to develop HLX-1502 for both plexiform and cutaneous subtypes of NF1.

HLX-1502 has anti-proliferative activity in human Schwann cells derived from a plexiform neurofibroma (ipNF95.6), and significantly reduces nerve volume and tumour number in the *Postn-Cre+ Nf1fl/fl* mouse model of plexiform neurofibroma. The level of efficacy observed in these models is equivalent to selumetinib. In addition to activity in plexiform neurofibroma, HLX-1502 is active in *in vitro* models of cutaneous neurofibroma and malignant peripheral nerve sheath tumour. Notably, HLX-1502 does not inhibit the RAS/MAPK pathway. Given efficacy of HLX-1502 in the *in vivo* NF1 mouse model, we set to understand the long term effect of compound treatment on NF1 Schwann cells and the effect of compound wash out, in comparison to the standard of care selumetinib. Data from the *in vitro* washout experiment suggested that while cells treated over a prolonged period of time with selumetinib or HLX-1502 are able to re-grow following compound wash out, cells that have been treated with HLX-1502 re-grow more slowly. Clinical development planning is in progress, as well as efforts to further understand mechanisms behind the activity of HLX-1502 in NF1.

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Dietary Intervention Rescues a Bone Porosity Phenotype in a Murine Model of Neurofibromatosis Type 1 (NF1)

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Reduced bone mineral density and increased bone porosity are features of Neurofibromatosis type 1 (NF1) that are often considered secondary to its orthopedic manifestations. However, osteopenia has been reported in ~30% of children with NF1 — none of whom presented with either tibial dysplasia or scoliosis. The mechanisms underlying these systemic effects on bone are unclear but may involve changes to cellular metabolism. Our recent work found metabolic changes in NF1-deficient muscle resulting in intramyocellular lipid accumulation. Moreover, the muscle phenotype in the $Nf1_{Prx1}^{-/-}$ mouse (limb-targeted $Nf1$ knockout mice) could be rescued with a dietary intervention that modulates lipid availability and metabolism.

To test this hypothesis, bone specimens were analyzed from $Nf1_{Prx1}^{-/-}$ mice fed standard chow and a range of modified chows as well as wild type control mice. MicroCT analysis was performed on the cortical bone to look at standard parameters (bone volume, tissue mineral density, cortical thickness) and specific porosity measures (closed pores corresponding to osteocyte lacunae and larger open pores). $Nf1_{Prx1}^{-/-}$ bones were found to have inferior bone properties to wild type bones, with a 4-fold increase in the porosity attributed to open pores. These measures were rescued by dietary interventions including L-carnitine + medium-chain fatty acid supplemented chow previously shown to improve muscle histology function. Histological staining visualized these changes in bone porosity.

These data support the concept that lipid metabolism may have a mechanistic impact on bone porosity and quality in NF1.

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Therapeutic Targeting of PRC2-Driven MPNST Metastasis

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Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas with poor clinical prognoses. Up to 50% of MPNSTs metastasize, and the 5-year survival rate of 20-35% has not improved in recent years. The overall goal of this study was to identify and disrupt mechanisms of MPNST metastasis in preclinical models. The majority of MPNSTs have loss-of-function mutations in the polycomb repressive complex 2 (PRC2), a master regulator of gene expression. Dysregulation of PRC2 activity is linked to poor prognosis, metastatic disease, and chemotherapy resistance across multiple cancer types. We have recently used a combination of in vitro metastasis assays, 3D collagen studies, and orthotopic mouse models to identify PRC2-dependent mechanisms of metastasis in multiple paired, isogenic MPNST cells. By combining these approaches with time-lapse microscopy and analysis of patient samples, we determined that loss of PRC2 drives MPNST metastasis through remodeling of the extracellular matrix (ECM). Specifically, PRC2 deletion increases collagen-dependent invasion in vitro and drives lung metastasis in orthotopic mouse models. Additionally, clinical sample analysis demonstrates that PRC2 loss correlates with metastatic disease, increased fibrosis, and decreased survival in patients with MPNSTs. PRC2-driven metastasis is associated with increased expression of lysyl oxidases (LOX), a family of matrix-remodeling enzymes. Importantly, inhibition of LOX activity restricts PRC2-dependent metastatic phenotypes in vitro, identifying a potential therapeutic vulnerability in patients with PRC2-null MPNST. This deeper understanding of PRC2-dependent metastatic events has high potential to improve the clinical management of MPNSTs and has broad implications for PRC2 function across multiple cancers. Current studies focus on testing clinically relevant agents that inhibit PRC2-driven mechanisms in vivo to determine if PRC2 regulation of LOX enzymes is a targetable pathway to prevent MPNST metastasis.

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CENPF as a Biomarker and Therapeutic Target for NF1-Associated MPNST

A. Sheth, PhD, *Department of Pediatrics, Herman B Wells Center for Pediatric Research, IUSM Indianapolis, IN*

Purpose: Individuals with Neurofibromatosis Type 1 (NF1) are predisposed to the development of benign peripheral nerve sheath tumors called plexiform neurofibromas (PNF), a subset of which have the potential to transform into malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs are highly aggressive, treatment-refractory form of sarcoma and is the leading cause of death in NF1 patients. Predictive biomarkers to identify PNF with malignant potential are urgently needed, as are molecular targets for the development of effective therapies to treat MPNSTs. Our recent work profiling differential gene expression associated with progression of PNF to MPNST identified CENPF, a mitotic protein, as the top upregulated gene in MPNST relative to precursor lesions, suggesting a role for this gene in the malignant transformation of PNF. Overexpression of CENPF has been implicated in the progression and poor prognosis of various cancers but has not been interrogated in MPNST.

Methods: To assess CENPF expression across the PNF-MPNST continuum we utilized immunohistochemistry (IHC) and Western blot (WB) analysis to quantify CENPF protein levels in each tumor type.

To assess CENPF as a therapeutic target for MPNST, we performed *in vitro* cell viability and apoptosis assays (CellTiter-Glo and Caspase-Glo 3/7, Promega) in MPNST cell lines following genetic depletion of CENPF.

To determine whether progression of PNF triggers the production of CENPF autoantibodies that may be used as a serological marker of malignancy, we performed enzyme-linked immunosorbent (ELISA) assays of plasma collected from our *Nf1$^{flox/flox}$;Cdkn2a$^{flox/flox}$; PostnCre$+$* mice, which faithfully recapitulate the spontaneous development of PNF that progress to MPNST, to detect and quantify CENPF-specific antibodies in MPNST-bearing mice.

Results: Quantitative detection of CENPF protein in human tumor samples via IHC confirmed significantly increased CENPF levels in MPNST compared to precursor lesions. Further, we found that siRNA and shRNA-mediated silencing of CENPF significantly decreased the viability and increased apoptosis of human MPNST cell lines *in vitro.* We also observed CENPF-specific autoantibodies in the plasma of tumor-bearing Nf1$^{flox/flox}$;Cdkn2a$^{flox/flox}$; PostnCre$+$ mice.

Conclusions: Our findings suggest that CENPF is a potential predictive biomarker to implement in the screening of NF1 patients for early detection of MPNST, either through immunohistochemical evaluation of biopsy samples or through minimally invasive serological detection of CENPF autoantibodies. Further, CENPF may represent a therapeutic target for the treatment of MPNST.

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Encephalocraniocutaneous Lipomatosis Phenotype Associated with Mosaic Biallelic Pathogenic Variants in the NF1 Gene

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Purpose: To describe the molecular mechanisms linking encephalocraniocutaneous lipomatosis (ECCL) and neurofibromatosis type 1 (NF1).

Methods: In this case study, we report on a NF1 patient with a germline pathogenic variant in the *NF1* gene and an ECCL phenotype, suggesting ECCL to be part of a spectrum of malformations associated with *NF1* pathogenic variants. An anatomical hemispherectomy was performed for intractable epilepsy. Through genetic analysis of blood, cerebral tissue, and giant cell lesions in both jaws, we identified the germline *NF1* pathogenic variant in all samples and a second hit *NF1* pathogenic variant in cerebral tissue and both giant cell lesions (Table 1.)

Table 1. First and second hit NF1 variants

Tissue	First hit (germline *NF1* variant) c.5234C>G p.(Ser1745*)	Second hit (somatic *NF1* variant) c.3916C>T p.(Arg1306*)
Cerebral tissue, NOS (2 specimens)	Present, heterozygous	Not present
Cerebral tissue surrounding hamartomatous lesion (3 specimens)	Present, heterozygous	Present in 53%, 56% and 60% of cells
Hippocampus (2 specimens)	Present, heterozygous	Present in 57% and 60% of cells
Hamartomatous lesion (2 specimens)	Present, heterozygous	Present in 35% and 55% of cells
Giant cell lesion jaw	Present, heterozygous	Present
Plexiform neurofibroma	Present, heterozygous	Not present. Further genetic testing (NGS) for a different second hit variant was negative.

Table 1. **First and second hit *NF1* variants.** DNA was extracted from FFPE (formalin-fixed, paraffin-embedded) tissue by use of standard techniques. All DNA samples were screened for the following *NF1* variants: NM_000267.3: c.5234C>G p.(Ser1745*) and c.3916C>T p.(Arg1306*). Primers were tagged with M13 sequences to facilitate sequencing. The PCR products were bidirectionally Sanger sequenced on an ABI 3100 sequencer. We performed genetic analysis on cerebral tissue (NOS cerebral tissue, hippocampus and the hamartomatous lesion), the giant cell lesion of the jaw and the plexiform neurofibroma.NOS; not otherwise specified.

Results: ECCL is a sporadic congenital condition characterized by ocular, cutaneous and central nervous system involvement. Mosaic activating variants in *FGFR1* and *KRAS* have been reported in several individuals with this syndrome. In our patient with ECCL and NF1, we identified the the germline *NF1* pathogenic variant in all samples and a second hit *NF1* pathogenic variant in cerebral tissue and both giant cell lesions, resulting in somatic mosaicism for a biallelic *NF1* inactivation originating in early embryogenesis (second hit mosaicism or Happle type 2 mosaicism). The biallelic deficit in *NF1* in the left hemicranium explains the severe localized, congenital abnormality in this patient. Identical first and second hit variants in a giant cell lesion of both upper and lower jaws provide confirmatory evidence for an early embryonic second hit involving at least the neural crest.

Conclusion: We suggest that the ECCL phenotype may be part of a spectrum of congenital problems associated with mosaic *NF1* nullisomy originating during early embryogenesis. The biallelic *NF1* inactivation during early embryogenesis mimics the severe activation of the RAS-MAPK pathway seen in ECCL caused by embryonic mosaic activating *FGFR1* and *KRAS* variants in the cranial region. We propose that distinct mechanisms of mosaicism can cause the ECCL phenotype through convergence on the RAS-MAPK pathway.

Investigating the Loss of H3K27me3 via PRC2 as a Cause of Genome Instability in Malignant Peripheral Nerve Sheath Tumors

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Malignant Peripheral Nerve Sheath Tumors (MPNSTs) exhibit extensive genome instability with complex chromosomal aberrations, including gains, losses, and rearrangements of chromosomal regions leading to gene dosage imbalances. In about 80% of MPNST cases, Polycomb Repressive Complex 2 (PRC2) function is lost via biallelic mutations in *SUZ12* or *EED,* which results in a global loss of H3K27me3, a repressive chromatin mark. It has been found that H3K27me3 is required for Topoisomerase IIα (Topo II) to properly decatenate sister chromatids during mitosis. Topo II is needed to release supercoils and decatenate DNA for high fidelity chromosome segregation. A loss of either the chromatin tether domain of Topo II or ablation of H3K27me3 results in error prone anaphases including chromosome bridges, lagging chromosomes, and ultra-fine DNA bridges. The purpose of this study is to determine if the genome instability observed in PRC2 deficient MPNSTs might be caused by the inability of Topo II to decatenate sister chromatids via H3K27me3. We have found that immortalized human *NF1*-deficient Schwann cells with the loss of PRC2 have more aberrant anaphases and ultra-fine DNA bridges than controls. In addition, we have performed a drug screen with Topo II, Aurora B, and Haspin Kinase inhibitors to investigate if further disruption of the metaphase Topo II checkpoint exhibits synthetic lethality with PRC2 loss in immortalized human Schwann cells. This drug study has revealed that the loss of PRC2 causes the cells to become more sensitive to all three classes of drugs tested. To examine the mechanism of cell death after drug treatment we are performing flow cytometry to analyze the phases of the cell cycle and apoptosis. To further investigate this issue, we're utilizing a model system in which human immortalized induced pluripotent stem cell (iPSC)-derived *NF1*-deficient Schwann cells with and without concomitant genetic knockout or pharmacological inhibition of the PRC2 are used. We are performing spectral karyotyping and gamma-H2AX western blotting to determine if chromosomal abnormalities and DNA damage response activation accompanies loss of H3K27me3 abundance. In summary, we have found evidence that the inability of Topo II to target chromatin due to the loss of H3K27me3 after PRC2 inactivation could be related to the genome instability observed in MPNST. Completion of this study will determine if the loss of PRC2 is a factor in the acquisition of aneuploid genomes in MPNST and provide therapeutic rationale for drugs targeting the metaphase Topo II checkpoint.

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The PRC2-Dependent and -Independent Surface Proteome of Malignant Peripheral Nerve Sheath Tumors

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Malignant peripheral nerve sheath tumors, or MPNST, are soft tissue sarcomas that arise from plexiform neurofibromas. These tumors develop following a series of sequential genetic alterations, primarily *NF1* loss-of-heterozygosity, deletion of *CDKN2A/B,* and biallelic inactivation of *SUZ12* or *EED* constituting inactivation of polycomb repressor complex 2 (PRC2). Despite the well-defined genetic etiology of many cases, treatment options for MPNST are limited due to an immune-cold tumor microenvironment and a lack of targeted therapies. To aid discovery of viable treatment options for MPNST, our lab has undertaken surface protein profiling of several MPNST model cell lines and patient-derived xenografts (PDX) using a surface-protein-capture technique in combination with mass spectrometry. Proteins localized to the cell surface serve as the interface of the tumor cell with the surrounding extracellular matrix and tumor microenvironment as well as actualizing autocrine and paracrine signals from surrounding cells. The resulting surface proteome – or, "surfaceome" – profiles reveal a variety of proteins expressed throughout multiple MPNST model cell lines. We defined an MPNST consensus surfaceome by evaluating proteins that were expressed in a majority of the MPNST models assayed. In order to determine a clinically actionable set of surface markers from this consensus set, we narrowed our search to proteins within the top half of this set by expression. Remaining proteins were assessed for expression in normal human tissue using ProteomicsDB, where proteins expressed below a defined threshold in a majority of tissues were then checked for evidence of membrane localization in the Human Protein Atlas. Finally, this filtered list was prioritized for validation using a variety of criteria, including whether increased expression correlated with decreased survival in TCGA data, whether the genetic loci exhibited cancer cell dependency in CRISPR screen data, and if there were available antibody-drug conjugates. We aim to evaluate the efficacy of targeting these molecules by using antibody-drug conjugates to specifically attack tumor cells expressing these surface proteins. Many of these high-profile targets have been shown to be regulated by PRC2 at the RNA level as well, providing potential insight into MPNST formation from pre-malignant neurofibromas. Example targets include ITGAV, MET, EGFR, B7-H3, CD70 and EPHA2. These efforts will aid the elucidation of mechanisms driving MPNST malignancy as well as the discovery of targeted therapies for patients with MPNST. Additionally, it will provide a resource for other researchers to mine to aid in therapeutic development.

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Developmental Analyses of Skeletal Manifestations in Knock-In Mouse Model of Neurofibromatosis Type 1 p.M992del "Mild" Patient Mutation

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Background: Animal models are critical for understanding disease and testing new therapeutics. Patients with the p.M992del mutation display a relatively mild clinical phenotype associated with café-au-lait spots but no neurofibromas, with a subgroup of patients displaying Noonan-like features. Therefore, we hypothesize that mutant p.M992del neurofibromin is hypomorphic and retains sufficient functional activity to suppress tumor formation, but not sufficient activity in all NF1 related functions.

Methods: Heterozygous mice (Nf1$^{M992del/+}$ on FVB background) were created using CRISPR/Cas9 and a repair template to introduce a 3 bp deletion in the homologous region of mouse Nf1 gene. We intercrossed heterozygous mice (Nf1$^{M992del/+}$) to assess viability of homozygous (Nf1$^{M992del/M992del}$) mutants, as most Nf1 mutant alleles induce early embryonic lethality when homozygous. Nf1$^{M992del/M992del}$ animals and control littermates were assessed for growth parameters, body composition, skeletal structure (histology), bone density (uCT) and bone structure (X-Ray). Histological analysis of growth plates and cranial sutures was also performed. Embryos from intercrossed heterozygous mice (Nf1$^{M992del/+}$) were analyzed at E19.5.

Results: Analysis of genotype ratios revealed perinatal lethality of Nf1$^{M992del/M992del}$ pups (observed 101 pups born compared to 262.75 expected pups). Surviving Nf1$^{M992del/M992del}$ mice fail to thrive and are ~50% of the size by weight of littermate controls; only 61 of the 101 observed pups survived to weaning. Mice display impaired bone organization of the sternum and craniosynostosis. Mice surviving to 6 months also display bilateral suppurative otitis media (n=2/4) and pneumonitis (n=3/4). E16.5 Nf1$^{M992del/M992del}$ pups display double outlet right ventricle phenotype, and 6-month-old mice display mitral valve defects. Preliminary Western blots confirm the presence of mutant neurofibromin protein at levels consistent with control animals with no changes in pERK or pAKT in Nf1$^{M992del/M992del}$ animals.

Conclusions: Nf1$^{M992del/+}$ mice appear to be healthy with no overt phenotype, whereas Nf1$^{M992del/M992del}$ animals display perinatal lethality, with surviving animals severely runted. Further characterization of the skeletal phenotype is ongoing. Preliminary data from backcrossing the p.M992del mutation from FVB/NJ mice to C57BL/6J strain indicates genetic background plays a role in embryonic lethality as no viable homozygous mice have been identified on the C57BL/6J background.

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Electrical Stimulation from Underlying Neurons Affects NF1 Schwann Cell Behavior

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Neurofibromatosis Type 1 (NF1) is a complex genetic disorder characterized by the development of benign neurofibromas, which can cause significant morbidity in affected individuals. While the molecular mechanisms underlying NF1 pathogenesis have been extensively studied, the development of effective therapeutic strategies remains a challenge. This paper presents the development and validation of a novel biomaterial testing model to enhance our understanding of NF1 pathophysiology, disease mechanisms and evaluate potential therapeutic interventions. Our long-term goal is to develop an invitro model of NF1 to evaluate drug targets. We have developed an *in vitro* system to test the cellular behavior of NF1 patient derived cells on electroconductive aligned nanofibrous biomaterials with electrical stimulatory cues. We hypothesized that cells cultured on electroconductive biomaterial will undergo morphological changes and variations in cell proliferation that could be further enhanced with the combination of exogenous electrical stimulation (ES). In this study, we developed electrospun Hyaluronic Acid – Carbon Nanotube (HA-CNT) nanofiber scaffolds to mimic the axon's topographical and bioelectrical cues that influence neurofibroma growth and development. The cellular behavior was qualitatively and quantitively analyzed through immunofluorescent stains, Alamar blue assays and ELISA assays. Gene expression was quantified using qRT-PCR with GAPDH as the housekeeping gene. Schwann cells from NF1 patients appear to have lost their ability to respond to electrical stimulation in the development and regeneration range, which was seen through changes in morphology, proliferation and NGF release. Without stimulation, the conductive material enhances NF1 SC behavior by increasing elongation and proliferation. Wild-type SC respond to electrical stimulation with increased cell proliferation and NGF release. Electrical stimulation increased expression on NCAM, GFAP, Oct6 and Sox10 in WT SC indicating that stimulation makes SC more pro-regenerative. In NF1 SC, we see a significant increase in Sox10 expression when cells are cultured on conductive HA-CNT, however expression decreases following stimulation. These results correlate with the cell spreading and proliferation results. Using this system, we can better understand the interaction between axons and SC that lead to tumor formation, homeostasis and regeneration.

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Circulating Protein Signatures Detect MPNST in Patients with NF1

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Purpose: We previously published that copy number analysis with size selection of cell free DNA (cfDNA) non-invasively distinguishes plexiform neurofibroma (PN) from malignant peripheral nerve sheath tumors (MPNST)[1]. Our published assay, however, has limited sensitivity for early-stage disease when intervention would be most impactful. Previous publications have demonstrated utility for circulating proteins in detecting early-stage cancer[2,3]. We hypothesize MPNST, atypical neurofibroma (AN), and PN have unique plasma circulating proteomic signatures that can non-invasively detect malignant transformation.

Methods: Candidate proteins for proximity extension assay (PEA) panels were identified through *ad hoc* analysis of MPNST bulk RNAseq data[4] with forward selection using the Human Protein Atlas for genes predicted to encode proteins (a) secreted into blood and (b) previously associated with malignancy. Combinations of pre-validated and pre-optimized DNA-oligonucleotide labeled dual recognition antibody panels (Olink) were selected to include, at minimum, all candidate proteins. A total of 1,463 proteins were ultimately assayed. 40uL of plasma from 118 samples (healthy (n = 10), PN (n = 29), atypical neurofibroma (AN; n = 25) and MPNST (n = 54)) subsequently underwent PEA immunoassay with outputs in normalized protein expression (NPX), logarithmically related to protein concentration. Differentially expressed proteins were identified using one-versus-one disease state comparisons with ANOVA and post-hoc Tukey HSD (honestly significant difference) of NPX values. For feature reduction and to move towards a clinically feasible panel size, proteins significantly enriched in MPNST plasma ($p<0.05$, delta NPX >0) relative to PN were filtered for only proteins encoded by genes overexpressed in malignant cell and regulatory T-cell (Treg) clusters in the NF1 single cell tumor atlas (n=55 tumors) being developed in a separate, parallel effort. Filtered proteins' individual performance at identifying MPNST were assessed using Youden's Index and receiver operating characteristic (ROC) curve in oneversus- all (OVA) comparisons. Integrative protein performance was calculated using support vector machine (SVM) models (C-classification, C=1).

Results: In *ad hoc* analysis of bulk RNAseq data, 324 genes were over-expressed in MPNST tissue relative to normal. Forward selection identified 64 genes predicted to encode secreted proteins with 35 previously associated with malignancy. Using a minimum NPX difference of 0.8 and $p<.05$, analysis of variance of PEA identified differentially expressed proteins between disease states: 29 markers for PN vs healthy, 7 for AN vs PN, 65 for MPNST vs AN, and 134 for MPNST vs PN. Filtering statistically significant proteins in MPNST vs PN (delta NPX>0, $p<0.05$) for genes expressed in malignant cell and Treg scRNAseq clusters yielded a panel of 23 proteins. Individually, median filtered proteins' area under the curve (AUC) was 0.75 (interquartile range: 0.71-0.77). Performance improved using SVM models integrating all 23 proteins: specificity 1.0, sensitivity 0.76, accuracy 0.90, negative predictive value 0.84, positive predictive value 1.0.

Conclusions: In this study, we demonstrate that (1) NF1 disease states have distinct secreted protein profiles detectable in peripheral blood and (2) scRNAseq informed protein panels accurately and non-invasively detect MPNST. Validation is required in a cohort enriched for patients with small disease burden or minimum post-operative residual MPNST, however, this platform holds significant promise for cancer screening in NF1. Modeling will be extended to AN. Furthermore, given PEA's minimal plasma requirements, a single tube of blood could be used for multiple analytes to improve performance through multi-modal integration of orthogonal approaches. These efforts are currently underway.

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Development of a Human Neuroepithelial Stem (NES) Cell Model of Gliomagenesis in Neurofibromatosis Type 1 (NF1)

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Background: Gliomas are the most common tumor of the central nervous system in Neurofibromatosis type 1 (NF1), seen in approximately 20% of patients[1,2]. Most gliomas affect children and have a predilection for the optic pathway and brainstem[1,3]. While many gliomas are low-grade, there is a 50-fold increase in the risk of developing high-grade glioma (HGG) in NF1 patients compared to the general population[2]. Recent genomic studies have demonstrated a higher mutation burden in NF1-associated HGG compared to low-grade glioma (LGG)[4]. *A large proportion of HGGs harbor CDKN2A and ATRX loss, akin to the LGm6 subgroup of sporadic gliomas described in the Cancer Genome Atlas*[5]. However, the mechanism by which these mutations functionally drive the process of gliomagenesis in human cells in the context of NF1 loss is poorly understood. There is a critical need for human neural stem cell intermediates for functional interrogation of mutations found in NF1-associated HGG. Previously, we derived human neuroepithelial stem (NES) cells that represent the developing neural tube from patient-derived induced pluripotent stem (iPS) cells[6,7]. NES cells are long-term self-renewing neural stem cell intermediates that can be differentiated towards brainstem neuroglial lineages. They provide powerful tools for studying putative cells of origin of tumors arising in the brain stem and the functional effects of mutations on neural and glial proliferation, differentiation, and tumorigenicity.

Aim/Purpose: To develop NES cells with heterozygous NF1 mutations in the germline from patient-derived iPS cells and to define the functional effects of the two most common genetic aberrations seen in NF1-associated HGG: 1) NF1 loss of heterozygosity and 2) loss of CDKN2A. We hypothesize these mutations are sufficient to drive the proliferation of neuroglial progenitors and initiate the development of HGG.

Results: We describe the derivation of NF1-mutant iPS cell lines from patients with NF1 (figure 1), and our methodology for generating the first NES cell line with NF1 gene perturbation. We describe the phenotype of these cells, and the development of a unique platform to test genetic drivers of high-grade tumors such as CDKN2A.

Conclusions: Human neuroepithelial stem cell models may provide new insights into the origins of NF1-associated gliomagenesis and novel tools for drug discovery. Our long-term goal is to develop a novel platform for the identification of drug targets using human stem cell models of NF1-associated glioma.

Figure 1:
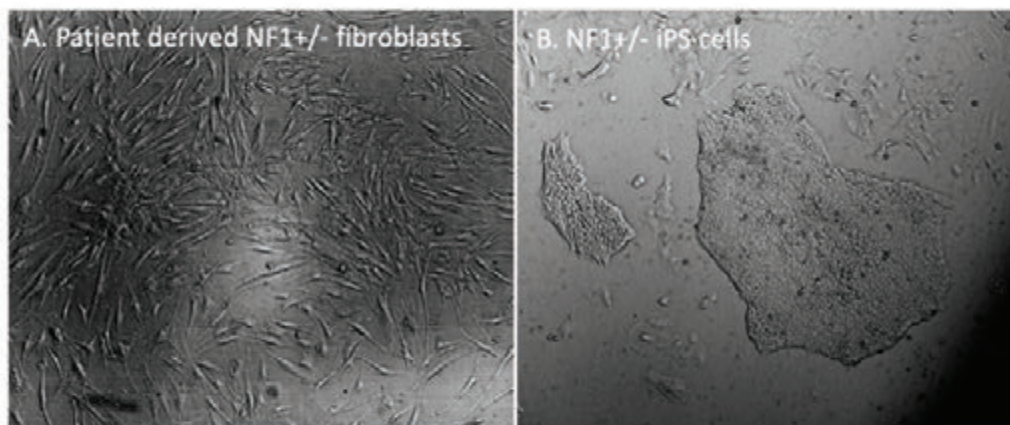
Figure 1. Patient-derived fibroblasts were established from skin samples taken during surgery for neurofibroma (A). Molecular reprogramming of fibroblasts to NF1+/- iPS cells.

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Identification of Drugs Targeting Epigenetic Regulators in an iPSC-Derived 3D MPNST Model

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Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas originating from cells derived from the neural crest (NC), often presenting as high-grade tumors and associated with a poor prognosis. They constitute the primary cause of NF1-related mortality. Timely resection remains the only curative approach. Despite ongoing efforts to discover effective treatments, there is a lack of effective therapies. The development of novel *in vitro* models that faithfully replicate MPNST genetics and biology could help identifying new therapeutic options. At a molecular level, the first steps in the progression towards an MPNST involves the sequential complete loss of the tumor suppressor genes (TSGs) *NF1, CDKN2A,* and PRC2 (*SUZ12* or *EED*). Additionally, *TP53* is inactive in about 25% of MPNSTs. Most NF1-associated MPNSTs exhibit a NC-mesenchymal phenotype, which contrasts with the NC-Schwann cell identity observed in plexiform neurofibromas and ANNUBPs.

We have developed iPSC-based 3D cellular models carrying the complete inactivation of *NF1, CDKN2A,* and PRC2 (3KO) that recapitulates the switch from a glial to a mesenchymal identity observed in MPNSTs. In addition, we also mutated 1 copy of the *TP53* gene in the 3KO cell line. Employing these 3D MPNST models with either 3 or 4 mutated TSGs, along with their isogenic controls, we conducted a high-throughput screening of an NCATS (National Center for Advancing Translational Sciences) epigenetics library comprising approximately 300 compounds. We tested the impact of these compounds on cell viability, spheroid size, and cell death in the MPNST 3D spheroids and cell lines. Some compounds are currently being tested in combination with MEK and CDK4/6 inhibitors. Results of some promising compounds will be presented.

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Characterization of a New Post Translational Modification of Neurofibromin: Atypical Structural Requirements for its SUMOylation

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Although extensive studies have been performed on Neurofibromatosis type 1 from a genetic and phenotypic point of view, NF1 still suffers from a lack of efficient targeted treatment. To develop new innovative therapies, it appears crucial to have a better understanding of Neurofibromin, Nf1, the protein encoded by the *NF1* gene, from a molecular point of view. We are especially interested in SUMOylation (Small Ubiquitin-related Modifier), a versatile and dynamic Post Translational Modification (PTM) involved in the regulation of nearly all cellular pathways and which is frequently defective in neurodegenerative disorders and correlates with resistance to cancer treatment when constitutively increased. We have previously shown that Nf1 partially colocalized with the ProMyelocytic Leukemia (PML) protein in PML nuclear bodies, which are hotspots of SUMOylation. Here, we demonstrate that the full-length isoform 2 and a SecPH fragment of Nf1 are substrates of the SUMO pathway, and we identify a well-defined SUMOylation profile of SecPH with two main modified Lysines. One of these sites, K1731, is highly conserved and surface-exposed. We show that well-described SUMOylation mechanisms are not required for K1731 SUMOylation. On the opposite, structure-guided mechanistic hypotheses combined with site-directed mutagenesis identify specific unusual structural elements of SecPH required for K1731 SUMOylation. Some of these elements are affected in reported NF1 pathogenic variants. This work describes for the first time the SUMOylation of Nf1 and depicts in details its mechanism providing one of the rare examples of SUMOylation dependent on the tertiary rather than primary protein structure surrounding the modified site, opening the path to a better understanding of Nf1 from a molecular point of view. This new perspective is highlighted in a second/an accompaning poster (HB) whereby SecPH SUMOylation studies of NF1 specific missense variants helped us to decipher the molecular mechanism underlying their pathogenicity.

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Analyses of FOXM1 in NF1-Associated Malignant Peripheral Nerve Sheath Tumors (MPNSTs)

Ellen Voigt, *The University of Iowa*

Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) are sarcomas that arise either spontaneously or through transformation of benign tumors, called plexiform neurofibromas (PNFs), in patients with Neurofibromatosis Type 1 (NF1). Besides the initiating loss of *NF1,* most MPNSTs have inactivated *INK4a* and *ARF* tumor suppressor genes. Understanding the driver mutations that cooperate with those changes to promote malignancy remains a high priority in the field. One potential driver, FOXM1, has functional links with both p16INK4a and ARF signaling and is an oncogenic transcription factor that promotes malignant progression and drug resistance in other cancers. It has not been well studied in MPNSTs. Consequently, we seek to understand the role of FOXM1 in these deadly tumors and we hypothesize it cooperates with *INK4a/ARF* loss to promote MPNST transformation.

Methods: FOXM1 mRNA and protein expression was evaluated in patient matched PNFs, ANNUBPs, and MPNSTs via RNA-Seq and IHC. *INK4a/ARF* status was determined by FISH and qRT-PCR. IHC evaluated expression of other proteins of interest, such as PD-L1, a known transcriptional target of FOXM1. For *in vitro* studies, FOXM1b/c overexpression (OE) or FOXM1 shRNA knockdown (KD) was conducted in normal human Schwann cells (NHSCs). These FOXM1-OE or FOXM1-KD cells are being CRISPR/Cas9 edited for *NF1* with or without simultaneous inactivation of *INK4a, ARF,* or both *INK4a/ARF.* Cell proliferation, survival, and transformation status will be measured.

Results: In patient matched tumor sets, *FOXM1* mRNA was significantly elevated in MPNSTs relative to PNF/ANNUBP precursor lesions. At the protein level, FOXM1 expression rose dramatically in a stepwise manner from normal nerve to PNFs, ANNUBPs, and MPNSTs. Increased expression of FOXM1 corresponded with heightened activation, as measured by upregulation of FOXM1 transcriptional target genes in MPNSTs versus PNFs. Moreover, high FOXM1 expression in MPNSTs correlated with loss of *INK4a* and *ARF,* as well as with elevated levels of tumor-promoting RABL6A (RAB-like GTPase) and PD-L1 (programmed death ligand 1) proteins.

Conclusions & Ongoing Studies: FOXM1 expression and transcriptional activity are greatly increased in human MPNSTs compared to benign precursors from the same patients. Novel correlations between *INK4a/ARF* loss and PD-L1 upregulation with FOXM1 overexpression in MPNSTs were observed that may be of physiological and clinical importance, warranting deeper investigation. Ongoing studies in cells and mice will determine 1) if elevated FOXM1 collaborates with *INK4a* and/or *ARF* loss to transform *NF1*-deficient human Schwann cells, and 2) the requirement of FOXM1 for MPNST pathogenesis.

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Exploring the Role of Cutaneous Innervation in the Development of cNFs in NF1

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Cutaneous neurofibromas (cNFs) are nerve sheath tumors that develop in 99% of NF1 patients at the nerve endings. They appear at puberty, often numbering in the thousands. Despite their benign nature, cNFs can induce pain and pruritus, considering them the main burden and suggesting that skin innervation could play an important role in the pathophysiology of cNFs.

In this study, we explored the role of skin innervation in the development of cNFs in NF1 patients and in the recently designed *Nf1*-KO mouse model. First, we performed quantitative sensory testing (QST) in 21 NF1 patients by comparing a cNF area to the contralateral healthy skin area (HLS). The cNF and HLS tested were then harvested and their innervation explored and quantified by immunohistochemistry (IHC) using a panel of specific neuronal markers. The following observations were made: (i) 14/21 of NF1 patients showed a reduced response to mechanical stimulation in cNF vs. HLS areas, while the response to thermal stimulation varied between patients, (ii) for all NF1 patients, PGP9.5+ abnormally dense innervation in cNF vs. HLS was observed, mainly in the lower dermis, (iii) in all cNFs analyzed, no axon-detached Schwann cells (SCs), nor naked axons were observed, (iv) CGRP-positive fibers (A-delta and C fibers) and TH-positive fibers (C-LTMR fibers) counted as minor populations in densely innervated cNFs areas. Characterization of other neurons innervating cNFs is in progress. Interestingly, the same observations were made in *Nf1*-KO mice developing mature cNFs, making this model valuable for deciphering the role of nerves in the initiation and development of cNFs and potentially as a new actionable target for their treatment.

To further characterize the sensory neurons located in the dorsal root ganglia (DRG) and innervating cNFs, we performed their retrograde labelling in *Nf1*-KO mice, by injecting fluorescent dyes such as Cholera Toxin B (CTB) or Fast Blue (FB) into cNFs areas. This strategy will help us to: identify, characterize and quantify the sensory neurons innervating the cNFs and to set up a model for their functional analyses. Preliminary data showed encouraging results in labelling the neurons either by the CTB or the FB. Furthermore, the 3D IHC of traced DRG neurons is accomplished using the clearing reagent of RapiClear®.

By accomplishing this project, we expect to gain knowledge in cNF related cutaneous innervation and the mechanism driving the development of cNFs and ultimately uncover potential pharmaceutical targets for cNF prevention and/or cure.

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Profiling the Immunotypes of the Tumor Microenvironment in Human NF1-Associated Peripheral Nerve Sheath Tumors

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Background: Malignant peripheral nerve sheath tumor (MPNST), an aggressive soft tissue sarcoma, is the leading cause of mortality in patients with neurofibromatosis type (NF1). NF1 is a neurocutaneous genetic condition with a predisposition to develop benign and malignant tumors. About half of affected individuals develop plexiform neurofibromas (PN), benign tumors with a 10% risk of transforming to MPNST. Further, atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) are pre-malignant entities with specific histopathologic features that predict a higher risk of malignant transformation. Despite many clinical trials, overall survival in patients with MPNST has remained stagnant and most succumb to their disease; thus, novel therapeutic approaches are urgently needed. MPNST are made up of transformed Schwann cell precursors, which do not grow and survive in isolation but rather interact with intra-tumoral immune cells, and little is currently known about the interaction of immune-modulating cells and the primary tumor cells in MPNST. A better understanding of the MPNST immune ecosystem will undoubtedly aid in the development of strategies to activate the immune system against the tumor.

Purpose: Our goal is to elucidate the composition of the tumor immune microenvironment (TIME) of MPNST to discover insights on the role that tumor-infiltrating immune cells play in malignant transformation of NF1-associated PNST.

Experimental Design: Utilizing fresh and formalin-fixed, paraffin-embedded tissue from patients diagnosed with NF1-PNST, we dissected the TIME by using immunohistochemistry, multiparameter flow cytometry (MFC), and comparative transcriptomic studies.

Results: Immunophenotyping confirmed increased infiltration of immune cells during malignant progression, notably demonstrating a predominance of infiltrating myeloid cells, particularly CD163^{+} tumor-associated macrophages (TAM). The T cells within MPNST exhibited signs of tumor activation, characterized by high PD-1 expression. Additionally, MPNST specimens demonstrated elevated levels of immunosuppressive TAM, as indicated by their heightened PD-L1 expression. The proportion of CD163^{+} myeloid cells within the TIME correlated with poorer progression free survival. Notably, H3K27me3 status differentiated immunotypes in MPNST.

Conclusions: The transformation to malignancy in PNST is characterized by an immunosuppressive microenvironment. Our findings suggest that CD163^{+} TAM with high expression of PD-L1 contribute to the immunosuppressive microenvironment observed in MPNST. Therapeutics that alter this immune population may unleash an anti-tumor immune response.

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ABSTRACTS

NF2-Related Schwannomatosis (*NF2*-SWN): Basic Science

Understanding the Role of Apelin-Mediated Angiogenesis in NF2-Associated Tumors

Srirupa Bhattacharyya, PhD, *Massachusetts General Hospital*

Purpose: NF2-associated meningiomas and schwannomas are often highly vascular making antiangiogenic therapy a promising strategy for these tumors. However, treatment with the classical antiangiogenic agent Bevacizumab (Avastin), a vascular endothelial growth factor (VEGF) inhibitor, has shown improvement in some NF2-related schwannomas, while a majority of NF2-associated meningiomas have remained nonresponsive supporting the existence of other underlying angiogenic mechanisms. Interestingly, in our previous transcriptomic work, as part of the Synodos for NF2 study, we identified increased expression of *APLN,* encoding the potent angiogenic peptide apelin, in cellular models of *NF2*-null meningioma raising a possibility that inhibiting apelin signaling may help overcome the resistance to Bevacizumab.

Methods: Extending our previous transcriptomic studies, we performed qRT-PCR of *APLN* and carried out immunoblotting to validate the presence of the apelin receptor APLNR/APJ in additional meningioma lines. We also examined the effect of exogenous apelin stimulation in two immortalized *NF2*-null meningioma lines by testing phosphorylation status of key downstream substrates of apelin/APLNR signaling. Furthermore, we generated a 3D *in vitro* meningioma spheroid model, which was co-cultured with human umbilical vein endothelial cells (HUVECs) to mimic vascularization and assess the influence of secreted apelin along with phenotypic rescue using MM54, a pharmacological apelin inhibitor.

Results: Increased expression of *APLN* in MN lines KT21-MG (grade III malignant) and MN1-LF (grade I with atypical features) was confirmed using qRT-PCR, and immunoblotting validated the presence of APLNR/APJ receptor in all cell lines tested. Stimulation with exogenous apelin in Ben-Men-1 (grade I) and MN1-LF cells revealed activation of several downstream APJ signaling substrates that are shown to play a major role in angiogenesis and proliferation. Notably, in our 3D meningioma co-culture model, HUVECs showed a more prominent morphology with formation of robust primitive tube-like structures surrounding and overlapping the spheroid surface, reminiscent of a crude vessel-like appearance, and treatment with apelin inhibitor MM54 led to a pronounced decrease in these vessel-like structures.

Conclusion: Our study establishes the importance of the angiogenic peptide apelin in NF2-associated tumors. Moreover, our results demonstrate, for the first time, successful generation of a 3D meningioma/HUVEC co-culture system to model NF2 tumor angiogenesis and suggest a role for apelin/APJ signaling in NF2 meningioma vascularization.

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TEAD Inhibitors in Combination with Brigatinib Prevent *NF2*-Deficient Meningioma Growth

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Purpose: The predominant modality of treatment for *NF2*-deficient meningiomas and schwannomas is surgical resection. Our group's previous work focused on the efficacy of TEAD inhibition in *NF2*-deficient Periostin-CRE; *NF2*fl/fl schwannoma mouse models (L, Laraba *et al.* 2023). Further *in silico* analysis, identified the tyrosine kinase inhibitor brigatinib as a top candidate for use in combination with TEAD inhibitors (VTs), for the treatment of *NF2*-deficient schwannoma and meningioma.

Methods: *In vitro* evaluation of grade 3 immortalised cell lines (NCH93 and KT21-MG1) were used to evaluate the efficacy of brigatinib as a monotherapy and in combination with TEAD inhibitors. Alongside the use of the orthotopic meningioma xenografts, established by the unilateral injection of KT21-*Luc* or NCH93-*Luc* cells into the skull convexity of immunocompromised Nod-Scid-Gamma (NSG) mice. Following tumour establishment, mice receive clinically relevant doses of VTs and/or brigatinib by daily oral gavage. Tumour growth was monitored *in vivo,* longitudinally by measuring luciferase activity in xenografted mice.

Results: Here we demonstrated, the efficacy of TEADi in combination with brigatinib in NCH93-*Luc* and KT21-*Luc* meningioma xenografts. Our data shows a potent inhibition of xenograft growth following treatment with TEADi VT2, brigatinib and in combination, when compared to the control group (Figure 1). Proliferation was evaluated by EdU assay, showing a significant reduction in KT21 and NCH93 proliferation both *in vitro* and *in vivo*. Furthermore, preliminary TUNEL analysis showed an increase in apoptosis, alongside the reduction in proliferation. Next, we will assess this combination therapy in primary meningioma cells as well as in the Periostin-CRE; *NF2*fl/fl schwannoma mouse model. Furthermore, target engagement of brigatinib will also be evaluated by western blot in all of these models.

Conclusions: VTs and brigatinib in combination are efficacious in reducing proliferation and tumour growth in *NF2*-deficient cell lines and tumour growth in pre-clinical meningioma models.
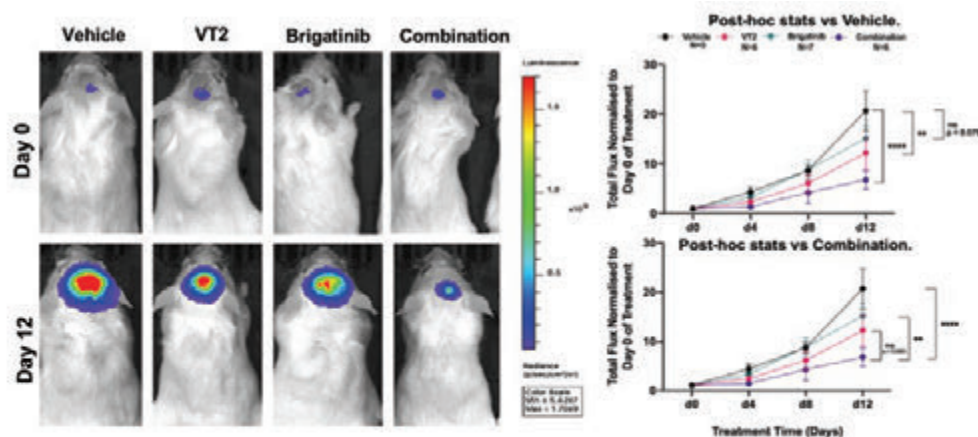
Figure 1. IVIS Bioluminescence Imaging of NCH93-Luc Xenograft Luciferase Activity in NSG Mice. After 18d of tumour establishment, mice were treated with either vehicle, 30mg/kg VT2 and or 50 mg/kg of brigatinib for 12 days. The combination treatment prevented tumour growth. Two-way Anova with multiple comparison (adjusted by Dunnett's test) for tumour growth. Vehicle vs VT ** P < 0.01; Vehicle vs Brigatinib 0.0797; Vehicle vs Combination **** P < 0.0001; Combination vs VT 0.0893 and Combination vs Brigatinib ** P < 0.01.

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Developing an AAV-Based Gene Therapy for Neurofibromatosis Type 2

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Aims: Neurofibromatosis type 2 (NF2) is a tumour-prone disorder caused by hereditary or spontaneous mutations in the NF2 tumour suppressor gene. Affected patients are predisposed to development of multiple tumours in the CNS, eyes and skin, which result in a wide range of life-limiting health problems and shortened lifespan. NF2 is incurable and current management involves regular monitoring of tumour development, surgery and radiation therapy to reduce tumour burden and symptom alleviation. CRISPR-Cas9 gene editing technology presents an attractive avenue for gene therapy to correct monogenic genetic diseases such as NF2. We aim to apply an AAV-based CRISPR-Cas9 gene editing strategy to restore the NF2 gene and achieve a curative outcome.

Methods: We have developed a recombinant dual AAV-based CRISPR-Cas9 genome editing system to restore a functional copy of the NF2 gene precisely at the NF2 locus. To achieve this, an optimal single guide RNA (sgRNA) was selected from *in vitro* sgRNA screening in HEK293 cells as well as in immortalised human Schwann cells (a type of glial cells in which NF2 mutations cause schwannomas) by AAV transduction.

Results: Homology independent gene editing experiments have been performed using the selected sgRNA and NF2 isoform 1 and demonstrated successful integration in the targeted site with ~10% efficiency in immortalised human Schwann cells. Different experimental conditions are being evaluated to increase editing efficiencies and these will be further tested in NF2-deficient human Schwann cells and *in vivo* in a PDX (patient-derived xenograft) mouse model. Success of gene therapy will be assessed *in vitro* and *in vivo* by functional experiments to measure cell proliferation, associated molecular markers and signal transduction signatures.

Conclusion: NF2 is a life-limiting and -threatening genetic disorder likely amenable to gene therapy, especially at the early life stage when tumour burden is minimal. CRISPR-Cas9 gene editing technology in combination with an effective AAV-based delivery system provides an alternative treatment strategy and potential cure for NF2.

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Genetically Engineered Minipigs Model Manifestations of NF2-Related Schwannomatosis

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Purpose: The majority of schwannomas demonstrate genomic alterations in the *NF2* gene. There are two major hurdles in the development of safe and effective treatments for schwannomatosis; first, the mouse models do not fully recapitulate the disease seen in patients and their full predictivity of clinical efficacy is still under testing. Second, due to a small patient population, the ability to recruit enough drug treatment-naïve patients for clinical trials is becoming increasingly challenging. We hypothesized that a germline *NF2* pathogenic variant will predispose heterozygote swine to development of NF2-related tumoral and non-tumoral manifestations.

Methods: Genetic studies have demonstrated that a *NF2* pathogenic variant p.R57* (c.169C>T) predisposes to multiple schwannomas, meningiomas and ependymomas at an early age. Comparison of human, swine, and mouse *NF2* genes show similarities in gene and protein structure, with >99% amino acid identity between swine and human NF2 coding sequences. *NF2*$^{+/-}$ founder minipigs were obtained using TALEN-mediated gene-editing of Ossabaw fibroblasts, followed by nuclear cloning and embryo transfer into female surrogates. *NF2*$^{+/-}$ minipigs were evaluated for clinical criteria used to diagnose NF2.

Results: We have developed NF2-related schwannomatosis (NF2) minipigs that exhibit spontaneous and cell-type-specific LOH, a critical step for tumor development in NF2 patients and a hallmark of NF2 that has not been observed in rodent models. We found that *NF2*$^{+/-}$ minipigs develop tumoral and non-tumoral manifestations of NF2, including juvenile posterior subcapsular cataracts that are typical NF2-related ocular lesions.

Conclusions: These findings provide proof-of-concept of the feasibility of generating a large animal model of NF2, which could represent a breakthrough in the field from a genomic, pathophysiologic, pre-clinical and therapeutic perspective.

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Trametinib-Induced Upregulation of PDGFRb and HSP27 in Human Schwannoma Model Cells are Attenuated with BRD4 Inhibition

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NF2-related schwannomatosis (NF2) predisposes individuals to benign schwannomas, meningiomas, and ependymomas. Currently, there is no FDA approved therapy for treatment of NF2, so patients are limited to surgery, radiation, and/or enrollment in clinical trials for kinase inhibitors. Kinase inhibitor monotherapies are successful for less than 15% of patients for a short period of time until disease progresses or treatment limiting adverse effects occur. Activation of one or multiple alternative survival and/or proliferation signaling pathways enables tumor cells to overcome inhibition of the targeted pathway. Currently, a phase 2 clinical trial on selumetinib in patients with NF2 tumors is ongoing (NCT03095248). Previous research conducted by our lab found no efficacy for selumetinib in human schwannoma model cells and identified trametinib as a superior MEK inhibitor for NF2-related schwannomas. However, we also demonstrated that in vivo, trametinib treatment leads to the development of resistance in mouse schwannomas identified through re-expression of phospho-ERK [1].

We conducted transcriptome and proteome analysis of human schwannoma cells treated with trametinib for 24 hours. Enrichment of differentially expressed genes and proteins via DAVID revealed upregulation of extracellular matrix receptor interactions, PDGFRb signaling, and transmembrane signaling, while cell cycle, DNA regulation, and DNA binding were downregulated. PDGFRb and HSP27 were identified as proteins of interest, and both mRNA and protein expression were increased 24 hours after trametinib treatment. Trametinib and BRD4-inhibitor JQ1, an investigational compound, synergized to reduce cell viability in preliminary drug combination screenings. Six BRD4 inhibitors in clinical trials were evaluated in two human schwannoma model cell lines. BMS-986158, currently in phase 2 clinical trials (NCT02419417, NCT05372354), was the most effective with a GI_{50} below 0.5μM. Evaluation of trametinib alone and in combination with BMS-986158 using live-imaging assay revealed that the combination is superior in reducing cell proliferation over either monotherapy in three human schwannoma model cell lines. In two of three cell lines, caspase-dependent apoptosis was induced with the combination treatment. BMS-986158 reduced the trametinib-related elevation in HSP27 and PDGFRb mRNA and protein levels compared to trametinib alone.

This study demonstrates the ability of human schwannoma model cells to induce rapid transcriptomic and proteomic remodeling in response to MEK inhibition with trametinib. Combining trametinib with a BRD4 inhibitor improved overall efficacy of the treatment and induced cell death in two human model shwannoma cell lines. Finally, the combination reduced the adaptive response of our human schwannoma cells induced by trametinib alone.

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Parallel *In Situ* and *In Vitro* Analysis of an NF2-Related Paraspinal Schwannoma

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Purpose: Little is known about how interactions between schwannoma cells and the tumor microenvironment contribute to pathogenesis and/or modifies drug response. Herein, we conducted experiments in parallel to define the microenvironment and in vitro drug response patterns of an *NF2*-related paraspinal schwannoma.

Background: *NF2*-related schwannomatosis (NF2) is a genetic predisposition to schwannomas in cranial, spinal, and peripheral nerves. Many pathogenic variants have been defined for the *NF2* gene, but truncating variants result in particularly aggressive NF2 phenotypes. A 16-year-old patient with such a mutation presented to clinicians in 2022 with ataxia from a paraspinal tumor at the T6/T7 neural foramen. The tumor was resected, and samples collected for *ex vivo* study.

Methods: The tumor was studied by immunohistochemistry (IHC) using a panel of schwannoma-, immune cell-, and extracellular matrix (ECM)-defining markers. Multiple tumor areas were sent for transcriptomic analysis for comparison with human model NF2-schwannoma cell lines; others were sent for methylation studies to assess global DNA modifications. Several primary and human telomerase reverse transcriptase (hTERT)-immortalized cell lines were derived from other sections of the tumor, which were then screened using high-throughput assays and a panel of drugs of clinical interest or targeting critical Schwann cell survival or proliferation pathways.

Results: IHC demonstrated expression of classic schwannoma markers and infiltration of monocyte-lineage cells. Additional staining demonstrated an abundance of ECM protein laminin and its receptor beta-1 integrin. Drug screens revealed similar sensitivity patterns between the primary cells derived from unique sections of the original tumor. Of these, selected monotherapies (CUDC-907, dasatinib, and BRD4-inhibitor BMS-986158) or synergistic combinations (trametinib and BMS-986158) either significantly inhibited cell growth or induced cell death. Transcriptomic pathway expression analysis of all tumor areas and NF2-schwannoma cell lines demonstrated conserved pathways relevant to schwannoma physiology including ErbB, Hippo, PI3K, MAPK, and Ras pathways. Data from methylation studies is currently pending.

Conclusions: These precision medicine-inspired experiments 1) demonstrate the *in situ* physiology of an aggressive *NF2*-related paraspinal schwannoma, and 2) define the drug sensitivities of the isolated schwannoma cells. Strong staining for laminin and beta-1 integrin were noted by IHC, and the isolated primary and hTERT-immortalized schwannoma cells were particularly sensitive to dasatinib, an inhibitor of kinases (SRC and FAK) activated downstream of this cell-extracellular matrix adhesion pathway. This parallel analysis deepens our understanding of how tumor microenvironment influences anti-schwannoma drug sensitivity patterns and could elucidate potential target pathways toward effective therapeutic development for *NF2*-related schwannomatosis.

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The Impact of Extracellular Matrix Remodelling on Tumour Immunity in NF2-Related Schwannomatosis Vestibular Schwannoma

Miriam Hernandez-Meadows, MS, *The University of Manchester, Manchester, UK*

Purpose: Characteristic histological features of vestibular schwannoma (VS) include cellular Antoni A tissue, which display zones of dense-elongated palisading nuclei termed verocay bodies, as well as microcystic Antoni B tissue. The extracellular matrix (ECM) is a diverse network of macromolecules, including collagens, proteoglycans, and glycoproteins, which surround cells providing structural support and necessary biochemical and mechanical cues to mediate tissue homeostasis. In this study we are investigating how different histological niches of VS are dynamically shaped by cell-matrix interactions and ECM remodelling, and the role of the ECM in VS tumour progression and immune escape.

Methods: Seventeen retrospective human *NF2*-related Schwannomatosis (*NF2*-SWN) VS cases in formalin-fixed paraffin-embedded blocks, were accessed through pathology at Salford Royal Hospital, Manchester, UK. Laser capture microdissection of neuropathologist-validated tumour regions of interest (Antoni A regions, Antoni B regions, immune infiltration, abnormal vasculature) was performed and samples were collected and processed for shotgun proteomics. To investigate the spatial landscape of VS, and how the ECM may influence the phenotype and compartmentalisation of immune cell populations within distinct tumour regions, *NF2*-SWN VS sections were stained with 41 antibodies targeting ECM, lymphoid, myeloid, vessels, and Schwann cells. Stained slides were then imaged using a Hyperion Imaging Mass Cytometry System.

Results: More than 1000 unique proteins were identified by non-biased proteomics and PCA analysis suggests clear distinction in proteomic signatures within Antoni A and Antoni B tissue. Notably, Antoni A tissue was enriched for basement membrane specific proteins compared to Antoni B. Hyperion imaging mass cytometry revealed the complex topography and spatial organisation of the ECM, with evidence of distinct tumour niches characterised by heterogeneous and focal ECM deposition. High dimensional multi-plex ECM and immune cell analysis also revealed significant correlation between the type and organisation of ECM with immune cell localisation in *NF2*-SWN VS tumour regions.

Conclusions: Our results show clear changes in ECM and immune-cells across different histological niches of *NF2*-SWN VS. We aim to perform mechanistic investigations to determine how this impacts spatial interactions between ECM and immune cell recruitment, differentiation and activation, and the contribution to *NF2*-SWN VS pathology. The evidence from this study suggests that investigating the components of the ECM in VS could reveal novel biological targets and direct future treatment strategies.
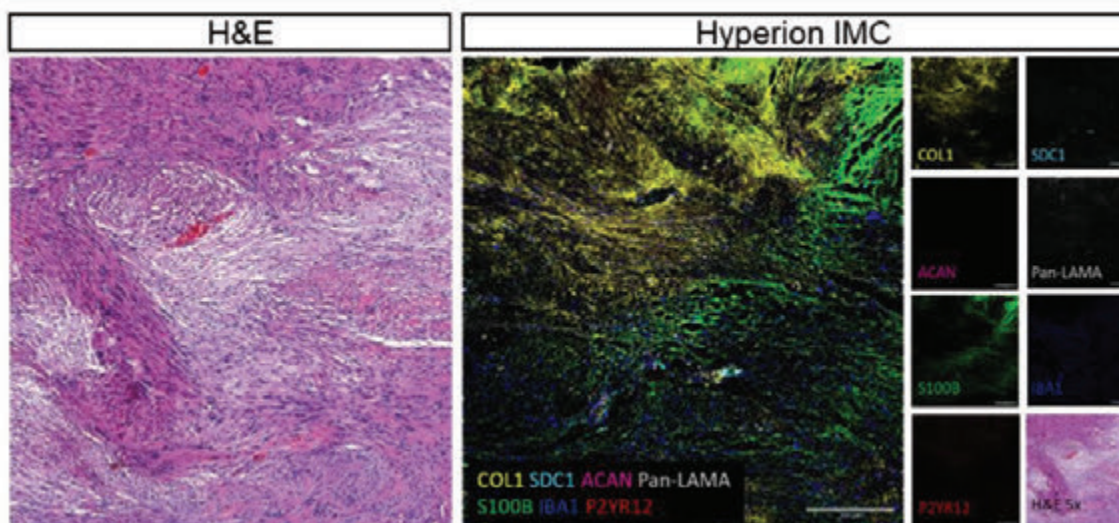
Figure 1: H&E and high dimensional imaging of the transition zone between Antoni A and Antoni B tissue with focal deposition of ECM (COL1, SDC1, ACAN, and Pan-LAMA), macrophage(IBA1), microglia(P2YR12), and Schwann cell(S100B) localisation. Scale bar represents 100μm.

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Investigating the Effect of Hippo Downstream Effectors YAP and TAZ on Satellite Glial Cell Function in NF2-Related Schwannomatosis

Amy Hewitt, BSc, *University of Plymouth*

This study investigated how dysregulation of Hippo signalling regulates satellite glial cell (SGC) function and survival in the NF2$^{fl/fl}$ PostnCRE mouse model.

In the NF2$^{fl/fl}$-CRE+ model, schwannoma tumours form in the dorsal root ganglia (DRG), vestibular ganglia and vestibular nerve. We have observed a progressive loss of DRG neurons at 3, 5 and 9-month timepoints. In NF2/YAP and NF2/TAZ double nulls, the loss of YAP and TAZ slows tumour progression but only the loss of TAZ significantly decreases DRG neuronal loss. In the DRG, SGCs wrap around neuronal cell bodies in sensory ganglia, regulating neuronal homeostasis. This study used DRGs from 9-month Schwann-cell specific Periostin-CRE-NF2$^{fl/fl}$ mice (Gehlhausen et al., 2015), NF2/YAP-nulls (Zhang et al., 2010) and NF2/TAZ-nulls (Azzolin et al., 2014) to investigate whether the loss of Merlin dysregulated SGC function, how YAP/TAZ contribute to SGC dysfunction, and how SGC dysfunction may contribute to DRG neuronal loss.

Kir4.1 and fatty acid binding protein 7 (FABP7) were used as markers of SGC function. Kir4.1 is the main ion channel used by glia to maintain potassium ion homeostasis for membrane polarisation. FABP7 is a fatty acid transporter which contributes to neuronal homeostasis. Neurofilament was a counterstain for the visualization of neuronal cell bodies associated with Kir4.1/FABP7-positive SGCs. Both Kir4.1 and FABP7 SGC expression was lost in the Merlin-nulls, although Kir4.1 to a greater degree. This demonstrates how SGC dysfunction ensues in Merlin-null SCGs, indicating a role for Merlin in SGC-neuronal homeostasis. Kir4.1 expression was maintained in the absence of YAP/TAZ (though not at wildtype level) whereas FABP7 expression was lost in both. This suggests YAP/TAZ dysregulate different SGC functions in opposing ways, potentially propagating tumour progression. When Kir4.1 and FABP7 were co-stained, FABP7 expression was retained in SGCs that had lost Kir4.1. This suggests loss of homeostatic SGC function occurs prior to neuronal death as FABP7 is expressed in Kir4.1-negative SGCs. Abnormalities within the DRG in Merlin-null mice also result in fibre loss within both the dorsal roots and the sciatic nerve.

This study demonstrated how Merlin loss results in SGC dysfunction, highlighting a role for Merlin in neuronal homeostasis. It also observed the effect the additional loss of YAP/TAZ has on SGCs dysfunction, by either subsequent retention of function (Kir4.1) or further functional loss (FABP7), demonstrating the role YAP/TAZ play in schwannoma tumour growth.

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Control of the Blood-Nerve Barrier and Inflammation in Schwannoma Tumours

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This study was conducted to improve the understanding around schwannoma tumour growth. We are interested in investigating the mechanisms governing the breakdown of the blood-nerve barrier (BNB), as well as the roles tumour-associated macrophages play in driving tumour growth.

Experiments have thus far utilized two *in vivo* mouse models for schwannoma. The first is the *NF2fl/fl;POCRE;Raf-TR* model. This model utilizes a drug-activated Raf kinase to induce schwannoma formation on an *NF2*-null background (Figure 1). We're using this model to study blood-nerve barrier integrity during schwannoma tumour formation.
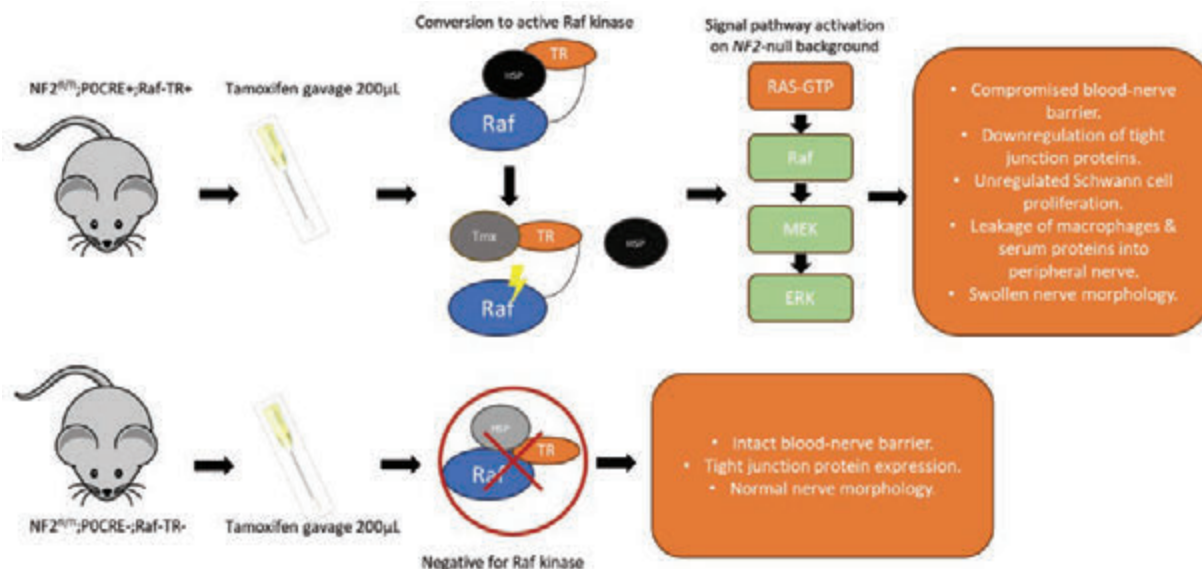
Figure 1: The *NF2fl/fl;POCRE;Raf-TR* mouse model and phenotypes associated with blood-nerve barrier breakdown.

The second model, the *NF2fl/fl;PostnCRE* model, uses a *CRE* recombinase system to biallelically deactivate *NF2* within Schwann cells to allow for spontaneous schwannoma formation within vestibular and dorsal root ganglia (VG and DRG respectively). This model is being used to study the interactions between tumour-associated macrophages and schwannoma cells during tumour development. This has been done using the colony-stimulating factor 1 (CSF-1) receptor inhibitor PLX5622 to deplete macrophages in tumour-bearing animals, followed by subsequent analysis of schwannoma proliferation.

Current results have identified glucose transporter 1 (GLUT1) as a potential biomarker for schwannoma development and BNB breakdown due to its loss of expression within endoneurial microvessels during tumour growth. We have further identified these microvessels themselves undergo significant increases in density with schwannoma formation. Additionally, we have identified significant influx of monocyte-derived macrophages into the peripheral nerve environment after BNB breakdown, with a 65% increase in macrophage density after a 21-day tamoxifen gavage treatment in *NF2fl/fl;POCRE;Raf-TR* animals.

In our *NF2fl/fl;PostnCRE* model studies, using 300ppm PLX5622-infused rodent chow, we have observed an average depletion of peripheral macrophages of 65% after a 21-day treatment timeline. Analysis into tumour cell proliferation identified a trend towards decreased proliferation in tumour-bearing animals on PLX5622 chow, but was statistically not significant, suggesting schwannoma cells do not rely on macrophages for aid in proliferation activity.

Additional Authors: Professor Oliver Hanemann, Dr Sylwia Ammoun, Professor David Parkinson

Inhibition of Focal Adhesion Kinase (FAK) Impairs Tumor Development in a Murine Model of NF2 Vestibular Schwannoma

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Neurofibromatosis type 2 (NF2) is an autosomal dominant cancer predisposition syndrome characterized by the development of bilateral vestibular (VS) and spinal schwannomas secondary to the functional loss of Merlin in Schwann cells or their precursors. While largely benign, these tumors can cause significant morbidity due to compromise of auditory, vestibular, facial, and vertebral nerve function. Surgical resection remains the standard of care, but is associated with significant morbidity. Thus, the identification of novel targets and development of pharmacotherapies is a critical unmet need. We previously identified Brigatinib as a promising agent for the treatment of VS and found it to inhibit the activity of Focal Adhesion Kinase (PTK2/FAK). Here we demonstrate that the genetic ablation of *FAK* impairs tumor development and preserves hearing in a murine model of NF2 vestibular schwannoma. Notably, these findings were accompanied by alterations in the immune microenvironment including decreased infiltration of immune cells and cytokine signaling. Accordingly, pharmacologic inhibition of FAK with VS-4718 in *Nf2$^{f/f}$ cre+* mice produced similar results with modest, but significant reduction in both tumor volume and number. Our findings suggest that inhibition of FAK may be efficacious for the treatment of NF2-associated vestibular schwannoma and warrants future investigation in prospective clinical trials.

Additional Authors: Li Jiang, Henry Mang, Waylan Bessler, Xiaohong Li, Qinbo Lu, Marisa Ciesielski, Constance Temm, Shaomin Qian, Alyssa Flint, Steve P. Angus, D. Wade Clapp

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HDAC Inhibition with Fimepinostat in Preclinical Models for NF2-Related Schwannomatosis

Anna Nagel, PhD, *University of Central Florida, College of Medicine*

NF2-related schwannomatosis is a genetic condition characterized by the growth of bilateral vestibular schwannomas. Due to their critical localization and slow but progressive growth and damage to hearing, anti-tumor therapy is needed but is currently unavailable. Surgical resection or debulking of the tumors is the most common treatment, however it often causes adverse side effects including deafness, facial paralysis. Ongoing research focuses on finding an effective pharmacological therapy, unfortunately clinically tested drugs control tumor growth temporarily in less than 15 % of trial patients.

Here, we tested activity of fimepinostat, a dual HDAC/PI3K inhibitor (aka CUDC-907) on human model merlin deficient Schwann cell (MD-SC) lines and a panel of NF2-related vestibular and non-vestibular patient-derived tumor cells. We confirmed its activity with live cell imaging coupled with an apoptosis marker activity readout (cleaved caspase-7). To interrogate cell death pathways, we performed apoptosis protein array assays (T=16, 24, and 48H), and analyzed the transcriptome, acetylome, and proteome. Next, we assessed its *in vivo* efficacy using a sciatic nerve allograft model. Merlin-deficient mouse Schwann cells were created by *in vitro* Ad-Cre inactivation of *Nf2$^{flox2/flox2}$* primary mouse SCs, transduced with firefly luciferase, and injected into nerves of immunodeficient mice. Growth was monitored every 7 days using IVIS optical imaging to measure luminescence flux. Animals were treated with either a vehicle (30% Captisol) or fimepinostat at 75mg/kg for three weeks on a 5/7-day schedule. Grafts were removed at 28dpi and weighted to assess final tumor growth.

All model MD-SCs and patient-derived tumor cell lines responded to fimepinostat with IG50s between 0.3 and 6 nM. 100 nM fimepinostat reduced growth of model MD-SC by 73-80%, patient derived vestibular schwannoma cells by 58%, and patient-derived spinal schwannoma cells by 40%. All cell lines induced apoptosis after 24-72 hours of fimepinostat treatment. In the in vivo study, we observed lower luminescence signal in treated animals at 28dpi. Resected tumor weight was significantly reduced by 61% (p=0.025) in fimepinostat treated group. Apoptosis protein arrays identified key players in cell death induction: p21, survivin, XIAP, and claspin. Transcriptomic analysis identified a number of differentially expressed genes, mostly enriched in apoptosis process, cell death, and cell cycle control. The acetylome analysis identified novel proteins that might serve as targets for future therapies.

Together, we demonstrate the effectiveness of HDAC inhibition against slow growing vestibular and non-vestibular schwannomas. Fimepinostat induces apoptosis and modulates acetylation of non-histone proteins. The exact pathway of cell death induction is not yet clear and is under study.

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Back to Base-ics: CRISPR Gene Therapy for NF Single Nucleotide Substitutions

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CRISPR/Cas9 has greatly expanded our capabilities to treat inherited conditions. Over 30% of NF1 and NF2 mutations are single base substitutions, many of which are amenable to CRISPR base editing (CRISPR-BE). This technology has the potential to treat a broad range of individual mutations in a bespoke manner. To demonstrate proof-of-concept, we selected an NF2 patient mutation (*NF2* c.169C>T) as a prototype for gene therapy correction.

A clonal human cell line harboring the NF2 c.169C>T pathogenic mutation was made using a traditional CRISPR-Cas9 HDR approach. Gene repair strategies for this mutation were then designed *in silico* using rGENOME and cloned into adenosine base editing Cas9 expression plasmids. Plasmids for expressing CRISPR-BE and a sgRNA guide were transfected into the *NF2* c.169C>T cell line model and the repair efficiency measured from genomic DNA by sequence analysis. Initial assessment revealed an 80-85% correction of the C>T transversion with no evident bystander effects (edits in adjacent nucleotides).

In parallel, an *Nf2* c.169C>T mouse model was generated. The sequence around the mutation site was "humanized" enabling sgRNA guides to work in both humans and knock-in mice. The heterozygous $Nf2^{169C>T/+}$ is currently being phenotypically characterized for spontaneous tumors in aged mice. This mouse model will be a valuable research tool to test CRISPR-BE repair *in vivo.* Our approach will feature a dual-AAV system and test a range of Schwann cell specific and AAV capsid cocktails.

This same systematic workflow is also being used for cell-line generation and testing for NF1 mutations.

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Spatial Transcriptomics of Spinal Ependymoma in NF2-Related Schwannomatosis

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Background: Spinal ependymoma (SP-EPN) is a central nervous system (CNS) tumor that affects 50% of patients with NF2-related schwannomatosis (NF2)[1,2]. The only effective treatment for end-stage SP-EPN is surgery, but this is associated with high risk of injury to the sensorimotor spinal tracts and paralysis. There is a critical need to understand the cellular origins of this tumor so that disease models of tumor progression can be generated for drug development. Recent genomic studies with bulkRNA sequencing suggest the molecular signature of SP-EPN matches that of ependymal cells (EPCs)[3]. However, large-scale genomic studies can often misrepresent rare stem cell populations within the tumor, such as radial glia cells (RGC)[4,5].

Aim/Purpose: The objective of this study was to examine the spatial heterogeneity within spinal ependymoma (SP-EPN) in a patient with NF2-related schwannomatosis (NrS).

Methods: We performed spatial transcriptomics (ST) on SP-EPN resected from a patient with NF2-related neurofibromatosis using the 10X Genomics Visium CytAssist Spatial Gene Expression platform. The final libraries were sequenced on the Illumina NovaSeq 6000. 28-bp reads including spatial barcode and UMI sequences and 50-bp probe reads were generated with Illumina NovaSeq 6000 at the Center for Medical Genomics at Indiana University School of Medicine. Space Ranger 2.1 was used to process the raw files following sequencing. The R package Seurat 4.9.9.9041 was used for data analysis following preprocessing with spaceranger. Sctransform was used to normalize the spatial transcriptome dataset. Clusters were identified using "FindClusters" and "FindNeighbors" functions.

Results: The SP-EPN sample exhibited cellular heterogeneity with diffuse expression of astrocytic and EPC markers, and smaller pockets with RGC or stem cell markers, as well as overlap between progenitor cell and mature cell markers (Figure 1). These findings suggest that there may be a developmental hierarchy within the EPC lineage in SP-EPN tumors, which may stem from aberrant RGCs.

Conclusion: Spatial transcriptomics performed on a SP-EPN tumor from a patient with NF2-related schwannomatosis demonstrates marked cellular and molecular heterogeneity within the tumor. Our preliminary data suggests SP-EPN may be a disease of gliogenesis and that a developmental hierarchy may exist in the SP-EPN with RGC-like cells and more mature progenitors in the EPC lineage. Albeit a single proof of concept, these results offer important studies to pursue including single-cell analytics and animal studies targeting embryonic radial glia cells and the ependymal cell lineage.

Figure 1. Spatial transcriptomics of spinal ependymoma (SP-EPN) from a NF2 patient shows RG markers GFAP, Nestin, SOX2, SLC1A3 and FABP7 spatially represented in a smaller clusters of tumor cells. Each dot is 55 micrometers in diameter. Scale – fold expression (red = high, yellow = intermediate, blue = low)

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Macrophage Distribution in Developing Schwannomas is Biased Towards Defined Markers of Tumor Heterogenicity

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Schwannomas exhibit surprising intrinsic heterogeneity and develop in a complex microenvironment consisting of multiple cell types, making the development of effective therapies challenging. Understanding this tumor heterogeneity is crucial to improve clinical responses. Macrophages are abundant components of most schwannomas, but not much is known about their pathological role in the disease or how they are recruited to and interact with tumor cells. In this study we aim to investigate how intrinsic heterogeneity within schwannomas influences macrophage recruitment into tumor lesions and explore the role of Schwann cell-derived extracellular vesicles (EVs) in influencing macrophages inflammatory states.

To capture both intrinsic and extrinsic heterogeneity in developing schwannomas, we employed a multiplex immunofluorescence imaging approach. We first defined and optimized a panel of five biomarkers to study intrinsic and extrinsic schwannoma heterogeneity. Using this panel, we examined biomarker distribution in paraffin-embedded dorsal root ganglia (DRG) arrays from *Postn-Cre:Nf2fl/fl* mice. We used machine learning-based single cell analysis to measure the proportion and spatial distribution of each cell population within the DRGs. Our data show that macrophages are abundant in developing schwannomas and exhibit a biased spatial distribution relative to markers of intrinsic heterogeneity that we recently identified. Ongoing efforts aim to identify macrophage-recruiting factors produced by tumor cells that cause this biased recruitment. In complementary work, we used an in vitro approach to ask whether tumor-macrophage interactions in schwannoma are mediated by EVs, and specifically whether tumor-derived EVs effect macrophage inflammatory states. EVs were isolated through ultracentrifugation from Nf2-deficient Schwann cells in two different phenotypic states that mirror intrinsic heterogeneity and were further characterized. EVs from the two population of Schwann cells show similar size and present the classical EV markers. We are currently working to assess how EVs isolated from different Schwann cell states alter macrophage inflammatory states.

Our data provide important insight into macrophage distribution within developing schwannomas, indicating a tendency of these cells to distribute near specific tumor cell subpopulations. Our ongoing efforts will continue to define how tumor cells and tumor cell-derived EVs impact macrophage recruitment and involvement in the tumor microenvironment.

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Targeting Aldehyde Dehydrogenases in NF2-Null Meningioma and Schwannoma

Libby Williams, BSc, *University of Plymouth*

Dysregulated Hippo pathway signalling has been demonstrated by us to drive overexpression of aldehyde dehydrogenase 1A1 (ALDH1A1) in NF2-null meningioma and schwannoma (Laraba et al., 2022). ALDH1A1, and the related isoform ALDH1A3 have been identified as markers of cancer stem cell activity and have been correlated with poor prognosis and chemoresistance in several cancers (McLean et al., 2023, Tomita et al., 2016). This project aims to clarify the role of aldehyde dehydrogenase (ALDH) enzymes in meningioma and schwannoma tumours, and evaluate their potential as future therapeutic targets.

The Aldefluor assay was used to measure ALDH enzymatic activity. Distinct populations of ALDH^{high} cells were identified in low- and high-grade meningioma cell lines. Using fluorescence-activated cell sorting (FACS) to isolate and culture these cells, the ALDH[high] group demonstrated significantly stronger colony-forming ability than their ALDH[low] counterparts (Figure 1).
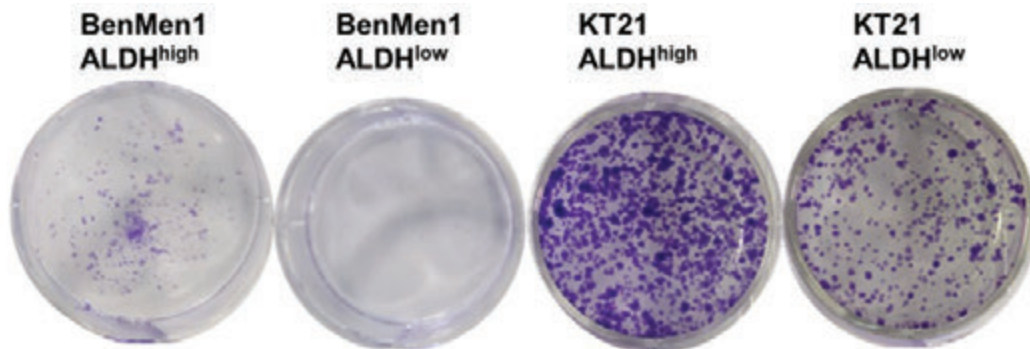
Figure 1. ALDH^{high} cells efficiently drive colony formation. Meningioma cells were fluorescence sorted using an Aldefluor kit. The highest and lowest 10% of cells by ALDH activity were isolated and re-plated. Cells were allowed to grow for 10 days, stained with crystal violet/methanol solution, and photographed.

In vitro experiments have revealed that the ALDH inhibitors nifuroxazide, DIMATE, and ABD 0171 effectively reduce the proliferation of meningioma and/or schwannoma cell lines. Use of nifuroxazide *in vivo,* in the NF2^{fl/fl} Periostin-CRE mouse schwannoma model, has shown a significant reduction in tumour growth in dorsal root ganglia and vestibular ganglia schwannomas.

In summary, this project has revealed a previously unstudied minority population of cells in meningioma that exhibit high ALDH enzymatic activity and are significantly more capable of forming colonies *in vitro.* Encouragingly, ALDH inhibitors tested thus far have shown strong promise at halting the growth of these tumours *in vitro* and *in vivo.* Experiments planned for the near future include the evaluation of ALDH inhibitor drug efficacy in an orthotopic model of meningioma in immunodeficient mice, examining the functional impact of ALDH1A3 knockdown *in vitro,* and on continuing the use of FACS to further clarify the potential link between ALDH[high] activity and cancer stem cell-like characteristics in meningioma and schwannoma. Additionally, we plan to investigate the effect of drug treatments on the production of ALDH-regulated metabolites in tumour cells, including retinoic acid signalling readouts.

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Understanding the Development and Evolution of Heterogeneity in Schwannoma

Emily Wright, *Massachusetts General Hospital, Harvard Medical School, Boston, MA*

Schwannomas are caused by inactivation of the NF2 tumor suppressor gene, and few cooperating mutations have been identified. Despite the lack of genetic variability, schwannomas exhibit remarkable histological, clinical, and therapeutic heterogeneity that has limited the development of effective non-surgical treatment options. In previous studies we found that loss of the NF2 tumor suppressor merlin drives intrinsic heterogeneity in schwannoma through the adoption of distinct programs of autocrine ligand production and polarized signaling. We identified biomarkers of these heterogeneous cell states and validated that they co-exist and drive heterogeneity in a novel 3D model and in mouse and human schwannoma tumors. Using bulk RNAseq analysis of *Nf2*-deficient Schwann cells we have identified core signatures and growth factor/cytokines specific to each state.

The goal of this study is to use the tools we have developed to investigate how intrinsic heterogeneity evolves and influences the microenvironment in schwannoma, and how heterogeneity is affected by drug treatment. We have used our 3D model, in which heterogeneous spheroids develop from single *Nf2*-deficient Schwann cells, to mechanistically investigate how intrinsic heterogeneity initiates and evolves at the earliest steps. We have developed a quantitative, multispectral imaging pipeline using machine learning-based single cell image analysis that is compatible with antibody- and RNAscope-based immunofluorescence. By applying this imaging pipeline to a validated genetically engineered mouse model in which schwannomas develop synchronously on all dorsal root ganglia we are building a multiparametric framework to understand how intrinsic and extrinsic heterogeneity coordinately evolve over time and change in response to different drugs that are being clinically evaluated in schwannomatosis patients. We are particularly interested in how intrinsic heterogeneity in developing schwannomas interfaces with the immune, nerve, and vascular compartments of the tumor environment. Our data already suggests that macrophages are recruited to schwannoma tumors at early stages and in a spatially biased fashion. Together, these studies will allow us to build a comprehensive model of schwannoma heterogeneity and understand how to combat it therapeutically.

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Schwannomatosis: Basic Science

LAST	FIRST	POSTER	TITLE
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Schwannomatosis: Basic Science

Dissecting the Molecular Mechanisms Driving DGCR8-Associated Schwannomatosis

Clara Nogué, Biotechnologist, MSc in Translational Medicine, PhD Student in Genetics, *Bellvitge Biomedical Research Institute (IDIBELL)*

DGCR8 germline pathogenic variants (GPVs)[1] predispose to familial euthyroid multinodular disease (MND) in conjunction with peripheral schwannomas. Until recently, GPVs in the microprocessor DICER1 were the main cause of fMND. *DGCR8* gene also encodes for a key component of the microprocessor and lies on chromosome 22q together with the other schwannomatosis genes. Our molecular study of 13 DGCR8-schwannomas by WES revealed a mutational pattern mirroring the one described for LZTR1-schwannomas[2], which allowed us to propose the model for DGCR8-schwannoma consisting of six hits, three steps[3] (Figure1). Remarkably, in 4/13 (30,8%) DGCR8-schwannomas, *DGCR8* was the only gene with two inactivating mutations.

Our aim is to profile the DGCR8-phenotypic spectrum and define the tumorigenesis model associated to DGCR8-schwannomas. So far, we search for patients with peripheral schwannomatosis and thyroid nodules with no germline alterations in *LZTR1/SMARCB1*. Overall, five patients have been recruited from which three have been tested resulting in a positive case. The remaining cases are under study.

To characterize the molecular profile of the 13 DGCR8-schwannomas, methylation analysis was performed and further compared to a public data collection[4]. At the methylation level, DGCR8-schwannomas formed an individual cluster independent from DGCR8-MNDs and closer to other schwannoma subtypes highlighting some similarities but also the uniqueness of *DGCR8* in their molecular profile (Figure2). Preliminary data revealed overexpression of RAS effectors in DGCR8-schwannomas[1]. To further investigate this effect, we are expanding the expression and miRNA expression profiling of DGCR8-schwannomas to compare them to SMARCB1/LZTR1-mutated schwannomas.

To validate our findings *in vitro,* we have engineered the Schwann cell line hTERT ipn02.32lambda using CRISPR/Cas9 and generated *DGCR8* heterozygous *(E518K/wt)* and *DGCR8* hemizygous *(E518K/-)* clones (Figure3), which reproduce a focal DGCR8 allelic loss. DGCR8 heterozygous and hemizygous clones showed an upregulation in DGCR8 RNA levels compared to parental status highlighting a potential autoregulative mechanism. At the protein level, both DGCR8 and its partner DROSHA also followed an auto-regulative loop. Effect on RAS expression levels, growth and survival properties of DGCR8 deficient cells are being characterized. To recapitulate the complete loss of 22qchrm observed in tumors, we are currently setting up a CRISPR technique (MACHETE)[5] that enables efficient megabase deletions. This will allow comparison of a focal *DGCR8* deficiency with the added loss of other schwannoma genes. This project will deliver a multi-omic profile of DGCR8-schwannomas and profit from our *in vitro* tools to identify the key players in this novel tumorigenesis pathway.

Figure 1.
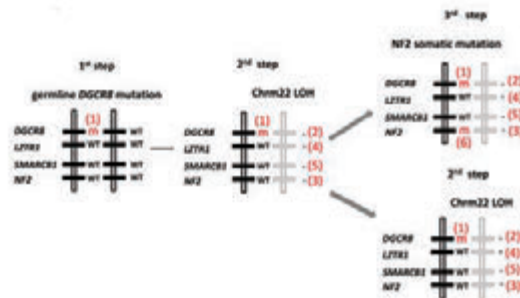
Figure 2.

Figure 3.
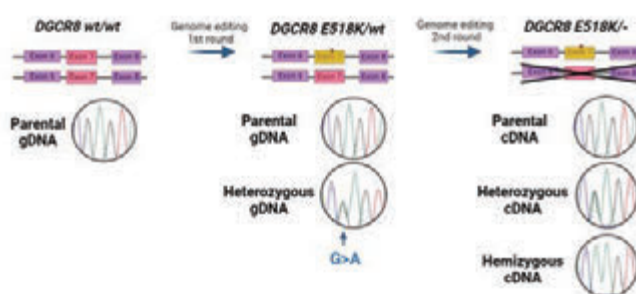
Figure 1. The six hit, three step model of schwannomatosis in DGCR8 schwannomas. The first hit corresponds to the germline pathogenic variant; in a second step a loss of chrm22q will delete the four genes accounting for 5 hits and a third step a somatic mutation in the remaining wt copy of NF2 will constitute the sixth hit. From Nogué et al, Acta Neuropath, 2022.

Figure 2. Unpublished data. T-SNE visualization of the 10K most differentiated CpGs from different tumor types, color-coded according to subtype. DGCR8-mutated schwannomas aggregate together differentiating from other schwannomas (SMARCB1-mutated, LZTR1-mutated, negative for these genes) and normal brain, ATRT-SMARCB1-mutated tumors and DGCR8-mutated thyroid lesions.

Figure 3. DGCR8 mutated cell lines. Genomic and cDNA sequences confirming genome editing of Schwann cells.

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Conditioned Medium from Painful Non-NF2 Schwannomatosis Tumors Increases Pain Behaviors in Mice

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The majority of schwannomatosis (SWN) patients experience debilitating pain. Yet, it is not known why only some schwannomas cause pain or whether distinct mutations in SWN-related genes, (SMARCB1 or LZTR1) differentially influence pain signaling pathways. We established cell lines from SWN tumors resected from patients with varying degrees of pain and bearing mutations in different SWN-related mutations. Compared with conditioned medium (CM) collected from "nonpainful" SWN tumors, CM from "painful" SWN tumors contained elevated levels of specific inflammatory cytokines (IL-6, IL-8, VEGF), and evoked greater neuronal responsiveness to noxious TRPV1 and TRPA1 agonists in vitro. Healthy mice were then exposed to SWN CM and painful responses were measured. We found differing responses to evoked and acute pain based on SWN-related gene mutations. Mice received an intraplantar injection with painful CM or non-painful CM and were immediately observed for painful symptoms such as licking and flinching of the affected paw. The injected mice were then subjected to Von Frey testing to examine responses to mechanically evoked pain. Mice injected with painful LZTR1 mutant CM demonstrated an increase in acute pain behaviors but were not hypersensitized to light touch. Painful SMARCB1 mutant CM sensitized mice to mechanical stimulation compared to non-painful tumor CM and control media at low forces, but this effect waned over time. CM from a painful tumor with no detectable mutation in either gene caused the greatest increase in response to low mechanical forces compared to non-painful CM and lasted for 2 days post-injection. These experiments provide the basis for expanding in vivo testing of additional painful and non-painful CMs with different mutation statuses as we aim to better understand the nature of the type of pain endured by SWN patients.

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Extended Genetic Analysis to Increase Rate of Detection of Pathogenic Variants Leading to Schwannomatosis

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The present study had the main objective of establishing a methodology to increase the rate of detection of pathogenic variants (PVs) in schwannomatosis cases. The schwannomatoses are tumour predisposition disorders predominantly resulting from PVs in *NF2, SMARCB1* or *LZTR1.* Rate of detection of PVs varies across schwannomatosis types, with higher rates (~70-90%) for familial cases but much lower rates (30-50%) for sporadic cases, partly due to mosaicism. Previous reports indicate there may be additional PVs to be discovered within non-coding regions of schwannomatosis genes[1]. Additionally, recent reports implicating other genes[2-4] highlight the value of extended testing to other potentially relevant loci.

We designed a next-generation sequencing (NGS) panel to screen people with schwannomas without a known PV identified by clinical genetic testing. Enrichment probes (Agilent) were used to target the full gene region of *NF2, SMARCB1, LZTR1, DGCR8* and *CDKN2A,* along with the coding region of *ERBB2,* following a report of hybrid neurofibroma/schwannoma tumours with somatic variants which were specific to non-*NF2*-related schwannomatosis[4]. Paired-end sequencing was performed using the NovaSeq 6000 platform (Illumina), with a minimum sequencing depth of 1000x on a cohort of 116 people with schwannomas. Tumour DNA was available for 29 individuals within the cohort, some of whom were suspected to have mosaic *NF2*-related schwannomatosis.

We identified a previously reported somatic variant in *ERBB2* (c.2329G>T; p.Val777Leu) was present in three independent tumours from one person. The variant was not present in blood, and no confirmed germline PVs were identified. We also identified non-coding variants of uncertain significance (VUS) in the known schwannomatosis genes, which require further analysis. Tumour-guided analysis of *NF2* variants did not identify mosaicism in blood using a standard bioinformatics pipeline.

In conclusion, extended genetic testing successfully detected a somatic *ERBB2* variant in three independent tumours from one schwannomatosis patient in our cohort. This variant was previously observed in non-*NF2*-related hybrid tumours. Therefore, its presence in schwannomas from our cohort may indicate *ERBB2* variants are not restricted to hybrid tumours. Further characterisation of non-coding VUS may confirm additional PVs. Finally, we expect the quality of sequencing data (up to 3000x across all targeted regions in majority of samples) will, in the future, allow us to identify low-level mosaic variants after optimisation of the bioinformatics analysis.

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Facilitating Computational Research and Data Sharing in the Schwannomatosis Community

Sasha Scott, PhD, *Sage Bionetworks*

Schwannomatosis is a rare, understudied syndrome that causes the development of nerve sheath tumors. While schwannomatosis is often caused by germline mutations in *SMARCB1* and *LZTR1*, the genomic landscape and genetic modifiers of this disorder are poorly understood. Recent efforts to study the genomics of schwannomatosis patients have increased our understanding of the disorder and generated a relative wealth of available data. However, the number of computational biologists in the field remains limited, which can make -omics and other big data analyses particularly challenging.

We established the Schwannomatosis Open Research Collaborative (SORC) with the goal of identifying genomic features that contribute to disease etiology and heterogeneity in schwannomatosis while also bringing more computational researchers into the NF community. We have built resources that decentralize computational biology research, allowing us to engage and collaborate with researchers from around the world. As a part of this effort, we are announcing the launch of SORC computational partnerships, in which we will work with selected researchers to design and complete genomic analyses, providing the necessary genomics expertise and computational resources.

We recently initiated the first SORC computational partnership using publicly available schwannomatosis data from the NF Data Portal (nfdataportal.org) to further explore genes that were previously found to have recurrent mutations in non-NF schwannoma samples. It is plausible that these genes of interest are involved in tumorigenesis.

We are soliciting collaborations with NF community members who have genomic data that needs to be analyzed or have ideas for analyses that utilize publicly available schwannomatosis data but do not have the resources to complete these analyses. If you are interested in working with SORC as part of a computational partnership are invited to contact SORC at nf-osi@sagebionetworks.org to discuss your idea. Additional information about SORC and the computational partnership effort can be found by scanning the QR code.

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Characterization of the Transgenic Animal Models *Danio Rerio* and *Caenorhabditis Elegans* to Examine Pathophysiology Associated with *SMARCB1* Variants

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Introduction and Aim: SMARCB1 is a ubiquitously expressed nuclear protein and a core subunit of the BAF chromatin-remodeling complex. *SMARCB1* variants have been associated with different diseases including tumor predisposition syndromes (Rhabdoid Tumor Predisposition Syndrome and Schwannomatosis) and developmental disorders (Coffin-Siris Syndrome). We propose to develop and characterize two transgenic animal models, the nematode *Caenorhabditis elegans* and the teleost *Danio rerio,* to examine the pathophysiology associated with *SMARCB1* variants.

Methods: In both model organisms we analyzed the expression of SMARCB1 orthologues. In *C. elegans,* the expression of the ortholog *snfc-5* was analyzed by q-RT PCR, from the egg stage to the adult. In *D. rerio* there are two paralogs: *smarcb1a* and *smarcb1b,* whose spatial and temporal expression was analyzed both *in vitro,* by q-RT PCR, and *in vivo* with an *in-situ* hybridization.

Finally, we employed CRISPR/Cas9 genome editing to obtain our transgenic animals: we have *smarcb1a-/-, smarcb1b-/-* and *smarcb1ab+/-* lines in *D. rerio.* In *C. elegans,* we created three independent lines harboring mutations associated with distinct human phenotypes. In *C. elegans* we performed preliminary experiments to characterized the transgenic lines, as brood size and longevity; moreover we are studying nociception and pain perception. In *D. rerio,* we are performing phenotypical observation, survival curves and histological analysis.

Results: In *D. rerio* the two isoforms were detected from the first stages of the embryonic development and also in all tissues. With an *in-vivo* analysis, we observed that *smarcb1* reaches the highest levels of expression in the central nervous system, highlighting a central role in this context. In *C. elegans* instead, the single isoform *snfc-5* is expressed from the egg stage to the adulthood. Our data suggest that *smarcb1* is a maternal determinant in both species. The phenotypical analysis of our transgenic lines, suggest that SMARCB1 plays an important role in survival, embryonic development and tissue maintenance.

Conclusions: Together, our findings suggest that SMARCB1 plays key roles in different pathways and developmental stages. Hopefully, the integration of all this multidisciplinary approach could help us to identify novel therapeutic targets for the future.

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Effect of Selumetinib Treatment on Pain Medication Utilization in Pediatric Patients: A US Claims Database Analysis

Ayo Adeyemi, PhD, *Alexion Pharmaceuticals*

Purpose: Selumetinib, an oral MEK1/2 inhibitor, received U.S. Food and Drug Administration approval in April 2020 for pediatric patients (aged ≥ 2 years) with neurofibromatosis type 1 (NF1) and symptomatic, inoperable plexiform neurofibromas (PN). PN may develop anywhere in the body, can cause pain, and may impair quality of life. This study aimed to evaluate real-world changes in pain medication utilization (PMU) post-selumetinib initiation.

Methods: This descriptive, non-interventional, retrospective cohort study used Merative™ MarketScan® Research Databases to identify patients aged 2–18 years with continuous enrollment 6 months before and after first selumetinib prescription (index), and ≥ 2 selumetinib prescription fills (4/10/2020– 12/31/2022). PMU was assessed. A paired generalized estimating equation (GEE) model with exchangeable correlation structure estimated difference in PMU (≥ 1 prescription fill of pain medication) pre- and post-selumetinib initiation. GEE was adjusted for sex, age, and Charlson Comorbidity Index.

Results: Of 90 eligible patients, the mean (standard deviation) age at index was 12.0 (4.3) years, 65.6% (n=59) had NF1 and PN diagnoses, 65.6% (n=59) were male, and 67.8% (n=61) had commercial insurance. General baseline indicators of pain included dorsalgia (16.7%), muscle weakness (15.6%), and abdominal pain (13.3%). PMU decreased by 38% post-index (adjusted odds ratio [OR] 0.62; 95% confidence interval [CI]: 0.30–1.28; p=0.198), mostly driven by a reduction in gabapentin and opioid use. Post-index, gabapentin and opioid utilization decreased by 67% (adjusted OR 0.33; 95% CI: 0.10–1.09; p=0.070) and 41% (adjusted OR 0.59; 95% CI: 0.19–1.90; p=0.379), respectively. In selumetinib-adherent patients (patients with ≥ 80% proportion of days covered [days covered/days in timeframe]; n=63), PMU decreased by 54% (adjusted OR 0.46; 95% CI: 0.18–1.16; p=0.100) versus no difference in non-adherent patients (adjusted OR 1.00; 95% CI: 0.30–3.37; p=1.000).

Conclusion: These data demonstrated a reduction in PMU in pediatric patients 6 months post-selumetinib initiation.

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Medication Adherence and Persistence on Selumetinib Treatment in Pediatric Patients: A US Claims Database Analysis

Ayo Adeyemi, PhD, *Alexion Pharmaceuticals*

Purpose: Selumetinib received U.S. Food and Drug Administration approval on April 10, 2020 for the treatment of pediatric patients aged ≥2 years with neurofibromatosis type 1 (NF1) and symptomatic, inoperable plexiform neurofibromas (PN). The primary objective of this study was to evaluate real-world adherence, persistence/discontinuation, and re-initiation among pediatric patients treated with selumetinib.

Methods: This descriptive, non-interventional, retrospective cohort study used Merative™ MarketScan® Research Databases to identify patients aged 2–18 years with continuous enrollment ≥6 months before (baseline period) and ≥6 months after (follow-up period) first selumetinib prescription (index) and ≥2 selumetinib prescription fills (4/10/2020–12/31/2022). Proportion of days covered (PDC; days covered/days in timeframe) was assessed to evaluate adherence (PDC ≥80%). Time-to-discontinuation (TTD; days from first fill to last day covered) and time-to-re-initiation of selumetinib (after a gap of ≥84 days) were determined using Kaplan–Meier analyses.

Results: Overall, 90 patients with ≥2 selumetinib fills were identified (mean [standard deviation] age, 12 [4.3] years); 65.6% (n=59) had NF1 and PN diagnoses, 65.6% (n=59) were male, and 67.8% (n=61) had commercial insurance. Mean PDC was 83.6% and 70% of patients were adherent. Younger patients (aged 2–5 years; n=8) were generally more adherent than those aged 6–11 (n=29) and 12–18 years (n=53; PDC ≥80%: 87.5% of n=8 vs. 72.4% of n=29 vs. 66.0% of n=53, respectively). Patients were 10% less likely to be adherent than those 1 year younger (odds ratio 0.90; 95% confidence interval: 0.80–1.0; p=0.063). At 6 months post-index, the probability of discontinuation was 14.5% (median TTD 2.25 years). Approximately 20% (n=7) of patients who discontinued later re-initiated, most (~80%) within 6 months of discontinuation.

Conclusion: Most patients were adherent to selumetinib treatment and the probability of adherence decreased with age. Approximately 20% of patients who discontinued selumetinib subsequently re-initiated, mostly within 6 months.

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Retrospective Study of MRI and PET-CT Findings in Malignant Peripheral Nerve Sheath Tumours in Neurofibromatosis-1 Patients in the Manchester Complex NF-1 Service

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Purpose: In 2009 NHS England appointed two Highly Specialized Services (HSS) in England for complex NF1. The Manchester HSS has retrospectively reviewed the available MR and PET-CT imaging, of patients who were diagnosed with malignant peripheral nerve sheath tumours (MPNST) since 2009. The literature highlights radiological findings that may distinguish between MPNST/atypical neurofibroma or benign neurofibroma. These include loss of target sign, T1 signal heterogeneity, perilesional oedema, irregular margins and SUV max >3.5. We sought to investigate the presence or otherwise of such imaging findings in a relatively large cohort of NF1 patients who developed MPNST.

Methods: Patients with biopsy proven MPNST were identified from the HSS database. Restrospective analysis of the imaging using local picture archiving and communications system (PACS) of these patients was undertaken. The following radiological characteristics were retrospectively evaluated on imaging performed pre-diagnosis in the areas where these patients eventually developed their MPNST: largest dimension, T1 and STIR signal (homogenous/heterogenous), presence of target sign, perilesional oedema, intra-tumoral cystic change, margin (regular/irregular) and PET specific uptake value (SUV) where available.

Results: A total of 63 patients were diagnosed with MPNST between the dates of April 2009 and February 2023. 53 patients had MR imaging suitable for analysis. We identified significant variability in size of MPNST ranging from 19mm to 292mm, with mean size of lesion pre-diagnosis of 70mm in long axis. Significant variability was observed in other morphological characteristics such as T1 and STIR signal heterogeneity. Absence of target sign was observed in 46 patients (87%), perilesional oedema identified in 15 patients (28%) and irregular margins identified in 9 patients (16%). PET CT was available in 13 patients, with SUV max ranging from 4.2 to 16.1.

Conclusions: MPNST imaging findings are widely variable and can have similar appearances to benign neurofibromas. Absence of target sign, perilesional oedema/irregular margins and increased SUV may be indicators of MPNST but further work is required to compare imaging findings with benign neurofibroma in the same cohort.

Figures:
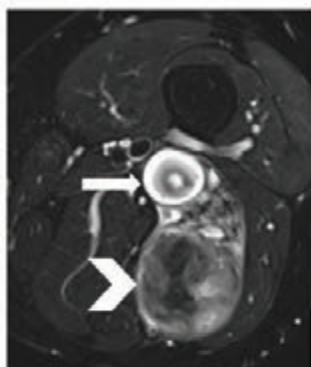
Figure 1. Axial T2 fat saturated MRI of distal thigh in 44 year old patient with NF1. Lesion related to distal left sciatic nerve with significant signal heterogeneity and loss of target sign (arrow head) proven to be MPNST. Typical benign appearing neurofibroma with presence of target sign seen adjacent (white chevron).
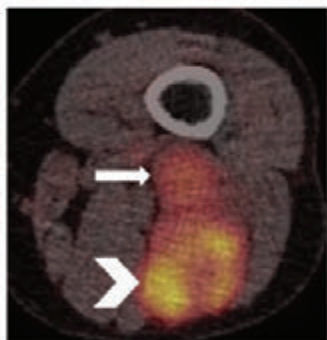
Figure 2. Trans-axial fused PET-CT image at corresponding level to MRI, demonstrates significant FDG avidity involving the more posterior lesion with SUV of 11.4 supportive of a diagnosis of MPNST (white chevron). Less avidity in the deeper lesion (white arrow).

Fatigue in Children and Adolescents with Neurofibromatosis Type 1

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Purpose: To describe fatigue levels in children and adolescents with NF1 and investigate the association between fatigue, other neurodevelopmental outcomes and quality of life.

Methods: Participants were drawn from a larger cross-sectional study examining sleep and cognition in children with NF1. Participants with NF1 and typically developing (TD) controls were 6-15 years of age. All participants completed a comprehensive neurocognitive assessment. The Pediatric Quality of Life (PedsQL) Multidimensional Fatigue scale (MFS) was administered to parents and children. Parents also rated children on general health-related quality of life (PedsQL core), pain (PedsQL NF1 module), sleep disturbance (Sleep Disturbance Scale for Children), executive functions (Behavior Rating Inventory of Executive Functions), autism traits (Social Responsiveness Scale Version 2), ADHD symptoms (Conners 3) and internalizing symptoms (Behaviour Assessment System for Children 3).

Results: Data of 81 children with NF1 and 44 TD controls have been analysed. Group differences were examined using independent-sample t tests or equivalent nonparametric tests. Analyses revealed that parents of children with NF1 reported significantly greater levels than those of TD controls on all on all measures of fatigue including total fatigue, cognitive fatigue and sleep fatigue ($p < .001$). Children with NF1 also report significantly higher levels of total fatigue and cognitive fatigue ($p < .01$) but not sleep fatigue when compared to TD controls. Spearman correlations revealed higher levels of parent-reported fatigue was associated with higher levels of pain, sleep disturbance, inattention, hyperactivity, ASD traits, internalising symptoms, executive dysfunction and poorer quality of life (all, $p < .001$) in children with NF1.

Conclusion: Children with NF1 experience significantly higher levels of fatigue compared to unaffected children. These higher fatigue levels are associated with a number of other clinical and neurodevelopmental outcomes. The nature of these relationships, including mediational factors, will be explored with the full dataset from the study. Increased fatigue levels in children with NF1 are significantly correlated with quality of life, suggesting fatigue as a potential target for future intervention.

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Incidental Fractures Found During Re-Staging Evaluations in Children with Neurofibromatosis Type 1 on the Phase I/II trial of the MEK Inhibitor Selumetinib

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Background: The ongoing multi-center Phase I/II trial of selumetinib for children with inoperable plexiform neurofibromas (PN) (SPRINT, NCT01362803) requires serial magnetic resonance imaging (MRI) to assess tumor burden every 4-6 cycles (1 cy=28 days) while on treatment. Since study enrollment began in 2011, several participants were found during routine restaging evaluations to have fractures on MRI that were asymptomatic and not previously suspected. Adults and children with NF1 have been described to have lower bone mineral density (BMD) and increased fracture risk compared to the general population, however the frequency of incidental fractures has not previously been reported. As whole body MRI (WBMRI) becomes more common in children with NF1 as part of the observation and management of their PN both on and outside of clinical trials, incidental fractures may be encountered more often. Here we report the incidence, location, and management of these fractures from participants on the SPRINT trial.

Methods: All participants enrolled on the SPRINT trial were included (Phase I/II). All adverse events were reviewed through a data-cut-off (DCO) of 1/5/2024 and all fractures were noted, regardless of severity or attribution. We defined an incidental fracture as a fracture found on imaging obtained for reasons other than assessing the skeleton (eg. MRI of plexiform neurofibroma) without known history of injury or symptoms. The duration of selumetinib treatment at the time of fracture (number of cy), fracture location, and any interventions were noted. Dual-energy X-ray absorptiometry (DEXA) was obtained prior to treatment for participants enrolled at NCI.

Results: Nine of the 99 participants (9%,4= female) enrolled on SPRINT had ≥1 incidental fracture during selumetinib treatment, for a total of 11 fractures. Of these, 8 were found during routine MRI restaging and the remainder (n=3) were found on CT or x-ray obtained for clinical indications unrelated to fracture outside of the trial. Seven of the 11 fractures were not adjacent to the PN being followed for the study. Of these, 5 were found on restaging WBMRI and 2 were found on x-ray. At the time of DCO, the 99 participants had undergone a total of 6,295 cy of treatment (approximately 482 patient years) and 1,644 re-staging MRIs. The median age at time of incidental fracture was 13.4 yrs (range 7.8 to 23.1 yrs), and median duration of treatment at the time of fracture was 38 cy (range 4 to 118). Fracture locations included sacrum (n=3), vertebral (n=2), iliac crest (n=1), pubic ramus (n=1), humerus (n=1), tibia (n=1), calcaneus (n=1), and foot (n=1). A possible history of trauma (e.g. a fall) was identified retrospectively in 4 cases (36%). Selumetinib was held for 3 of these fractures (range 8 to 46 days) while workup was obtained, but none of the fractures required fixation or further intervention. Baseline DEXA obtained for 7 of the 9 participants with incidental fracture were abnormal in 4 (abnormal (z-score ≤ -2).

Conclusion: Incidental fractures were identified in 9 of 99 participants on SPRINT. Notably, a significant number of incidental fractures were distant from the target PN (64%) and only discovered through use of WBMRI (45%). The impact of selumetinib on incidental or other fracture risk in people with NF1 cannot be assessed from this data, but as more people with NF1 get routine WBMRI imaging incidental fractures may be discovered more frequently. Reassuringly, in this small cohort, no intervention was needed beyond observation to manage these incidental fractures.

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Mental Health and Pain Management Health Care Utilization in Patients with Neurofibromatosis 1 in Ontario, Canada

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Objectives: To compare the use of mental health care, as well as services for pain management in people with Neurofibromatosis type 1 (NF1) compared to the general population.

Methods: We created a registry of individuals with confirmed NF1 attending pediatric and adult NF clinics in the province of Ontario, Canada, between 1990 and December 31, 2020. We linked the registry to administrative databases held at ICES, using de-identified unique identifiers. Index date was birth year or eligibility for the Ontario Health Plan. NF1 individuals were matched 1:5 to general population controls, based on date of birth, sex, income quintiles and rurality. At the end of the study window, we compared prevalence claims with mental health diagnostic codes, for outpatient care hospitalizations, and emergency department (ED) visits. We also compared billing codes for pain interventions (e.g. nerve blocks) as well as opioid prescriptions.

Results: 1,205 individuals with NF1 were matched to 6,025 controls; 50.8% were female. Mean follow up time was 19.6 ± 8.7 years in NF1, and 18.8 ± 8.5 years in controls. At the end of the study window, mean age was 26.2 ± 16.9 years (median 23, IQR: 14-36).

During the study window, NF1 patients had more outpatient visits for mental health diagnoses compared to controls (78.8% vs. 60.3%, $p<0.001$). When looking at specific diagnostic codes for visits, NF1 patients had more visits for anxiety and mood disorders (50.1% vs. 42.8%, $p<0.001$), behavioral and developmental disorders (42.7% vs. 21.4%, $p<0.001$) and social problems (20.5% vs. 8.9%, $pe<0.001$) There were no differences in visits for substance abuse or psychotic disorders.

There were no differences in the rate of ED visits due to any mental health diagnoses (RR: 1.0, 95%CI: 1.0-1.01); however, NF1 patients had more ED visits due to deliberate self-harm than controls (mean visits: 4.62 vs. 1.32, $p=0.01$, RR: 3.45(2.04-5.8). There was no difference in the proportion of patients with hospitalization due to mental health diagnoses (3.2% vs. 2.7%, $p=ns$).

More patients with NF1 had outpatient visits for pain management (37.7% vs. 22.6, $p<0.0001$), and more patients with NF1 received a prescription for opioids compared to controls (35.7% vs. 21%%, $p<0.0001$).

Conclusions: Individuals with NF1 in Ontario, Canada, have a high need for specialized mental health and pain management services. The higher use of opioids in this population warrants special attention, although there were no differences in the prevalence of substance abuse in this cohort.

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Evaluation of Standard of Treatment Outcomes for Cutaneous Neurofibromas (CNFs) in People with Neurofibromatosis 1 (NF1): An International Pilot Study — Paris Site

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Purpose: The primary objective of this pilot study was to explore and identify measures that hold significance for both patients and physicians regarding cutaneous neurofibromas (cNF).

Introduction: Cutaneous neurofibromas (cNF), a hallmark feature of NF1 can cause significant physical and psychological burdens. No medical treatment currently exists to cure or prevent cNF. Nevertheless, ongoing clinical trials are exploring innovative therapeutic approaches. Robust outcome measures are required to assess potential interventions for cNF in clinical trials, in order to enhance the quality and comparability of research alongside routine patient evaluations.

Methods: Patients and clinicians (dermatologists/NF1 specialists) were recruited from NF1 skin clinics across two sites (Paris and Sydney). Patient reported outcome measures (PROMs) and blinded clinician assessments were conducted at baseline, 3 (M3)- and 6-months (M6) post CO_2 laser (Paris) and Nd:Yag (Sydney) treatment. Outcome measures from both sites were ranked to identify a minimum dataset. Findings from the Paris site are reported.

Results: 32 patients were treated and assessed in Paris (mean age 43,7 years, 14 male). Patients experienced a statistically significant improvement in their overall quality of life related to cNF at 3- and 6-months post treatment, mainly in the "emotions" domain (Figure 1).

Patients experienced statistically significant improvements in the visibility of cNF at both the M3 and M6 marks (Table 1). Satisfaction scores remained consistently high at both M3 and M6 (8.5/10 (SD 1.6) and 8.6/10 (SD 1.32), respectively). Patients generally considered their cNFs problematic, with appearance, number, and size being the most bothersome aspects. However, improvements were noted post-treatment, particularly in appearance. Scar assessment revealed generally positive outcomes, with low scores for pain, pruritus, stiffness, thickness, and skin irregularity, though a majority noted differences in scar color compared to surrounding skin. No significant differences were found between scores at M3 and M6 post-treatment.

Patients expressed satisfaction with the aesthetic outcome, with mean scores of 3.95/5 and 4.05/5 for 2D photographs taken at M3 and M6 respectively (p= 0.5297) (Table 2). Clinicians assigned lower GAIS scores at both M3 and M6 compared to baseline (p=0.007 and p=0.002 respectively), with further improvement noted at M6 compared to M3, a change significant for clinicians (p=0.000) but not for patients (p=0.336). Patients initially rated higher severity, number, and size of cNF compared to clinicians (p=0.001, p=0.005, and p=0.000 respectively), but both patients and clinicians observed improvements in these parameters at M3 and M6.

Conclusion: Table 3 summarizes the proposed minimal data set of selected instruments which emerged as a result of our pilot study.

Table 1- Follow-up at 3- and 6-months post treatment

n=25	Month 3		Month 6		Wilcoxon Signed-Rank Test	
Question/variable	Mean	SD	Mean	SD	Average of differences ($\bar{x}d$)	p value
Satisfaction from treatment NRS (score out of 10)	8.52	1.64	8.60	1.32	0.08	0.822
How much of a problem are your cutaneous neurofibromas to you? 5-point Likert-scale (1 Very mild, 5 Very severe)	3.68	0.99	3.60	1.04	-0.08	0.484
In the past four weeks, how much has each of these features of your cutaneous neurofibromas bothered you? 5-point Likert-scale (1 Very mild, 5 Very severe)						
Appearance	3.44	1.16	3.40	1.35	-0.04	0.715
Number	3.28	1.40	3.56	1.26	0.28	0.144
Size	3.08	1.08	2.88	0.95	-0.2083	0.183
Color	2.46	1.28	2.60	1.26	0.125	0.835
Itch	1.68	1.03	1.96	1.00	0.25	0.183
Pain	1.52	0.82	1.80	1.04	0.28	0.193
Thickness	2.80	1.15	2.64	1.08	-0.16	0.326
Compared to before your treatment as part of this research study, do you feel that the problems you had with the cNFs that were treated are Worse (1-3), no change (4), better (5-7)?	5.48	1.42	5.04	1.46	-0.44	0.236
Compared to before your treatment as part of this research study, do you feel that the following cNF characteristics have changed: 7-point Likert-scale (Worse (1-3), no change (4), better (5-7)						
Appearance	5.04	1.79	5.28	1.43	0.24	0.226
Number	4.75	1.87	4.96	1.49	0.25	0.248
Size	4.79	167	4.88	1.45	0.08333	0.773
Color	4.78	1.38	4.68	1.38	-0.08696	0.813
Itch	4.50	1.35	4.56	1.26	0.08333	0.588
Pain	4.79	1.28	4.6	1.32	-0.1667	0.551
Thickness	4.88	1.54	4.68	1.52	-0.2	0.502
Concerning the treated areas, do you feel that the appearance of cNFs that were treated is: 7-point Likert-scale (Worse (1-3), no change (4), better (5-7)	5.83	1.58	6.00	0.66	0.1304	1
Assessment of scars 10-point Likert-scale for each question						
Have the scars been painful the past few weeks?	0.72	1.72	0.68	1.31	-0.04	0.958
Have the scars been itching the past few weeks?	1.67	2.58	1.24	1.81	-0.375	0.775
Is the scars color different from the color of your normal skin at present?	4.80	3.06	4.32	3.00	-0.48	0.123
Is the stiffness of the scars different from your normal skin at present?	2.64	2.98	2.12	2.44	-0.52	0.287
Is the thickness of the scars different from your normal skin at present?	2.08	2.63	2.56	2.65	0.48	0.208
Are the scars more irregular than your normal skin at present?	2.28	2.48	2.52	2.62	0.24	0.378
What is your overall opinion of the scar compared to normal skin?	4.21	3.09	2.68	2.36	-1.4583	0.088

NRS: Numeric Rating Scale

Table 2 - Patient and clinician assessments of cNF severity and aesthetic improvement of images collected via 2D photography at baseline, and 3- and 6-months post-treatment.

	M0		M3		M6	
GAIS 5-point Likert scale (1: Exceptional improvement, 5: Worsened patient)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Patients (n=21)	NA	NA	2.33	0.94	2.14	0.64
Clinician (n=4)	NA	NA	2.99	0.91	2.79	0.93
Patient vs Clinician Mann-whitney u p-value	NA		**0.007**		**0.002**	
Wilcoxon Signed-Rank Test p-value	M0 vs M3		M0 vs M6		M3 vs M6	
Patient	NA		NA		0.336	
Clinician	NA		NA		**0.000**	
cNF severity 5-point Likert scale (0: Clear ; 5: Very severe)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Patients (n=21)	3.52	0.75	1.90	1.22	1.81	1.25
Clinician (n=4)	2.71	1.04	2.05	1.20	1.95	1.27
Patient vs Clinician Mann-whitney u p-value	**0.001**		0.7622		0.804	
Wilcoxon Signed-Rank Test p-value	M0 vs M3		M0 vs M6		M3 vs M6	
Patient	**0.000**		**0.000**		0.6078	
Clinician	**0.000**		**0.000**		**0.036**	
cNF number 10-point Likert scale (0: Absent ; 10: Very numerous)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Patients (n=21)	7.33	2.33	5.33	3.54	4.62	3.29
Clinician (n=4)	5.35	3.03	3.71	2.89	3.43	2.84
Patient vs Clinician Mann-whitney u p-value	**0.005**		**0.044**		0.088	
Wilcoxon Signed-Rank Test p-value	M0 vs M3		M0 vs M6		M3 vs M6	
Patient	**0.002**		**0.001**		0.090	
Clinician	**0.000**		**0.000**		**0.001**	
cNF size 10-point Likert scale (0: Absent ; 10: Very numerous)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Patients (n=21)	5.33	1.80	3.24	2.41	3.05	2.54
Clinician (n=4)	3.90	1.66	2.42	1.91	2.36	1.96
Patient vs Clinician Mann-whitney u p-value	**0.000**		0.072		0.242	
Wilcoxon Signed-Rank Test p-value	M0 vs M3		M0 vs M6		M3 vs M6	
Patient	**0.002**		**0.001**		0.397	
Clinician	**0.000**		**0.000**		0.344	

Table 3 – The proposed minimal data set of instruments as a result of our pilot study

Patient Reported Experience Measure (PREM) / Patient Reported Outcome Measure (PROM)	Clinician Reported Outcome Measure
Clinical Assessment	**Clinical Assessment**
Overall:	Overall:
Visibility* (5-point Likert-scale)	Visibility* (5-point Likert-scale)
Change over time* (Patient global impression of change PGI-C*)	Estimated total number of cNF* (5point Likert-scale)
Targeted area or target cNF	Targeted area or target cNF
cNF severity (5-point Likert scale)	cNF severity (5-point Likert scale)
cNF number* (10-point Likert scale)	cNF number* (10-point Likert scale)
cNF size‡ (10-point Likert scale)	cNF size‡ (10-point Likert scale) + measurement using 3D photography
Cosmesis* (GAIS)	Cosmesis* (GAIS)
	Number* of treated cNF for interventional treatments
Daily Life Impact	
Health related quality of life* (cNF-skindex)	
Patient Satisfaction	
Satisfaction with treatment* (10-point Likert scale)	
Satisfaction with aesthetic result* (5-point Likert scale)	
Perception of Health	
Perceived severity†	
- Overall	
(How much of a problem are your cutaneous neurofibromas to you? 5-point Likert-scale)	
- Targeted area	
cNF severity (5-point Likert scale)	
Wound healing+	
Overall opinion regarding scars	

Clinical Assessment, Daily Life Impact, Patient Satisfaction and Perception of Health are the four domains of the core outcome domain set for cutaneous neurofibromas

*Subdomain pertaining to the "A" ring of the core outcome domain set

‡ Subdomain pertaining to the "B" ring of the core outcome domain set

† Not applied in this current study

+Subdomain pertaining to the "C" ring of the core outcome domain set

cNF, cutaneous neurofibromas

GAIS, global aesthetic improvement scale

Figure 1 – cNF skindex at baseline, 3- and 6-months post CO$_2$ laser treatment of cutaneous neurofibromas

cNF Skindex (n=26)

Mean (SD)	M0	M3	M6	Paired t test	M0 vs M3	M0 vs M6	M3 vs M6
Total	46.77 (25.71)	40.46 (26.28)	40.77 (25.76)	Total	**0.04655**	**0.03954**	0.8768
Symptoms	9.35 (6.97)	8.23 (8.92)	7.46 (7.16)	Symptoms	0.4925	0.1729	0.4694
Emotions	23.46 (10.42)	19.42 (11.20)	20.46 (10.99)	Emotions	**0.006189**	**0.02257**	0.2002
Functionning	13.96 (12.19)	12.83 (12.65)	12.85 (11.91)	Functionning	0.3782	0.2181	0.9728
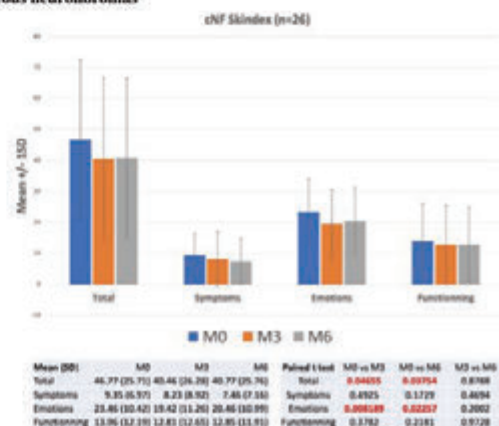
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Application of Artificial Intelligence in Cutaneous Neurofibroma Research: An International Collaborative Approach

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Purpose: To develop artificial intelligence (AI) approaches to analyse 2D and 3D images of cutaneous neurofibroma (cNFs).

Background: The clinical manifestations of NF1 can have a significant impact on quality of life related to disfigurement, with benign cutaneous neurofibromas (cNFs) present in almost all adults and numbering in their thousands in some individuals.[1-5] Many new therapies are in development including oral, topical and locally ablative treatments.[6,7] Successful assessment of new treatments requires robust outcome measures and an understanding of the natural history of CNFs.

Clinical research into the natural history and treatment of CNFs is ongoing at several International Centres. This includes natural history studies (eg Johns Hopkins University, Stanford), GWAS CNF severity studies (e.g. Newcastle University Cutaneous Neurofibroma consortium project), and treatment outcome and longitudinal imaging studies (e.g. Sydney University outcome measure and imaging reliability studies). Research from these centres has built large repositories of CNF imaging including pre and post treatment imaging and longitudinal imaging using 2D and 3D imaging methods including whole body 3D imaging (Canfield WB360). Manual analysis of this data is not feasible due to the large number of lesions and images. Artificial intelligence (AI) has shown promise in the diagnosis, imaging, treatment planning, and drug discovery of multiple cancers, such as the lung, breast, and prostate cancer via enabling analysis of large datasets.

Results: Collaboration with AI colleagues across sites at Johns Hopkins, Sydney and Queensland Universities have used traditional machine learning algorithms (thresholding and clustering) and modern deep learning algorithms (EfficientNet, UNet and Meta's Segment Anything Model (SAM)) for lesion segmentation. Training of the deep machine learning models have utilised public datasets of labelled melanoma lesions and expert labelled public and research images of CNFs.

Figure 1. A semi-automated pipeline to assist expert labelling of images developed at JHU

Figure 2. Successful segmentation of CNFs using AI trained model from Queensland University using UNet.

Figure 3. Automated detection of CNFs using AI trained model from Sydney University successfully segmenting clustered CNFs.

Figure 1
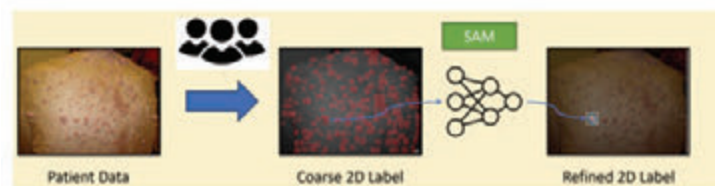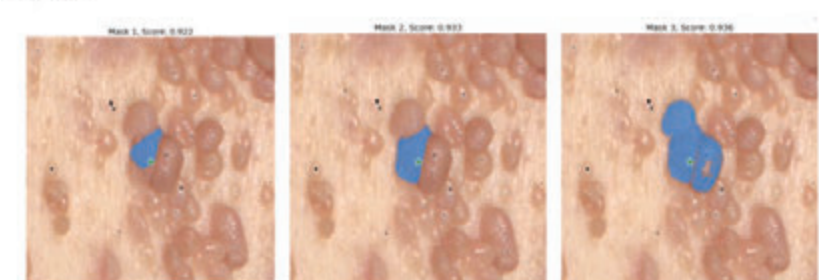
Conclusion: Independently, sites have worked to solve components of the requirements needed to develop the AI tools necessary for analysis of the large CNF research datasets to enable reliable and efficient application of digital imaging for defining cNF natural history and therapeutic outcomes. Ongoing collaboration is addressing the technical challenges to analysing and integrating digital data sets captured through various approaches to achieve detection, registration and measure of change.

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Figure 2

Figure 3

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A Case of Neurofibromatosis Type 1 and Glioblastoma with Positive Treatment Response to Selumetinib

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant condition which predisposes patients to tumors, such as optic glioma, pilocytic astrocytoma, neurofibroma, breast carcinoma and leukemia. This is due to dysregulation of the RAS/MAPK pathway as a result of the NF1 mutation. The incidence of malignant intracranial tumors such as glioblastomas are exceedingly rare with only 24 reported cases of glioblastoma in NF1 patients[1].

Case Presentation: We present a case of a 51-year-old male with a history of pancreatic neuroendocrine tumor (resected in 2012) and NF1. He has had resection of possible plexiform neurofibroma on his vocal cords at three years old and spinal tumor removed at 13 years old. He also has numerous superficial cutaneous neurofibromas. In May 2019, he developed progressive right facial numbness and pain. MRI brain showed enhancement concerning for tumor in the pons for which he underwent a right retro-sigmoid craniotomy in July 2019 with 70% tumor removal. Pathology showed glioblastoma, IDH wild type, MGMT promoter methylated, positive for NF1, ATRX retained, and CDK N2A loss. He underwent concurrent chemoradiation followed by maintenance temozolomide for 6 cycles. He was monitored on surveillance until December 2020. He presented to our institution in January 2023. MRI brain showed hyperintense mass in the anterior right pons on T2 weighted sequences with new enhancement. MRI spine also showed multiple tiny neurofibromas in the cauda equina. He was then started on selumetinib (MEK inhibitor) because the pathology showed glioblastoma with NF1 mutation. MRI brain about 2 months after treatment showed mild interval increase in enhancement with stable FLAIR and T2 weighted signal changes. He was started on temozolomide (150-200 mg/m2 at 5 days on, 23 days off regimen) for 6 cycles. The patient underwent 12 months of selumetinib therapy with MRIs showing significant decrease in T2 hyperintensity and enhancement in the ventral pons.

Conclusion: Selumetinib is a MEK1/2 inhibitor approved for treatment of refractory neurofibromas in children with NF1. The presented case highlights the challenging management of glioblastoma in NF1 patients. The positive response observed with selumetinib in this patient underscores the potential therapeutic efficacy of MEK1/2 inhibitors in the context of glioblastoma associated with NF1.

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Using Multilingual AI-Driven Techniques in Understanding NF1 Diagnostic Journey Globally

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Purpose: The goal of this study was to provide a deeper understanding of the diagnostic journey of patients with neurofibromatosis type 1 (NF1) and identify differences among countries from 3 regions — Europe, Asia, and Latin America.

Methods: The research was based on a real-world evidence approach using artificial intelligence (AI) and machine learning. The technology can analyse large volumes of unstructured data and extract meaningful information (natural language processing), such as first symptoms that led to NF1 diagnosis, visited healthcare professionals (HCPs), and differential diagnoses. The data are based on real anonymised patient stories in which a patient or a caregiver mentioned NF1, gathered from open patient forums, social networks, and other sources of user-generated content (Table 1).

Results: Hyperpigmentation was the most common first symptom observed (31-82% of cases), but some countries had their own characteristics (Figure 1). For instance, in Argentina almost 30% of cases were familial NF1 cases, not necessarily symptomatic. In most countries neoplasms were one of the causes for diagnosis (6-6.7% of cases).

Regarding the first HCPs seen by patients with NF1 (entry point), paediatricians (33-56% of cases) and neurologists (13-22%) were mentioned in most countries (Figure 2). Conversely, surgeons and dermatologists were prevalent as entry points for South Korea and Malaysia, respectively.

Despite significant differences in the healthcare systems and NF1 awareness among countries, there was a similar pattern in age of diagnosis. The peak of diagnosis occurred from 1 to 5 years of age (24-41% of cases) in all countries, and with 22-37% of cases with diagnoses before the age of 1 (the number of unique mentions for each country varies from 17 in Malaysia to 93 in Sweden).

Magnetic resonance imaging (MRI) was the most frequently mentioned medical test (~40% of mentions in all countries) and genetic testing was the second most frequent with 17-32% of mentions (the number of unique mentions for each country varies from 108 in Malaysia to 17,387 in South Korea).

Conclusions: Application of AI-driven technology in healthcare has shown its promise and importance. This analysis provided valuable insight into the diagnostic journey of patients with NF1 from different angles and highlighted the specific characteristics of some countries; these insights were particularly valuable in the absence of local field force. These findings may help address knowledge gaps and fast track NF1 diagnosis.

Region	Europe		Asia		Latin America	
	Sweden	Romania	South Korea	Malaysia	Argentina	Mexico
Number of posts	5,667	4,762	65,741	3,092	6,394	5,168
Number of patients with NF1*	435	357	5,474	63	1,196	813
Share of digitally active patients**	12.4%	5.6%	31.6%	0.6%	7.6%	1.9%
Average number of posts per patient	13.03	13.34	12.01	49.08	5.35	6.36

Table 1. Statistics for 6 countries. *Number of patients/caregivers who posted about their NF1 journey. **Percentage of patients who go online to discuss their problems and seek alternate opinions (calculated based on the reported NF1 prevalence of 1/3,000[1]). Data collection occurred between 2017-2022 for Sweden, 2016-2022 for Romania and Argentina, 2016-2021 for South Korea and Mexico, and 2010-2023 for Malaysia.

Figure 1. First symptoms that drove patients to seek a medical diagnosis. The number of unique mentions (n) on this topic for each country is indicated in each column. Hyperpigmentation includes cafe-au-lait-spots, spots on the skin, freckles, etc. Neurofibromas include plexiform neurofibromas. Family history includes familial NF1 cases (including asymptomatic cases). Neoplasms includes benign tumours, brain tumours, and other malignant neoplasms.
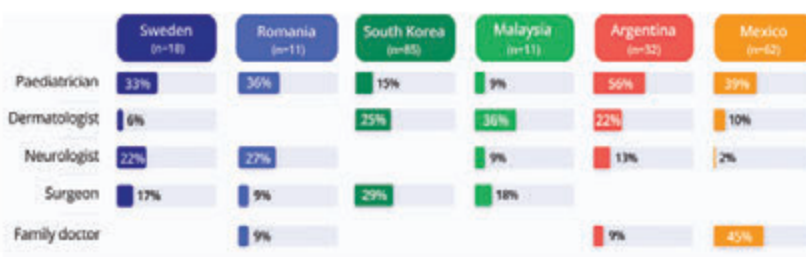
Figure 2. HCPs who were mentioned in diagnosing or suspecting NF1 and referred the patient to a relevant specialist. The number of unique mentions (n) on this topic for each country is indicated in the column.

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Adjuvant MEK Inhibition to Prevent Rebound Growth Following Partial Resection of Plexiform Neurofibromas

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Background: Plexiform neurofibromas (PNs) are benign peripheral nerve sheath tumors that occur in patients with neurofibromatosis type I (NF1). While they are benign in nature, these tumors can cause significant morbidity leading to functional impairment, pain, and disfigurement. Management of PNs is challenging. Complete surgical resection is often not possible due to tumor growth along vital structures, and rebound growth is frequently experienced following subtotal resection (STR) of PNs.[1] The mitogen-activated protein kinase (MAPK) pathway has been implicated in the growth of PNs, and the use of MAPK enzyme (MEK1/2) inhibitors has been shown to be an effective therapy in the treatment of PNs.[2,3]

Objective: To describe our institutional experience using a short course of adjuvant MEK1/2 inhibitors in the treatment of pediatric patients with PNs following STR to prevent rebound growth.

Methods: A single-institution retrospective record review of pediatric patients with NF1 who underwent STR of their PN.

Results: A total of 43 patients with 59 separate PNs had STR of their PNs. Fourteen PNs were treated with resection alone, ten PNs received adjuvant mechanistic target of rapamycin (mTOR) inhibitors following STR and 35 PNs were treated with a six-month course of adjuvant MEK1/2 inhibitors following STR. The number of patients requiring additional treatment with surgical resection or medical therapy was 11 of 14 patients (78.6%) in the resection only group, 8 of 10 patients (80%) in the adjuvant mTOR inhibitor group and 6 of 25 patients (28.6%) in the adjuvant MEK1/2 inhibitor group. The mean time to additional treatment was 22 months for the resection only group and 19 months for adjuvant mTOR inhibition group. The mean follow-up time for patients receiving adjuvant MEK1/2 inhibition was 29 months. Skin rash was the most common adverse effect seen with adjuvant MEK1/2 inhibition, and one patient experienced a dose-limiting thrombus.

Conclusions: A short course of MEK1/2 inhibitors following STR of PNs is effective in preventing rebound growth when compared to STR alone or adjuvant mTOR inhibitors following STR. Treatment is well tolerated and should be considered as adjuvant therapy in pediatric patients.

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Multiparametric MRI-Based Plexiform Neurofibroma Subtypes Definition Correlated with Different Malignant Transformation Potential

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Introduction: Grading the risks of benign plexiform neurofibromas (PNFs) developing malignant peripheral nerve sheath tumors (MPNSTs) will aid in treatment and follow-up decisions for neurofibromatosis type 1 (NF1) patients. However, studies on this topic are rare. We aimed to define a MRI-based PNF classification system to reflect the malignant transformation potential and guide the individualized management.

Methods: 175 NF1 patients (136 for study cohort and 39 for validation cohort) underwent total-body PET/MRI were retrospectively included who. A K-means clustering algorithm was applied to the maximum standard uptake value (SUVmax), and the morphological characteristics were then analyzed. The apparent diffusion coefficient (ADC) was also calculated as additional evidence. Fleiss' kappa was adopted for interobserver agreement evaluation.

Results: Eight PNF subtypes were defined (Figure 1 and Figure 2) and classified as Grade I, II, and III, indicating a high to low risk of developing MPNSTs (SUVmax: Grade I vs Grade II, $p<0.05$; Grade I vs Grade III, $p<0.001$; Grade II vs Grade III, $p<0.001$). The interrater association for subtype definition was almost perfect (kappa = 0.959; 95% CI: 0.942, 0.976). The patients' age and nerve-based location of PNF lesions were also statistically associated with the malignant transformation potential ($p<0.01$, $p<0.001$).

Conclusion: We developed a new MRI-based PNF classification system for different malignant transformation potentials. The results also corroborate previously described findings that NF1-related MPNSTs were normally deeply located and detected in adults.

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Genotypic Spectrum of NF1 from a Chinese Children Hospital

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Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant condition, with a birth incidence of approximately 1:2000–3000, caused by germline pathogenic variants in NF1, a tumor suppressor gene encoding neurofibromin, a negative regulator of the RAS/MAPK pathway. By far, thousands of mutations of NF1 gene had been reported, but the hotspot regions of the NF1 gene and obvious genotype-phenotype correlations were still absence. The aim of this retrospective study was to define the mutational spectrum in our hospital.

Methods: We included NF1 patients between November 2018 and August 2023 from our center who underwent genetic testing for NF1 variants. Genotypic spectrum of NF1 analyses were performed, focusing on variation types and involved neurofibromin domains.

Results: A total of 74 patients were enrolled, comprising 30 males and 44 females, with a median age of 5.5 years. Truncating variants, splicing variants and single amino acid variations accounted for 44/74(59.5%), 14/74(18.9%) and 11/74(14.9%) respectively. Five patients had a whole NF1 gene deletion (Table 1). We found 15 of these variants had not been previously reported: 9 frameshift, 2 nonsense, 2 splice, 1 missense and 1 inframe variants (Table 2).

We identified recurring variants occurring two or more times in the cohort, including nonsense variants p.R304* (5 occurrences from 3 family) and p.R440* (2 occurrences).

We also analyzed the distribution of mutation sites in NF1 patients. 11 out of 74(14.7%) patients had variants affecting the C-terminal domain (CTD), 10 (13.5%) in the GTPase activating protein-related domain (GRD), 6(8.1%) in the HEAT-like repeats domain (HLR), 5(6.8%) had variants in the cysteine-serine rich domain (CSRD), 4 (5.4%) in the Sec14-Pleckstrin Homology (PH) domain, 3 (4%) in the protein kinase C domain (PKC),and 1 (1.4%) in the Cyclin T binding domain (TBD)(Table 3).

Conclusions: This study expands the spectrum of mutations in the NF1 gene.

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Table1 Distribution of mutation types in patients

Mutation type	n (%)
Deletion	5/74 (6.7)
Truncating	44/74 (59.5)
frameshift	22/74 (29.7)
nonsense	21/74 (28.4)
copy number variations	1/74(1.4)
Splicing	14/74 (18.9)
Single amino acid variations	11/74 (14.9)
missense	9/74 (12.2)
inframe	2/74 (2/7)

Table 2 Characteristics of the novel NF1 variants identified; positions are based on GRCh37/hg19 NM_000267.3

Patient No.	Gender	Nucleotide change	Consequence	Exon	Domain	Inheritance
1	M	373_377delCGTGA	p.E126Kfs*6	4	PKC	NT
2	M	1722-2delA	Splice	intron15	-	De novo
3	M	2156del	p.I719Tfs*29	18	CSRD	De novo
4	M	2533del	p.C845Vfs*33	21	CSRD	De novo
5	M	3791A>T	p.E1264V	28	GRD	De novo
6	F	3921delT	p.I1307Mfs*2	29	GRD	De novo
7	F	3933delC	p.S1312Lfs*15	29	GRD	De novo
8	F	4747_4753delCCTATTT	p.P1583Ffs*18	35	Sec14-PH	De novo
9	M	5749+332A>C	Splice	intron30	-	De novo
10	F	6452C>G	p.S2151*	42	HLR	NT
11	M	6683_6685del	p.2228_2229del	44	HLR	De novo
12	F	7063-2_7064dup	p.S2355Rfs*32	48	CTD	NT
13	F	7090dup	p.R2364Pfs*22	48	CTD	NT
14	M	7537C>T	p.Q2513*	51	CTD	NT
15	F	7881del	p.V2628Cfs*30	53	CTD	De novo

Abbreviations: NF1, neurofibromatosis type 1; NT, not tested

Table 3 Distribution of mutation frequencies of involved domains in patients

Involved domains	n (%)
CTD (residues 2260–2818)	11/74(14.7)
GRD (residues 1198-1530)	10/74(13.5)
HLR (residues 1825–2428)	6/74(8.1)
CSRD (residues 543–909)	5/74(6.8)
Sec14-PH (residues 1560-1816)	4/74(5.4)
PKC (residues 97–243)	3/74(4)
TBD (residues 1095-1197)	1/74(1.4)

Impact of Selumetinib Treatment for NF1 PNs in Pediatric Patients: Perspectives from Patients and Patient Caregivers

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Background: Plexiform neurofibromas (PNs) affect approximately 20% to 50% of patients with NF1 and can lead to pain, disfigurement, and compression of vital structures (Nguyen et al., 2011; Tchernev et al., 2016; Miller et al., 2019). Selumetinib, a MEK1/2 inhibitor, is the first and only FDA-approved pharmacological treatment licensed for the treatment of symptomatic, inoperable PNs in children with NF1. The aim of this study was to assess the drivers for initiation of treatment as well as the impact of selumetinib treatment on the patient's quality of life from the perspective of both patients and their caregivers.

Methods: This was a qualitative study of children and their caregivers who were prescribed selumetinib for the treatment of their PN(s). Participating patients were age ≥ 9 years and taking Selumetinib for ≥ 6 months; caregivers were a parent or primary caregiver of a patient aged 2–18 who had been taking selumetinib for ≥ 6 months. Demographic and clinical background information were summarized using descriptive statistics.

Results: The study included 10 patients (mean age of 13.2; range 9-17 years) and 19 caregivers (mean patient age of 11; (range 3 – 17 years)) with an average selumetinib treatment duration 30.6 months (range 7 to 96 months). Reported treatment goals mainly focused on tumor size reduction (N=8, 42.1%); stabilization of PN(s) (N=7, 36.8%); and a decrease in PN-associated pain (N=6, 31.5%). In terms of treatment response, most caregivers (n=17, 89.4%) reported either stabilization or a reduction in size of PNs. Of the 11 caregivers reporting pain pre-treatment, all (100%) reported pain reduction post selumetinib initiation. All caregivers (N=19, 100%) reported some form of physical improvement, and over one third (N=7, 36.8%) reported improvements in the emotional, social, and learning domain following treatment initiation. Patients less frequently reported pre-treatment burden of PN but reports of post-treatment improvement was consistent with that of their caregivers.

Conclusion: Children with NF1 and PNs and caregivers are concerned about the presence of PNs and associated morbidities. Treatment goals primarily focused on tumor shrinkage/stabilization and reduction in PN-associated pain. Most caregivers reported tumor stability and/or shrinkage, reduction in pain, as well as well improvement in psycho-social aspects following selumetinib therapy initiation, and their children's responses were generally consistent regarding treatment benefits, despite understatement of pre-treatment burden of PN.

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Cancer Risk and Survival in Danish Individuals with Neurofibromatosis 1

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Aim: To assess cancer risk and survival in a Danish cohort of individuals with Neurofibromatosis type 1 (NF1) compared to a randomly selected group from the general population without NF1.

Study population: 2,753 individuals with NF1 were identified from the Danish National Patient Registry and the clinical database RAREDIS by the International Classification of Disease (ICD) version 8 743.49 and ICD-10 Q85.0, in the period 1977-2020. Age- and sex-matched comparisons were identified from the Danish Civil Registration System with a ratio of 1:10. All individuals were linked to the Danish Cancer Registry to obtain information on cancer diagnoses. Study participants with a cancer diagnosis registered before study entry (date of NF1 diagnosis) were excluded.

Methods: Cumulative risks were calculated using the Nelson-Aalen estimator with death as a competing risk. Survival after any first cancer was estimated among individuals with NF1 using the Kaplan-Meier method. To illustrate a comparable five-year survival between the two groups, when considering the distribution of age at cancer diagnosis, type of cancer, and year of cancer diagnosis, a weighted Kaplan-Meier method was utilized.

Results: Out of 2,107 individuals with NF1 and 20,926 comparisons, 418 (19.8%) and 1,117 (5.3%) individuals respectively were diagnosed with cancer. At age 75 years, 62% (95% confidence interval (CI) 51-72%) of men and 59% (95% CI 53-65%) of women with NF1 had at least one cancer diagnosis compared to 30% among both men (95% CI 28-33%) and women (95% CI 27-33%) without NF1 (Figure 1). The cumulative risk of gastrointestinal cancer was slightly increased for both men and women with NF1 compared to individuals without NF1 (Figure 2). Figure 3 depicts the five-year survival probability after any type of first cancer diagnosis in the NF1 cohort, which was 58% (95% CI 51-65%) for men and 64% (95% CI 57-70%) for women. Five-year survival was significantly lower in women with NF1 compared to women without NF1 (P-value 0.0015) but there was not a significant difference in the men (P-value 0.25) (Figure 4).

Conclusions: Individuals with NF1 had a higher cumulative risk of being diagnosed with any type of cancer at all ages compared to individuals without NF1, with a slightly increased cumulative risk of gastrointestinal cancers. There was a significant difference in the five-year survival probability after a first cancer diagnosis between women with and without NF1, which should be investigated further.

Figure 1
The cumulative risk of the first diagnosis of any type of cancer (excluding non-melanoma skin cancer) in individuals with and without NF1 stratified by sex. NF1-specific tumours such as optic nerve glioma and malignant peripheral nerve sheath tumours (MPNSTs) are also included. Age is the underlying time scale.
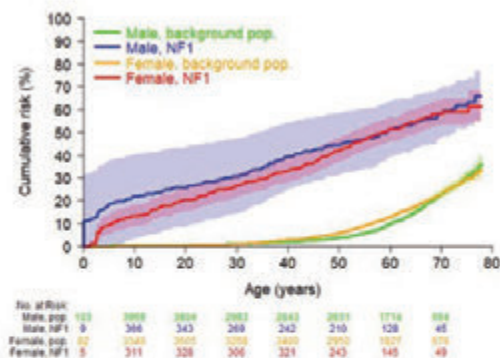
Figure 2
The cumulative risk of gastrointestinal cancer as the first cancer diagnosis in individuals with and without NF1 stratified by sex. Age is the underlying time scale. The cumulative risk is only depicted up to 5% in the figure.
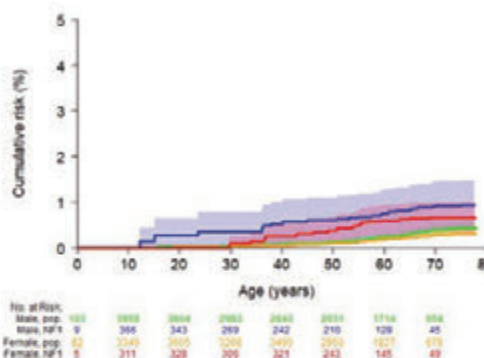
Figure 3
Five-year survival after a first diagnosis of any type of cancer in the NF1 cohort stratified on sex. There were 418 cancer diagnoses registered in the NF1 cohort, of these 219 in the 1,061 females and 199 in the 1,046 males included.
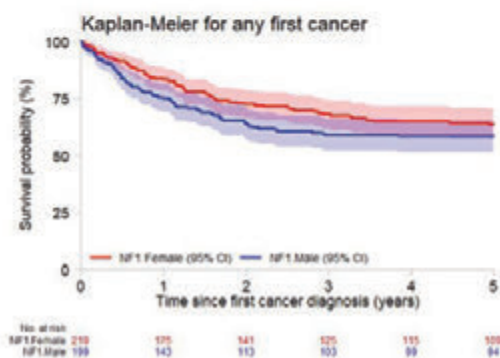
Figure 4
Five-year survival after a first diagnosis of any type of cancer in the NF1 cohort and the comparisons stratified on sex. The comparison group is weighted to mimic a matched design, where the distributions of cancer type, age at cancer diagnosis and cancer diagnosis year in the comparison group are similar to the distributions in the NF1 cohort.
A weighted log-rank test, comparing the NF1 group with the weighted comparison group, results in the p-values for women p-value=0.0015 and men p-value=0.26. These are the p-values comparing the curves shown in the figure.
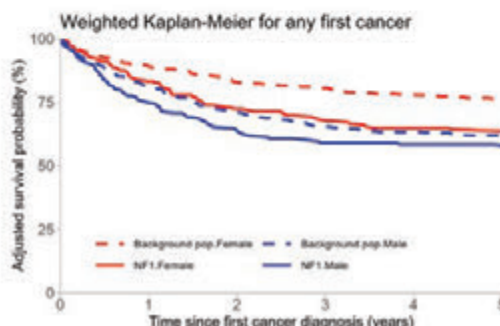
Structural Remodeling of Plexiform Neurofibroma During Puberty: A Case Report

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Purpose: This case report presents a 21-year-old male with neurofibromatosis type 1 (NF1) and a plexiform neurofibroma (PN) showing structural remodeling and an unusual accelerating growth pattern.

Methods: The participant in this case underwent whole-body magnetic resonance imaging (WB-MRI) as part of a longitudinal imaging and PN biomarker study (NCT05238909) at Ann & Robert H. Lurie Children's Hospital of Chicago in collaboration with the National Institutes of Health. The individual also consented to use of prior clinical imaging. Detailed review and analysis of clinical history and imaging was completed and described chronologically.

Results: The individual presented is a now 21-year-old male with NF1 and a known PN.   At age 3.6 years, he had magnetic resonance imaging (MRI) performed revealing a 35.5mL subcutaneous mass in the left flank with characteristics of multinodular PN. An area of hazy T2-hyperintensity was observed in the adjacent subcutaneous fat without soft tissue asymmetry or detectable tumor-like structural change. Also, a thin patch of faint bright signal in the skin over the left groin was noted. In the next 2 years, PN volume increased by 19.3ml per year. During the following 7 years, the average yearly growth was 37.4ml, partly due to the appearance of streaky ill-defined tumor within the subcutaneous fat. From age 12.5 years, near puberty onset, growth sharply accelerated to 142.4ml per year. The streaky infiltrate in the subcutaneous tissue became dense diffuse mass growing beyond the initially observed T2-hyperintense area, expanding to the skin surface, and wrapping around the left side of abdominal wall, hip, and lower back, while the multi-nodular component of the tumor gradually disappeared. By 21-years-old, the mass measured 1681ml, causing significant disfigurement.

Conclusions: There are two unusual aspects of this PN case. First, we observed a complete transformation from multi-nodular to diffuse structure. It is unclear if the diffuse PN developed as a result of the multi-nodular PN extending streaky narrow strands into the fatty subcutaneous tissue, or the multi-nodular and diffuse components represent two separate entities that eventually merged. Second, the PN growth rate accelerated during puberty. Puberty-related increase in size and number is characteristic of discrete cutaneous neurofibromas, but not of PN. However, published PN growth data is largely limited to tumors with well-defined borders. More information is needed about superficial skin infiltrating PN growth.

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Multiple MPNST and ANNUBP in NF1 – A Complex Patient

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Neurofibromatosis type I (NF1) carries an individual lifetime risk of malignant peripheral nerve sheath tumour (MPNST) of 8-13%, this is higher in those with high internal burden of neurofibromas or certain types of *NF1* pathogenic variant. Atypical neurofibromatous neoplasm with unknown biological potential (ANNUBP) is the term proposed in an NIH consensus overview to classify tumours with some features of malignant transformation, but which histologically fall short of MPNST. These may be a precursor to MPNST in NF1.

We present a complex case of a young woman under the care of the Highly Specialised Complex NF1 service in Manchester, UK.

Patient A is the first affected person in her family, with pigmentary features fulfilling NIH diagnostic criteria for NF1. RNA based testing identified a NF1 c.4340A>G p.(Gln1447Arg) missense variant of uncertain clinical significance. Known to have an asymptomatic optic pathway glioma and moya moya requiring revascularisation in childhood, she was referred to the Complex NF1 service. Aged 16, she had surgical excision of 2 growing symptomatic sub-cutaneous, histologically benign neurofibromas from her forearm and thoracic region.

Whole body imaging revealed a significant internal burden of disease, ongoing surveillance imaging was undertaken. Aged 20, PET scanning identified several lesions with increased metabolic activity on delayed imaging. Lesions of concern were sequentially surgically removed from various locations. Histological evaluation of these confirmed 4 ANNUBP and one low grade MPNST. Fortuitously, the patient highlighted a new, asymptomatic lesion on the mons pubic that was not present on the PET scan just prior to surgery. This was added to the planned excision. Unexpectedly, this revealed a high grade MPNST. Due to minimal clear margins, re-excision was followed by proton beam radiotherapy. Less than one year following treatment, aged 22, surveillance imaging detected concerning growth of another previously identified lesion. Of note, this was outside the previous field of proton beam. This was histologically confirmed to be another high grade MPNST following surgical excision. Further proton beam radiotherapy is currently being undertaken. This case highlights the importance of holistic care within a specialist multi-professional team, a low threshold for surveillance imaging in patients with NF1 and a known high internal burden, and the importance of prompt resection of rapidly enlarging lesions. Further work is required to identify factors that may pre-dispose individuals to this phenotype and systemic treatments that may reduce the risk of the development of new MPNST or transformation of in situ neurofibromas.

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A Systematic Review and Meta-Analysis of the Prevalence of Cutaneous Manifestations in Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1(NF1) occurs in one out of every 2500-3000 live births. There is, unfortunately, a wide spectrum of clinical manifestations among NF1 patients, even within a family that is affected by the condition. Therefore, the National Institutes of Health established seven diagnostic criteria upon which diagnosis relies on, with the manifestation of two of these criteria being imperative for confirmation. Cutaneous manifestations are the hallmark of NF1 including café au lait spots, freckling, and cutaneous neurofibromas. The objective of this systematic review and metanalysis is to highlight the prevalence of these cutaneous manifestations in NF1 which are presented early in the disease and could in many cases be the only presented signs when the case is due to a spontaneous mutation or with no anticipated family history. In addition, the association of nevus anemicus (NA) and juvenile xanthogranuloma (JXG) with NF1 have been mentioned repeatedly in younger children (<2y), therefore we aim to define the prevalences of these cutaneous manifestations in different age groups and provide valuable insights to evaluate suggestions on including these features in the diagnostic criteria for NF1.

Methods: We conducted a systematic search of NF1 cutaneous manifestations studies, with patients in all age groups, in OVID Medline, Embase and Cochrane Central Register. Studies were appraised with the Joanna Briggs Institute Prevalence Critical Appraisal tool. Pooled prevalence proportion of cutaneous manifestations, juvenile xanthogranuloma and nevus anemicus clinical features in overall NF1 population will be estimated through random-effects meta-analysis. These findings offer a broad overview of how frequently each feature occurs over time in the NF1 population. The prevalence of each feature will be pooled across various age groups, and a comparative analysis among these groups will be carried out (subgroup analysis) considering preschool and up to 10 years as children, pre/ and adolescence as 10-18 years and adults as patients >18years. In addition, prevalence of these features will be plotted against covariate mean age by conducting a meta-regression to evaluate the association of these clinical features with age.

Results: The search resulted in 4161 abstracts, and 8 duplicates were removed. After abstract screening, 177 studies were assessed trough full text screening. Of these, 52 studies were fully appraised, and data abstracted. Analyses are ongoing and the metanalyses of the prevalence of each specific cutaneous manifestation will be presented at the conference.

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Trial in Progress: A Phase 0/I/II Study of the Cyclin-Dependent Kinase (CDK)4/6 Inhibitor Abemaciclib for Neurofibromatosis Type 1 (NF1) Related Atypical Neurofibromas (ANFs)

Margaret Fagan, RN, *National Cancer Institute*

Background: Atypical neurofibromas (ANFs) are considered pre-malignant peripheral nerve sheath tumors (PNST) in NF1, and defined on histopathology by cellular atypia, increased cellularity, loss of neurofibroma architecture, and/or very infrequent mitoses. Molecularly, most ANF have loss of the cell-cycle regulator gene *CDKN2A* in addition to biallelic loss of *NF1* and do not seem to respond to the MEK inhibitors that shrink most PN. The CDK4/6 inhibitor, specifically abemaciclib, targets this pathway and is approved for use in breast cancer and other malignancies. Here we report the status of an ongoing Phase I/II study of abemaciclib for inoperable ANF in people with NF1 (NCT04750928) and the plan to amend the study to include a Phase 0 component.

Trial Design & Current Status: Participants ≥12 years old with NF1 and an ANF for whom surgical removal could cause significant clinical morbidity OR for which participant is unwilling to undergo surgical resection OR the presence of more than one distinct nodular lesion (DNL) including at least 1 biopsy proven ANF are eligible for the Phase I/II study. The Phase I portion of the study will assess the tolerability of abemaciclib in people with NF1 and will start at 75% of the adult recommended dose (150 mg PO BID). If the first 6 participants tolerate the 75% dose without a dose limiting toxicity, the dose will be increased to the adult recommended dose (200 mg PO BID). The phase II study will assess for tumor response in a Simon's minimax two-stage design. To date, two participants have been treated at phase 1, dose level 1 with no dose limiting toxicities reported.

Study Update: The Phase 0 amendment (pending IRB approval) will allow for enrollment of participants ≥ 18 years old whose ANF is resectable and planned to be removed for a clinical indication. These participants will receive 7-10 days of abemaciclib immediately prior to resection of the tumor. Tissue and plasma pharmacokinetics will be obtained and we will compare the effect of treatment on phosphorylated Retinoblastoma (pRb) in the tumor compared to the pre-treatment biopsy as well as other pharmacodynamic markers. The phase 0 study will use a 2-stage design with 3 participants in the first stage, with the primary endpoint defined as a ≥ 50% decrease in pRb (serine 807) as detected by immunohistochemistry between the pre-treatment and on-treatment tumor sample. If 1 out of 3 participants has a pharmacodynamic response in the first stage, we will expand by an additional 2 participants. If ≥2 participants have a pharmacodynamic response, then the trial will stop without recruiting further patients and this two-stage design will have 89% power to detect a 60% pharmacodynamic response rate across patients with the type I error rate of 0.02.

Summary: The Phase 0 amendment of the ongoing Phase I/II trial of the CDK4/6 inhibitor abemaciclib for ANF in NF1 will allow us to gain unique insights into the pharmacokinetics of abemaciclib in tissue and plasma and the pharmacodynamic impact of treatment on these tumors.

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High Grade Gliomas in Pediatric and Adolescent Patients with NF1 – A Review of Clinical, Genomic and Treatment Characteristics and Survival Outcomes

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Purpose: Neurofibromatosis type 1 (NF1)-associated pediatric high-grade gliomas (NF1-pHGG) arise either from the malignant transformation of low-grade lesions or de novo. Histologically and molecularly, they can be classified as high-grade astrocytoma with piloid features (HGAPs) or IDH-wildtype glioblastoma. In adult patients, the outcome has been reported to be very poor, and novel therapies are urgently needed. Limited data are available in children and adolescents with NF1-HGG. We describe the clinical and molecular characteristics, as well as treatment outcomes in patients with NF1-pHGG treated at Memorial Sloan Kettering Cancer Center (MSKCC) and New York University (NYU).

Methods: We retrospectively reviewed all patients under 21 years of age with a diagnosis of NF1 (updated 2021 criteria) and HGG (WHO grade III or IV) or HGAP (low grade glioma histology with concurrent *CDKN2A* homozygous deletion or *ATRX* loss) treated at MSKCC and NYU between 2005–2023.

Results: 7 patients were identified: 5 females and 2 males. The median age at diagnosis was 13.3 years (4.3–19.7 years). Tumor site was thalamic (n=2), optic pathway (n=2), hemispheric (n=2), cerebellar (n=1). In 2/7 patients, pHGG arose from transformation of a previously diagnosed pathologically proven LGG. Final pathological diagnosis was HGG (n=4), anaplastic astrocytoma with piloid features (n=3). Most common alterations identified were loss of function alterations in *NF1* (n=4), *CDKN2A* (n=4), *ATRX* (n=3), *TP53* (n=2) and *EED* (n=1); *MYCN* amplification (n=1) and *PTPN11* gain of function (n=1). All patients underwent received adjuvant focal radiotherapy and chemotherapy. Two patients (*MYC* amplified glioblastoma, *TP53* mutant glioblastoma) died within two years of diagnosis while one patient with HGAP died four years post diagnosis. 6/6 patients were treated with MEK inhibitors (MEKi) off-label either prior to (n=3) or immediately after upfront radiotherapy (n=3); no objective responses were noted. 4/4 patients were treated off-label with various anti-PD1 antibody based immune checkpoint inhibitors (ICIs), either alone (n=3) or in combination with anti CTLA4 inhibition (n=1). 3/4 patients had progressive disease. One patient with multiply recurrent/progressive disease experienced a minor imaging response and prolonged ongoing clinical benefit (>2 years) with anti PD-1 antibody monotherapy.

Conclusions: This patient cohort sheds light on the clinical and biological heterogeneity encountered in patients with NF1-pHGG. While there were no objective responses noted in patients treated with MEKi, treatment with ICI maybe beneficial in a subset of patients with NF1-HGG and should be evaluated in a prospective clinical trial, either alone or in combination with other modalities.

Social Functioning and Social Cognition in Preschool Children with NF1

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NF1 is known for its frequent neurocognitive deficits and psychosocial problems.[1-6] Thus, the affected children demonstrate difficulties in social functioning and elevated autistic symptoms have been described.[7] To date, most studies have assessed social functioning and social cognition in school-aged children with NF1.[8-9] Since preschool age is considered a key period for developing social-emotional competencies, the present study aims at evaluating social functioning and related cognitive abilities in preschool children with NF1 aged 3 to 6 years.

Social functioning was evaluated using the parent-reported outcome of the Strengths and Difficulties Questionnaire (SDQ). Social cognition was assessed through a subtest of the standardized Viennese Developmental Test (WET) which evaluates facial emotion recognition. Other cognitive abilities, such as general intelligence, verbal cognition and working memory, were evaluated using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and WET. Scores of the standardized assessments were compared with normative data. Attention was evaluated using the standardized German parent-questionnaire in preschool children (KOPKI 4-6). Furthermore, the bio-psycho-social model 'SOCIAL'[10] was applied to investigate the association between cognitive, internal and external factors, and social functioning in a linear regression model.

Results of preschoolers with NF1 ($n=50$, *mean age* $=5.04$ years) show significant deficits in social functioning ($p=.005$) and social cognition ($p=.005$) compared to the norm. In addition, general intelligence (IQ, $p=.003$), working memory ($p<.001$) and parent-rated attention ($p=.006$) are significantly reduced. Variance in social functioning was successfully explained by the SOCIAL model ($p=.005$), with attention and familial NF1 being the strongest predictors of social functioning deficits. The associations are presented in the following figure (numbers indicate beta coefficients, asterixis indicate significance, *p<.05; **p<.01).

The study demonstrates that deficits in social functioning, social cognition and general cognitive abilities are already present at preschool age. This is of high clinical relevance since social-emotional competencies represent an important developmental task at preschool age, and moreover, crucially affect further social and academic development.[11-13] Hence, the results highlight the importance of early neuropsychological diagnostic and psychosocial interventions to promote social-emotional development and overall quality of life in young children with NF1.

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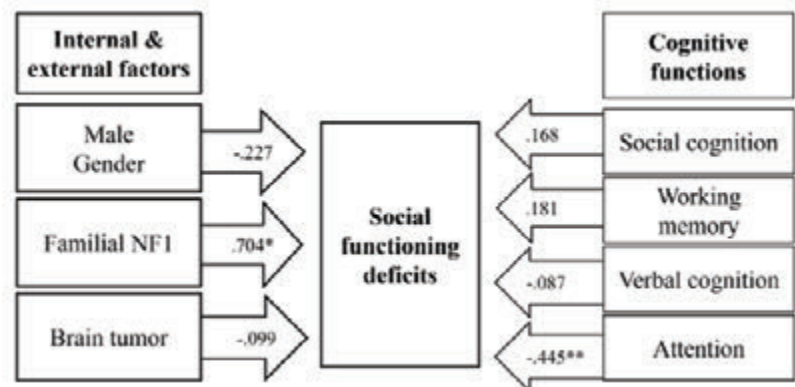
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Evaluation of Standard of Treatment Outcomes for Cutaneous Neurofibromas (CNFs) in People with Neurofibromatosis 1 (NF1): An International Pilot Study

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Purpose: The purpose of the study was to identify a minimum dataset for future research, clinical trials, and patient management.

Cutaneous neurofibromas (CNFs) have a significant impact on the emotional and physical wellbeing of people with NF1. However, treatments to date are limited to ablative interventions where treatment outcome measures are not standardised. The aim of this study was to identify a minimum dataset for the evaluation of treatment outcomes post-ablative therapy at sites in Paris and Sydney.

Methods: Patients and four clinicians (dermatologists/clinical geneticist) were recruited from NF1 skin clinics. A comprehensive set of evaluations were performed, including patient reported outcome measures, blinded clinician assessments, and photographic analysis of cNFs, to determine their sensitivity and accuracy. Observations were conducted at baseline, 3- and 6-months post-treatment (single Er:YAG laser or excision therapy). Outcome measures from both sites were ranked to identify a minimema dataset. Findings from Sydney are reported.

Results: 29 adult patients were recruited and treated with ablative therapy. Patients reported statistically significant improvement in total cNF-related quality of life (cNF-Skindex) at 6-months post-therapy, mainly in the functioning domain (Figure1). There was also a decrease in the number of patients who reported bothersome cNFs at 6-months post-treatment.

Patient and clinician assessment of a representative cNF from 2D photographs demonstrated significantly reduced severity [size, shape, volume, and texture] at 3- and 6-months post-treatment (p<0.05) (Table1). We also found significant reduction in patient and clinician perceived overall number of cNFs in a field of view (FOV) post-treatment as compared to baseline. Patients also reported average global aesthetic improvement in ratings of a representative cNF and the overall field of view. Patient scores were 'much improved' however, clinicians rated improvement less positively for a targeted cNF and FOV respectively at 3- and 6-months post-therapy.

Clinicians described small increases in redness (3-months), hypopigmentation (6-months) of cNFs from 2D photos at 3- and/or 6-months post treatment as compared to baseline, and patients described colour of scarring as most different to normal skin. Mean patient satisfaction scores were high (>80%) at 3-months and 6-months post-treatment.

Conclusions: Using data from the Paris and Sydney sites, we ranked outcome measures deemed most informative based on sensitivity to change and reliability. We agreed on a minimum dataset of outcome measures (Table 2), which will be validated in additional sites and will inform future guidelines, research, and clinical trials in the era of emerging drug therapies for the treatment of cNFs. Later time points would assist in distinguishing between wound healing and scarring in this cohort, an important assessment of treatment success.

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Figure 1: cNF-Skindex scores at 0- and 6-months post-treatment (n=20)

Table 1: Patient and Clinician assessment of a representative cNF from 2D photos

Patients n=14	0 months		6 months		Difference 0-6 months	Compared to SD at baseline	Significance P value
	Mean	SD	Mean	SD			
Size	3.43	0.85	2.57	1.09	-0.86	-1 SD	0.007**
Shape	3.36	0.93	2.36	1.08	-1.00	-1 SD	0.023*
Volume	3.38	1.04	2.31	1.43	-1.07	-1 SD	0.039*
Texture	3.23	1.17	2	1.24	-1.23	-1 SD	0.014*
Clinicians n=4; photographs 12 patients.	0 months		6 months		Difference 0-6 months	Compared to SD at baseline	Significance P value
	Mean	SD	Mean	SD			
Size	3.60	0.76	2.34	0.62	-1.26	-1SD	0.007**
Shape	3.46	0.74	2.29	0.58	-1.17	-1SD	0.004**
Volume	3.25	0.80	1.63	0.47	-1.62	-2SD	0.003**
Texture	2.91	0.67	1.72	0.45	-1.19	-1SD	0.026*
Hypo-pigmentation	1.16	0.18	1.81	0.69	+0.65	+1SD	0.052

Table 2: Recommended Minimum Dataset

Recommended Minimum Dataset	
Patient Reported Experience Measure (PREM) **Patient Reported Outcome Measure (PROM)**	**Clinician Reported Outcome Measure**
Clinical Assessment	**Clinical Assessment**
Overall	Overall
Visibility* (5-point Likert-scale)	Visibility* (5-point Likert-scale)
Change over time* (Patient global impression of change PGI-C)	Estimated total number of cNF* (5point Likert-scale)
Targeted area or target cNF	Targeted area or target cNF
cNF severity (5-point Likert scale)	cNF severity (5-point Likert scale)
cNF number* (10-point Likert scale)	cNF number* (10-point Likert scale)
cNF size* (10-point Likert scale)	cNF size† (10-point Likert scale) + measurement using 3D photography
Cosmesis* (GAIS)	Cosmesis* (GAIS)
	Number* of treated cNF for interventional treatments
Daily Life Impact	
Health related quality of life* (cNF-skindex)	
Patient Satisfaction	
Satisfaction with treatment* (10-point Likert scale)	
Satisfaction with aesthetic result* (5-point Likert scale)	
Perception of Health	
Perceived severity‡	
- Overall	
(How much of a problem are your cutaneous neurofibromas to you? 5-point Likert-scale)	
- Targeted area	
cNF severity (5-point Likert scale)	
Wound healing*	
Overall opinion regarding scars	

Dental Developmental Stages and Decayed, Missing, and Restored Teeth in Neurofibromatosis Type 1-Affected Children and Adolescents

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Background/Purpose: Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited tumor predisposition disease with a highly variable phenotype. The influence of the characteristic NF1 tumors (neurofibromas) on dentition has not yet been examined in detail. The aim of the study was to assess the dentition of NF1 children and adolescents, considering the symmetry of tooth development.

Material and Methods: The panoramic radiographs of 59 patients with a confirmed NF1 diagnosis were compared with 59 age-and-sex-matched controls. The stages of tooth development on the sides of the jaw, added to a score, were assessed. In addition, the number of filled or decayed teeth, and the number of retained or missing teeth were assessed.

Results: The tooth development of both study groups is symmetrical for almost all parameters and in the same developmental stage according to the sum score of the tooth development stages. Discrete developmental delays of teeth, in particular in the oral area of facial plexiform neurofibroma (PNF) are noticeable. NF1 patients' teeth showed less decay and more restorations than that of the control group. The facial PNF (FPNF) does not impair emergence of deciduous teeth.

Conclusion: Development of dentition of NF1 patients does not differ from the general population. However, FPNF with oral tumor components often prevent mesial movement of permanent molars and premolars, so these teeth do not develop contact (spacing), hardly emerge or may stay retained in bone. Oral PNF may have a low-retarding effect on some tooth root development (e.g., wisdom teeth). This effect is negligible when comparing the affected and unaffected sides of the jaw and is probably non-specific.

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Dysplasia of the Jaws and Temporomandibular Joints in NF1-Affected Children and Adolescents

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Purpose: Facial plexiform neurofibromas (FPNF) are a rare and often very conspicuous finding in neurofibromatosis type 1 (NF1). The soft tissue tumors can cover the regularly expected deformation of the facial skull bones due to their volume. Only isolated reports have been presented on early manifestations of jaw deformities in children. The aim of this study was to test the hypothesis that jaw changes can already be diagnosed in children on routine radiographs.

Material and Methods: 59 orthopantomograms of children and adolescents with NF1 and 59 age-and-sex matched controls were examined for deformities of the mandible and the temporomandibular joint (TMJ) (Female: 24, male: 35; age: 3-18 years (ys); mean age (Both groups): 10.32 ys (Females: 12 ys; males: 9.17 ys). NF-patients were classified according to presence (n=34; (57.63%), FPNF group) or absence of FPNF (n=25 (42.37%, disseminated cutaneous neurofibroma group (DNF group)).

Results: Patients in the DNF group had symmetrical jaw halves andregular shaped articular fossae. Deformations of the jaws and TMJ can only be detected in FPNF group. The findings are unilateral and always localized on the side of the FPNF. Combinations of findings (narrowing of the articular process, deep sigmoid notch between the mandibular processes, flattened articular fossa, open mandibular angle) are essential for the diagnosis.

Conclusion: If FPNF is suspected in children, oral investigation and radiological examination of the jaws should be performed. The combination of typical skeletal findings of the jaws and skull base can both promote diagnosis and reveal the need for treatment of dentoalveolar diseases and skeletal anomalies of the facial skull at an early stage.

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Neo-Ossification of an NF1-Associated Central Giant Cell Granuloma After Repeated Curettage

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Background/Purpose: NF1 is an autosomal dominant hereditary syndromic disease characterized by developmental disorders and a high incidence of neoplasia. In many entities, the histogenesis of NF1-associated neoplasms suggests that they originate from derivatives of neural crest cells. The craniofacial skeleton develops from the neural crest. A rare tumor-like lesion of the jaw, the central giant cell granuloma (CGCG), occurs sporadically, but is also detected in NF1. CGCG is a rare lesion in NF1. Recent studies have shown that autosomal dominant mutations can be detected in sporadic CGCG, which inactivate inhibitors of RAS. CGCG is considered a benign neoplasia. It has recently been shown that NF1-associated CGCG also has a somatic NF1 mutation in addition to the germline mutation. Long-term observations on the course of NF1-associated CGCG do not exist. The case report illustrates the course of treatment over several years.

Case Report: The germline mutation of the NF1 gene was detected in a patient who was 14 years old at the time of initial treatment. A somatic mutation of the NF1 gene was detected in multiple CGCG of the mandible. Surgical treatment consisted of soft tissue removal, marginal bone removal in the cavities, and defect filling with osteogenic material. The lesions soon recurred and required a second curettage. Radiographs a few months later showed marginal radiopacity of the roundish lesion, but still central radiolucency of bone. The patient was then lost to follow-up for several years. When the local findings were checked in the 25-year-old female, tomographs of the mandible showed that the lesions were neo-ossified. Residues of the bone replacement materials were visible in the tomograms.

Conclusion: NF1-associated CGCG can be treated by curettage. Bone replacement materials are integrated into the newly formed bone. Apparently, mechanical cleansing of the bone lesions from the tumor is sufficient to initiate ossification. Early recurrences should prompt short-term revisions of the local findings to disrupt the growth potential of the tumor cells and promote ossification. The report shows that bone-preserving surgery can be effective in the treatment of NF1-associated CGCG. However, it is currently not possible to estimate how long it will take for neo-ossification to occur. Regular checks should be carried out with this therapy.

Neurofibromatosis Type 1-Associated Plexiform Neurofibroma (FPNF): Topography of Lesions and Surgical Treatment

Reinhard E. Friedrich, MD, DMD, PhD, FEBOMFS, *Department of Oral and Craniomaxillofacial Surgery, Eppendorf University Hospital, University of Hamburg, Germany*

Purpose: Facial plexiform neurofibromas (PNF) are rare tumors. PNF are usually diagnosed in patients with neurofibromatosis type 1 (NF1). Facial PNF (FPNF) often grows invasively and destructively, which may complicate surgical treatment. Clinical data on surgical procedures NF1-associated FPNF are scarce. This study details FPNF treatment data from a nationally networked reference center for the treatment of NF1 patients.

Material and Methods: The localization and treatment data of 179 NF1 patients with FPNF were analyzed. Photographically documented tumors of the study area, further determined by imaging, were manually transferred to a facial scheme and digitized. The digitized facial tumor extensions of each patient were overlaid in a single image (Photoshop™). The completed file of the facial scheme contained the sum of the patients' tumor localizations. Then the frequency of tumor localization was indicated with a color code on schematic face drawings (heat map).

Results: The tumors showed no side preference of occurrence. The graphic representations illustrated the need for the treatment of orbital/periorbital and cheek regions. Tumors do not respect anatomical units. However, the division of the face according to dermatomes, especially the trigeminal nerve, offers inferences of tumor spread and guides treatment planning. The mean number of surgical measures per patient was 2.21 (median: 1). Extensive swelling, hematoma, and delayed wound healing were all common postoperative complications.

Conclusion: The color-coded, schematic overview of the frequency distribution of cutaneous tumor spread in NF1 patients with surgically treated FPNF illustrates the main fields needing surgical therapy. The imaging procedure appears suitable for estimating facial tumor growth and the documentation of the post-surgical course.

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Outpatient Clinic for Hereditary Bone and Soft Tissue Tumors for Patients with High Risk of Developing Sarcoma

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Purpose: Some malignant bone and soft tissue tumors occur in hereditary tumor syndrome such as Neurofibromatosis type 1 (NF1), Retinoblastoma (Rb), and Li-Fraumeni syndrome (LFS). The incidence of malignant tumors is high in these diseases. However, there are few facilities in Japan that provide a systematic medical treatment system. The Department of Orthopedics, Okayama University Hospital has established an outpatient clinic specializing in hereditary bone and soft tissue tumors (HBST). This outpatient clinic belongs to the NF1 Japan Clinic Network (NF1-JNET).

Methods: In September 2021, we established an outpatient clinic for HBST such as NF1, Rb, and LFS. The aim of this clinic is to provide (1) genetic counseling and genetic testing for patients and their families, (2) multidisciplinary surveillance for early diagnosis and treatment of malignancy, and (3) social support (such as medical expense subsidy systems, etc.). In addition, selumetinib was administered for plexiform neurofibromas (PN) in NF1. This presentation examines the number of genetic testing, the detection rate of malignant tumors, and the treatment outcomes of selumetinib.

Results: From September 2021 to January 2024, we provided genetic counseling to 93 patients (NF1: 77, Rb: 4, LFS: 5, others: 7). Genetic testing was performed in 38 patients (41%), and 32 patients (84%) had germline pathogenic variants. Some adults underwent annual whole-body MRI surveillance. Two cases of NF1 developed new malignant tumors and were treated (one case each of malignant peripheral nerve sheath tumor and leukemia). Furthermore, five cases of PN were treated with selumetinib, and all cases showed tumor shrinkage.

Conclusions: The establishment of a specialized clinic for HBST allowed for the consolidation of hereditary tumor syndrome such as NF1 and provided continuous follow-up. Selumetinib was effective against PN in NF1. Although the incidence of sarcoma is high in hereditary tumor syndrome such as NF1, the prognosis is expected to improve with the establishment of appropriate follow-up based on evidence.

Previously Unidentified Plexiform Neurofibromas in Children and Young Adults Detected by Whole Body MRI

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Purpose: To describe internal tumor burden in children and young adults with neurofibromatosis type 1 (NF1) with or without clinically known plexiform neurofibromas (PNFs) using whole-body magnetic resonance imaging (WB-MRI)

Methods: This is a prospective cohort study of individuals with NF1 who underwent WB-MRI between 2022 and 2023 at Ann & Robert H. Lurie Children's Hospital of Chicago. WB-MRI studies were acquired as part of the plexiform neurofibroma biomarker study in collaboration with the National Institutes of Health. Images were obtained from neck to toe. PNF size was measured by volumetric analysis and measurable PNF was defined as larger than 3 cm in longest diameter. PNF locations were classified into five anatomical locations. Each participant had a physical examination by a NF1 specialist on the day of scanning or within 3 months prior to MRI. Pictures were also taken for a subset of individuals. Medical and imaging data were collected using REDCap.

Results: Twenty-seven individuals with NF1 underwent WB-MRI (14 female and 13 males assigned sex at birth), 66.6% (18/27) were <18 years old and 33.3% (9/27) between 18 to 33 years old. The median age at WB-MRI was 15 years (range: 3-33 years). Fifteen patients (55.6%) had one or more internal PNFs, while the other 12 (44.4%) did not have any measurable internal tumors. A total of 27 PNFs were identified (mean, 1.8 tumors per individual); 16 were undetectable on clinical exam, and 9 of these 16 were identified in patients younger than 18 years of age at scanning. The most frequent locations of PNFs were neck (N=2), trunk (N=7), upper/lower extremities (N=4), and major nerve thickening (N=3). In one young adult without known PNF the WB-MRI raised concern for malignancy and the patient underwent biopsy that confirmed the diagnosis of malignant peripheral nerve sheath tumor (MPNST).

Conclusion: In a cohort of NF1 patients, 55.6 % had evidence of at least one PNF on screening WB-MRI. 59.2% of these PNF were previously unknown. The most common locations potentially missed during clinical evaluation were paraspinal and major nerve thickening tumors. Remarkably, screening MRI detected one MPNST in an asymptomatic patient without prior history of PNFs. This data supports the need to implement WB-MRI screening of individuals with NF1.

Challenges of Glomus Tumor Management in a Weight-Bearing Digit in a Pediatric Neurofibromatosis Type 1 Patient

Thomas J Geller, MD, *Johns Hopkins All Children's Hospital, St Petersburg Florida, Johns Hopkins University*

Purpose: The purpose of this study is to delineate the difficulties of Glomus Tumor management in Pediatric Neurofibromatosis-1. (NF-1)

Methods: Case review

Summary: Glomus tumors of the digits are a well-recognized complication of Neurofibromatosis type 1 in adult patients. The tumors may occur in various locations in up to 5 % of NF-1 patients, occurring more commonly in adult females during the 4th to 5th decade of life, and presenting with elements of a triad of localized tenderness, severe paroxysmal pain and sensitivity to cold exposure. Glomus tumors in general have a recurrence risk of up to 33 percent in published series of adult patients. They are rarely described in pediatric patients, with the first description of a digital glomus tumor in a child in 2010.

We review a case of an 11year old girl presenting to pain clinic with a severe causalgia in the distal second toe, who had incidentally been noted to have multiple café au lait spots. Her pain was aggravated by weight-bearing, cold air exposure, limiting her participation in school. When referred to the neurocutaneous clinic she had confirmatory genetic testing for NF-1. Her other NF-1 manifestations included, scoliosis, paraspinal neurofibromas, and small facial neurofibromas. Her magnetic resonance imaging of the foot revealed signal changes involving the distal second phalanx with surrounding reactive soft tissue changes of the middle and distal second subungual phalanx, but no underlying osseous erosion. Excisional biopsy-confirmed benign glomus tumor, but was obtained in a fragmented fashion. Her pain failed to respond to neuropathic pain medication and management. Despite resection she developed recurrence of pain within months of surgery and imaging revealed recurrent tumor.

Conclusions: The location of our patient's tumor, difficulties of complete resection without producing motor/sensory deficits or disfiguration, and her clinical course are typical of digital subungual glomus tumors, and she has required management in a chronic pain clinic setting with multiple modalities, upon parental deferral of repeat surgical intervention. The patient, now age 14 years, has expressed an interest in amputation of the distal toe if alternative procedures are ineffective.

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Comprehensive Approach to Adult Patients with Neurofibromatosis Type 1 at the Neurofibromatosis Unit of Italian Hospital in Buenos Aires, Argentina

Valiere Giménez Mauricio, MD, *Italian Hospital of Buenos Aires, Multidisciplinary Unit of Neurofibromatosis, Rare Diseases Program*

Introduction: A multidisciplinary strategy for the comprehensive management of patients with NF1 is presented by the Neurofibromatosis Adult Functional Center at the Italian Hospital of Buenos Aires *of Buenos Aires Argentina.*

Methods: The neurofibromatosis unit of the Italian Hospital of Buenos Aires proposes a comprehensive approach to patients with neurocutaneous diseases. At the initial consultation, the unit coordinator conducts a thorough interrogation regarding the patient's medical history and clinical manifestations. Subsequently, a complete physical examination is performed, and a presumptive diagnosis is established. Subsequently, the team coordinator proposes a specific approach strategy for each patient according to the manifestations and clinical presentation of each particular case. The team consists of specialists who evaluate all patients (neurologist, dermatologist, clinician, and neuro-ophthalmologist) and specialists who are only required for specific cases (orthopedic surgeon, neurosurgeons specializing in the peripheral nerve, pain specialists, psychiatrists, oncologists).

Results: There are a total of 24 patients under observation. The average age is 40 years old (ranging from 33 to 47). There are 13 women (54.2%) and 11 men (45.8%). Of the patients evaluated 83% presented café-au-lait spots as a diagnostic criterion, being the most frequent manifestation, followed by family history, present in 33.3% (8/24). From total patients to clinical evaluation 76.2% showed freckles and 47.6% had Lisch nodules. In NF1 patients with no family history, the most frequent association of clinical criteria was tibial pseudoarthrosis + café-au-lait macules and café-au-lait macules + plexiform neurofibromas. Regarding neurological manifestations 30.4% of patients presented with migraine-like headache characteristics. About 41.7% had neuropathic pain and 8.7% had seizures. Of the total patients 47.8%) had cognitive impairment detected in the bedside examination (MOCA test). Stroke event was detected in 16.7% of patients and one of them had a vertebral artery dissection.

Only one patient had hypertension secondary to pheochromocytoma. Regarding alterations in visual acuity 45% had myopia, while 50% had astigmatism. Scoliosis was detected in 40.9% of the affected individuals and only one had dystrophic scoliosis. Neoplasms were found in 33.3% (8 out of 24) and MPNST was diagnosed in two patients.

Of the 18 patients with superficial NF 33.3% had pain and of the 12 with deep plexiform neurofibroma about 58.3% related pain was associated.

Conclusion: We present this multidisciplinary approach scheme as a way to promote comprehensive care for patients with neurocutaneous diseases.

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Figure :
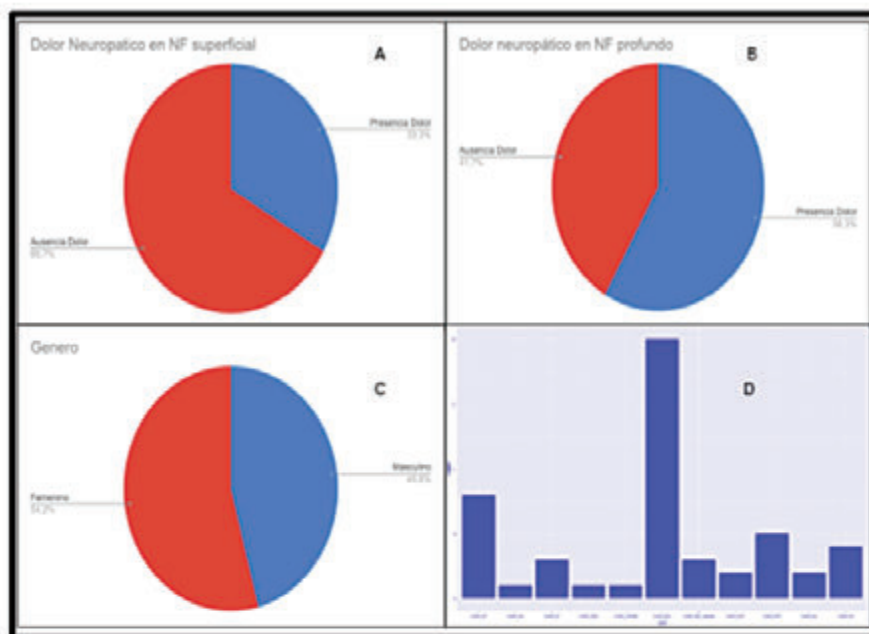
A) Percentage of patients with superficial plexiform neurofibromas and pain. Of the 18 patients with superficial NF, 7 (33.3%) had pain

B) Percentage of patients with deep plexiform neurofibromas and pain. Of the total number of patients with neurofibromatosis type 1, 63.2% presented with deep neurofibroma (12/19). Out of the 12 with deep NF, 7 have pain (58.3%).

C) Percentage of patients by sex. There are 13 women (54.2%) and 11 men (45.8%):

D) Percentage of Clinical Findings by Frequency That Led to Diagnosis. About 83 % (20/24) presented coffee with milk stains as a diagnostic criterion, being the most frequent manifestation, followed by family history, present in 33.3% (8/24). The most frequent diagnostic criteria combination was The most frequent diagnostic criteria combination was café-au-lait macules + Family history of NF1 at 26.1%:

Utilizing Multivariant Brain Analysis for Understanding the Effects of Neurofibromatosis 1 on Neuropsychiatric and Brain Outcomes

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Purpose: This study aims to elucidate the effects of Neurofibromatosis Type 1 (NF1) on neuropsychiatric and brain outcomes through multivariate brain analysis, considering its impact on genetic pathways and neurodevelopment.

Methods: We employed cognitive testing, T1-weighted and diffusion weighted MRI scans to compare neuroanatomical features between children with NF1 and Noonan Syndrome (NS) and typically developing (TD) children. We analyzed variations in cognition, neuropsychiatric conditions and white matter integrity, focusing on specific tracts.

Results: Our findings demonstrate that NF1 and NS, arising from pathogenic variants in the Ras-MAPK signaling pathway, are linked to hyperactivation of signaling pathways influencing neurodevelopment and increasing the risk of autism spectrum disorders (ASD) and other neuropsychiatric conditions. We observed significant neuroanatomical variations and deficits in cognitive flexibility in children with NF1 and NS compared to TD children. White matter integrity was extensively reduced in NS and NF1, with specific tract compromises in the superior longitudinal, uncinate, and arcuate fasciculi in NS and the corpus callosum in NF1.

Conclusions: The distinct tract-specific alterations in white matter integrity for NS and NF1 suggest precise targets for therapeutic interventions. Our study proposes using targeted brain-focused outcome measures for existing Ras pathway treatments, such as MEK inhibitors, offering new directions for treating and managing neuropsychiatric disorders linked to NF1 and NS.

Ganglion Involvement as an Independent Risk Factor for Malignant Transformation of Head and Neck Plexiform Neurofibromas in Neurofibromatosis Type 1

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Background: Individuals with neurofibromatosis type 1 (NF1) are confronted with a 10% lifetime susceptibility to malignant peripheral nerve sheath tumors (MPNST) which is the primary reason for premature mortality in these patients. Clinical evidence suggests that the majority of NF1-associated MPNST originate from preexisting plexiform neurofibromas (PNF). However, it remains uncertain whether the characteristics of PNF are associated with the risk of malignant transformation. Here, this study aimed to investigate the potential correlation between the various characteristics of PNF and the risk of malignant transformation.

Methods: In this retrospective study, patients with head and neck PNF who were hospitalized at Shanghai Ninth People's Hospital between June 2012 and July 2023 were included. Univariable and multivariable logistic regression analyses were performed to assess the associations between clinical features and the progression of PNF. Cox proportional hazards regression and parametric accelerated failure time survival models were employed to quantify the potential effects of variables on the time to malignant transformation.

Results: Of the 470 head and neck PNF, 19 (4.04%) had histological progression. Logistic regression demonstrated that ganglion involvement augmented the risk of malignant transformation in head and neck PNF (adjusted OR, 3.47 [95% CI,1.24 to 9.67]) while the association of PNF progression with growth pattern, tumor morphology, and nerve of origin was not significant. Notably, ganglion involvement significantly shortened the time to histological progression by 35.5% (HR, 8.79 [95% CI, 3.10 to 25.01]).

Conclusion: Ganglion involvement stands as an independent risk factor for the malignant transformation of head and neck PNF. Consequently, patients with this high-risk profile require closer surveillance for malignancies and proactive management of lesions arousing suspicions.

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Accelerated Long-Term Forgetting in Children with Neurofibromatosis Type 1

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Purpose: Investigations into memory among children with NF1 have been limited to the conventional timeline observed in standardised neuropsychological assessments, typically confined to a 30-minute delay. However, due to the shared structural and functional neuropathology between children with NF1 and those with accelerated long-term forgetting, the study was designed to investigate long-term verbal recall.

Method: This study aimed to explore accelerated long-term memory in children by introducing a novel word-list task called the Experimental Word Recall Task, assessing verbal memory at intervals of two-minutes, thirty-minutes, and seven days. Fifty-five children with NF1 (28 males, 27 females; mean full scale IQ=89.64; SD=11.35) and eighty-six neurotypical children (43 males, 43 females; mean full scale IQ=112.19, SD=11.48) participated, undergoing assessments of intelligence and verbal memory. Additionally, parents completed questionnaires, enabling the exploration of correlations between Attention Deficit/Hyperactivity Disorder (ADHD) symptomatology, executive functions, and long-term memory recall.

Results: Considering significant group differences in full-scale IQ ($p < .001$), statistical analyses covaried for full-scale IQ. The study identified accelerated long-term forgetting in children with NF1 at a seven-day delay ($F(1, 138) = 5.374$, $p = .022$), while no group differences were observed at typical time intervals investigated in standard neuropsychological assessments (i.e., two- and thirty-minutes). Notably, accelerated long-term forgetting did not exhibit a significant correlation with ADHD symptomatology or executive dysfunction.

Conclusion: The study suggests accelerated long-term forgetting in children with NF1 compared to neurotypical children, irrespective of levels of ADHD symptomatology and executive dysfunction. This insight challenges conventional perspectives on memory in children with NF1 and emphasises the importance of extending assessment timelines beyond traditional boundaries.

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Comparative Agreement, Feasibility, and Acceptability of Home-Based Telehealth Versus Face-To-Face Pediatric Neuropsychological Assessments: A Within-Person Crossover Study

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Objective: It is unclear whether pediatric telehealth-delivered neuropsychology (teleneuropsychology) assessments provide equivalent outcomes to those obtained face-to-face. This study reports results on (i) the comparative agreement, and (ii) the feasibility and acceptability of teleneuropsychology assessments in children and adolescents.

Method: Using a quasi-prospective repeated-measures AB:BA crossover design, participants (N = 36) with neurofibromatosis type 1, autism, and from the general population underwent both face-to-face and home-based teleneuropsychology assessments using a trained parent facilitator. Measures included Full Scale IQ from the Wechsler Intelligence Scale for Children Fifth Edition, Word Reading, Spelling, and Numerical Operations subtests from the Wechsler Individual Achievement Test–Third Edition, Comprehension of Instructions, Score!, Formulated Sentences, the Rey Complex Figure Test, and the California Verbal Learning Test. Children, parents/carers, and clinicians also completed a feasibility and acceptability survey.

Results: Predominantly high agreement between face-to-face and telehealth intelligence and academic scores were identified from intraclass correlation coefficients (ICCs), independent of age and retest period. ICC values were excellent for Full Scale IQ (0.94), good for all intelligence index scores, academic achievement subtests, verbal learning and expressive language (range, 0.76-0.89), moderate for verbal recall, comprehension of instructions and copy accuracy (range, 0.63 to 0.69), and poor for sustained attention (0.21). Reliable Change Indices revealed stability in tests scores for individuals across testing modalities. Teleneuropsychology assessment was satisfactory according to children, parents/carers, and clinicians, although in-person assessment was preferred.

Conclusions: Findings support the use of home-based teleneuropsychology assessment in pediatric populations.

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Assessing Predictive Associations Between Neurological Factors and Differentiated Attention Functions in Children and Adolescents with Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) is one of the most prevalent rare genetic diseases, confronting patients with numerous medical (neurological, cutaneous, osseous) and neuropsychological symptoms. To identify risk factors and allow for targeted neuropsychological intervention, potential associations between the heterogenous somatic and cognitive phenotype have been proposed.

The present study harnesses the benefits of machine learning analysis to investigate potential non-/linear associations between the medical and neuropsychological profiles of $n=236$ pediatric NF1 patients. A total of 14 models was constructed to predict objective, behavioral, and parent-rated measures of 7 distinct attention domains based on medical characteristics (eg. *CNS tumors, focal MRI signal intensity*).

While the young patients' attention scores differed significantly from the expected, normative values, only behaviorally observed measures of *sustained attention, hyperactivity* and *observed attention problems* could be predicted from the chosen biological factors. Additional information on the location of neurological alterations did not significantly improve the predictions. The most important predictors were *younger age* and *male sex,* with significant *age-sex interactions* in *sustained attention* and overall *observed attention deficits.*

These findings highlight the relevance of attention deficits in pediatric NF1 patients, especially in *young boys* and *adolescent girls.* However, the results indicate the absence rather than the non-linearity of associations between gross NF1-related neurological characteristics and attention skills. Therefore, it is recommended to prioritize *age* and *sex* over neurological characteristics as risk factors when planning neuropsychological interventions to prevent psychosocial consequences of attention deficits on the quality of life of young patients with NF1.

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Outcomes for Paediatric Patients Started on MEK Inhibitors in the North of England

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Aims of the study: Retrospective study of children with NF1 who were considered potential candidates for MEK inhibitors in the North of England.

Methods: Children with symptomatic inoperable (complete surgical resection not possible) plexiform neurofibromas known to the HS Complex NF1 service from 2017 to date were included. Since 2017, 55 children were eligible and discussed in the national MEK inhibitor MDT as per UK pathway or part of a trial. 20 children did not meet full requirements for approval. 8 children were approved but took part in a research trial; their data will not be presented here. 27 children met clinical requirements for MEK inhibitor treatment. 2 patients have not yet started treatment. The main symptom indicators were pain, disfigurement and potential threat to function.

Results: Since 2017, 25 children have been treated with MEK inhibitors. 15 were male and 10 female. The age range at discussion at the National MDT was 1 year to 17 years 1 month, mean age 7 years 9 months and median age 6 years 10 months.

Prior to May 2022, MEK Inhibitors were available in England on compassionate grounds. Since May 2022, NICE approved the use of Selumetinib in England for children 3 years and over. The target locations of the plexiforms were as follows: craniofacial, cervical/neck, spinal phenotype (neurofibromas affecting every spinal nerve root), paraspinal/lumbar//leg, paraspinal/lumbar, pelvis, thorax and foot.

MEK inhibitors controlled severe pain in 7 patients (3 spinal phenotype). Analgesia was stopped in 3 children.

MEK inhibitors for disfigurement were all craniofacial; 7 in total, 2 have had a combination of MEK inhibitors and surgery and 5 have had MEK inhibitors without surgery (1 of which is now listed for surgery).

MEK inhibitors for threat to function were in total 11; 4 for cervical cord compression and 2 for lumbar spinal cord compression and 5 for airway compromise (One child already had a tracheostomy with the plexiform compressing main bronchi.)

None of the children had to stop the MEK inhibitors permanently because of side effects.

Conclusions: MEK inhibitors can significantly reduce pain. Families and health professionals perceived craniofacial plexiforms as particularly disfiguring. Spinal cord compression was most prevalent in the cervical area. Surgery continues to play a vital role in the management of children with symptomatic plexiform neurofibromas. The emerging combined approach of surgery and MEK inhibitors highlights the importance of an MDT approach in the management of these children.

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Successful Outcome of the Use of MEK Inhibitors (Selumetinib) for a Symptomatic Inoperable Plexiform Neurofibroma of the Foot in a Young Child

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Aims: In 2009 NHS England commissioned two Highly Specialised Services for Complex NF1 in England based in Manchester and London.

In May 2022 NICE approved the use of Selumetinib for symptomatic inoperable plexiform Neurofibromas in children aged 3 years and over in England.

This report describes the favourable outcomes for a child who was started on the MEK inhibitor Selumetinib.

Methods/Case: A one-year-old female with complex NF1 (Whole gene deletion) was referred to the Manchester HSS service in 2015. Her history and outcomes are reviewed in this report.

She had a plexiform neurofibroma of the left foot. The child was reviewed regularly by the HSS who worked closely with her local tertiary specialists in Newcastle. Over a few years the plexiform showed progressive growth with pain, disfigurement of the foot and gait alteration. Two features were prominent; the first was of pain on walking, with reluctance to go to school or go out anywhere due to pain. The second was the lifelong difficulty for the family of having to buy the child two pairs of shoes (one size bigger than the other) to provide appropriate fitting footwear for both feet.

The local tertiary specialists and the Manchester NF1 HSS discussed the options in an MDT and following that with the family. It was felt that surgery would not offer any lasting improvement to the child's symptoms and could possibly make things worse.

In May 2022 Selumetinib became available in England and there was unanimous agreement that the child should be put forward as a suitable candidate at the National MEK inhibitor MDT. The main indicator would be pain.

This was approved in February 2023 and MEK inhibitors were started June 2023, the child 9 years of age at the start of treatment.

After just 6 months of treatment, a reduction in size of the plexiform and a complete resolution of pain was experienced. For the first time in her life the child could fit in same sized shoes and the family were delighted.

Conclusion: Plexiform neurofibromas of the foot are notoriously difficult to manage. Surgical interventions are limited and not often helpful. We demonstrate an excellent result from Selumetinib in this child, which suggests that this therapeutic option should be considered more often in this group of children.

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Successful Implementation of Mock Magnetic Resonance Imaging (MRI) in Children with Neurofibromatosis Type 1 Requiring General Anesthesia for MRIs

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Introduction: Magnetic Resonance Imaging (MRI) is frequently used in children with Neurofibromatosis type 1 (NF1). On clinical trials for people with NF1 at the National Institutes of Health (NIH), participants (pts) undergo MRIs every 4-6 months, with a duration of 45-150 minutes depending on body regions included. General anesthesia (GA) is often required for children given the scan length and/or underlying neurocognitive disorders. MRIs obtained under GA require pre- and post- safety evaluations, food restrictions, intravenous catheter insertion, and recovery time. Here we describe results of implementing Mock MRI for pts with NF1 and plexiform neurofibromas.

Methods: We retrospectively collected data on all pts with NF1 who had a Mock MRI at the NIH since March 2019 from the electronic medical record. Mock MRIs were performed by a Pediatric Specialist from the NIH Clinical Center Recreational Therapy Department after consult from the primary team. The therapist performed both initial and in-depth assessments of the pts and families needs (educational, developmental, neurocognitive) and then facilitated an individual session with the use of a non-functional MRI scanner and other age-appropriate educational preparatory materials. The session involved getting to know the equipment and doing a simulated scan. Throughout the session the therapist continually utilized strategies based on the patient's age, anxiety, level of engagement, and processing skills to facilitate the Mock MRI. The therapist taught coping and relaxation strategies during the practice session to support the pts' ability to tolerate the procedure. Information about the session was shared with the primary team and a plan for subsequent non-sedated MRI or additional supportive measures (e.g. repeat Mock MRI, oral anti-anxiety medication) was developed along with the pt and family.

Results: Eleven children and young adults with NF1 (age range 8-24 years, n=6 male) who had each previously required GA for MRI participated in a Mock MRI session. Two pts had an underlying diagnosis of a neurocognitive disorder (ADHD n=1, ADHD and autism, n=1) requiring medication, two pts reported "anxiety" without a formal diagnosis, and two pts were noted by the provider to fidget and have difficulty sitting still during history and physical exam. Two of the 11 pts requested a second Mock MRI session before attempting an unsedated MRI. After participation in at least one Mock MRI session, 10 pts (91%) successfully completed a clinically evaluable MRI without GA. Six pts received an oral anti-anxiety medication (e.g. lorazepam) to facilitate the unsedated scan. Only one pt has required GA for an MRI obtained after the Mock MRI sessions.

Conclusion: Mock MRI sessions facilitated by Recreation Therapy specialists for children and young adults with NF1 previously requiring GA for MRI scans, were feasible and allowed most pts to perform subsequent MRIs without GA successfully. Additional supportive measures such as repeat Mock MRI sessions and/or an oral benzodiazepine were helpful for some pts. The ability to obtain high-quality, evaluable MRIs without GA in these pts has potentially improved safety, decreased cost, and increased efficiency. Based on our single institution experience, Mock MRIs may be a useful tool in facilitating unsedated MRIs for children and young adults with NF1. Further prospective studies are needed to validate this.

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Evaluating Mitochondrial Respiration Dysfunction in Patients with Neurofibromatosis Type 1

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Purpose: Assess the mitochondrial respiratory efficiency in patients with NF1.

Introduction: Neurofibromatosis 1 (NF1) is one of the most common hereditary tumor predisposition syndromes. The clinical phenotype arises from mutations and deletions of the NF1 tumor suppressor gene encoding neurofibromin. Emerging evidence suggests that neurofibromin regulates cellular and organismal metabolism. The decreased respiratory quotients (ratio of eliminated CO_2 to consumed O_2) reported for NF1 patients implicate neurofibromin defects as a source of altered basal metabolic rate).[1] Similarly, knockout models have reduced respiratory quotients, and increased energy expenditures.[2-4] Further analysis of isolated cells lacking neurofibromin show lower basal and coupled respiration, lowered maximal and spare respiratory capacities, and elevated reactive oxygen species, underscoring the importance of neurofibromin on mitochondrial function and its absence causing deregulation of cellular and organismal metabolism related to RAS-pathway dysregulation.

Methods: 44 NF1 patients and 11 chaperons who did not have neurofibromatosis nor Schwannomatosis (control arm) were enrolled in the study. Ten milliliters of the subject's (patient or control) blood were collected for bioenergetics evaluation using Seahorse XF Pro extracellular flux- analysis in peripheral blood mononuclear cells within three hours of their blood collection ever 3 months for 3 times, i.e. at time of enrolment, 3 and 6 months. For NF1 patients, symptoms of pain and fatigue were measured through Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) and Numerical Pain Rating Scale (NRS-11) scales in the 3 visits of the study. Biomedical evaluation with liver function, creatinine kinase, hemogram, and echocardiogram were done.

Results: This study is still underway and results partially representative of the subject population. Figure 1 shows that patients with NF1 have a decreased ratio of oxygen consumption rate (OCR) to extracellular acidification rate (ECAR) when compared with controls ($p = 0.002$, Welch's t-test). Figure 2 shows that the values for OCR and ECAR in patients with NF1 are deviated to a less aerobic, more glycolytic metabolism than the control group. There was no correlation between OCR/ECAR results and the patient's age ($r(29) = 0.045$, $p=0.84$, Pearson test) or gender (t-test, $p=0.986$). Correlation between the OCR/ECAR ratio and NSR-11 or FACIT-F scores are currently underway. OCR/ECAR rations were stable between different collections, except for one patient who started treatment with a MEK inhibitor during this study.

Conclusion: Patients with NF1 have dysregulation in cellular respiration towards a less aerobic more glycolytic pathway. MEK-inhibitors might influence the efficacy of cellular respiration in patient with NF1.

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Figure 1: Oxygen consumption rate (OCR) to extracellular acidification rate (ECAR) ratio in the control in the NF1 patient groups
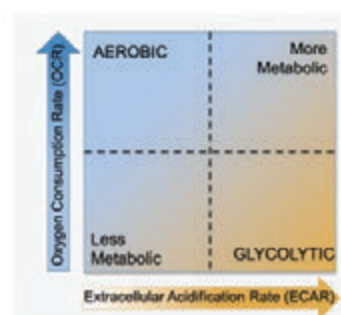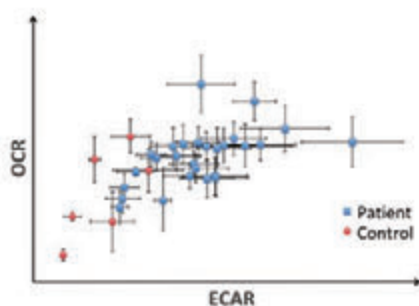
Figure 2: graph for OCR per ECAR for each sample for both groups (NF1 patients and controls) and its meaning regarding aerobic and glycolytic metabolism

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Age-Varying Associations Between Executive Functions and Academic Achievement in Children with Neurofibromatosis Type 1: Integrative Analyses of Data from Seven Institutions

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Purpose: Executive functions (EF) have been shown to predict academic achievement. Yet, we know little about how EF covary with academic achievement at different ages in individuals with neurofibromatosis type 1 (NF1). This study examines the age-varying associations between EF and academic achievement (math, reading, and writing) in children with neurofibromatosis type 1 (NF1).

Methods: Individual-level data of 730 children with NF1 (ages 4-18 years) from seven institutions (see Table 1 for detailed information) were combined following recommended integrative data analysis procedures. Academic achievement (math, reading, and writing) was assessed using the Woodcock-Johnson Tests of Achievement or Wechsler Individual Achievement Test. Executive functions (inhibitory control, flexibility, emotional control, working memory, and planning/organization problems) were assessed with age-appropriate versions of parent-reported Behavior Rating Inventory of Executive Function (BRIEF). Time-varying effect modeling (TVEM) was conducted to examine how executive functions were associated with math, reading, and writing in children with NF1 across ages. TVEM is a nonparametric statistical technique that can flexibly estimate the associations between variables as continuous functions of age. This technique produces curvilinear estimates of the intercept and slope functions with 95% confidence intervals (CIs), without imposing any constraints on the shape (e.g., linear, quadric) of the associations. The estimates are typically summarized graphically. Significant associations between variables are indicated by 95% CIs that do not include zero. Significant age differences are indicated by non-overlapping 95% CIs between specific age points.

Results: Math, reading, and writing scores were all significantly associated with better inhibitory control (math: ages 9-18; reading: ages 11-18; writing: ages 11-17), flexibility (math: ages 7-14; reading: ages 7-13 and 15-17; writing: ages 7-13), emotional control (math: ages 8-17; reading: ages 11-16; writing: ages 10-15), working memory (math: ages 7-16; reading and writing: ages 6-16), and planning/organization (math: ages 7-17; reading: ages 8-15; writing: ages 7-15) across middle childhood to middle/late adolescence. Some significant age trends in the magnitude of the links between EF and academic domains were observed. Specifically, the links between inhibitory control and math (Figure 1) and reading (Figure 2) became significantly stronger from early childhood to adolescence. The strength of the other EF-academic links remained stable across ages.

Conclusions: The relationship between EF and academic achievement varies across specific EF and academic domains and across developmental periods. The results are generally consistent with findings on the EF-academic achievement links in the general population. The findings suggest that EF interventions may be effective in improving academic achievement in children with NF1.

Figure 1. Age-Varying Associations between Inhibitory Control and Math
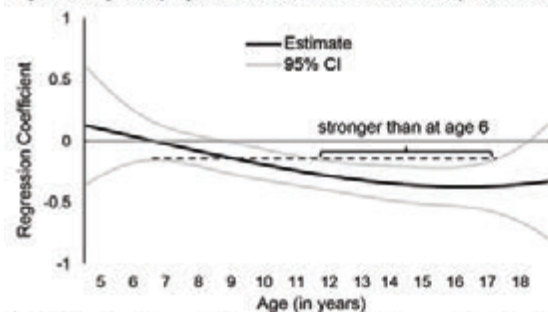
Note. Significant associations between variables are indicated by 95% confidence intervals (CIs) that do not include zero. Significant age differences are indicated by non-overlapping 95% CIs between specific age points. Results related to age 4 were not presented due to inadequate sample sizes, however, the data were retained in the analysis to enhance the robustness of estimated coefficients.

Figure 2. Age-Varying Associations between Inhibitory Control and Reading
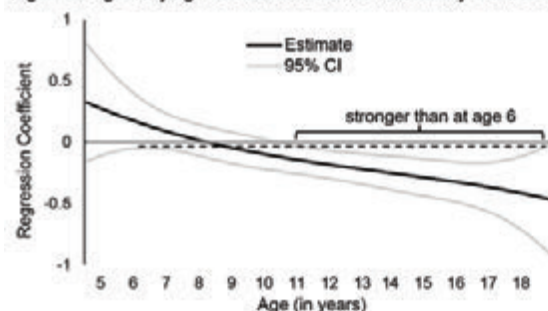
Note. Significant associations between variables are indicated by 95% confidence intervals (CIs) that do not include zero. Significant age differences are indicated by non-overlapping 95% CIs between specific age points. Results related to age 4 were not presented due to inadequate sample sizes, however, the data were retained in the analysis to enhance the robustness of estimated coefficients.

Table 1. Characteristics of Data for Each Site and the Combined Sample

Characteristic	National Cancer Institute, USA	Children's National Hospital, USA	Children's Hospital Colorado, USA	Murdoch Children's Research Institute, Australia	Children's Hospital at Westmead, Australia	Washington University in St. Louis, USA	University of Texas, MD Anderson Cancer Center, USA	Combined
Recruitment population	Convenience sample enrolled in a natural history study recruited nationally (clinicaltrial.gov number: NCT00924196)	Convenience sample recruited through patients seen for clinical evaluation in a pre-clinical appointment survey from a pediatric neuropsychology clinic	Convenience sample recruited through patients seen for outpatient neuropsychological evaluation	Convenience sample recruited on a sequential basis from a multidisciplinary NF1 Clinic	Convenience sample recruited on a sequential basis from a neurogenetics clinic	Convenience sample from NF1 outpatient clinic	Clinical referrals through the NF1 clinic	NA
Academic instrument	WJ-III	WJ-III, WJ-IV, WIAT-II, WIAT-III	WIAT-III, WIAT-IV	WIAT-II	WIAT-II, WIAT-III	WJ-III, WJ-IV	WJ, WJ-R, WJ-III, WJ-IV	NA
Executive function measurement	BRIEF-Preschool, BRIEF	BRIEF-Preschool, BRIEF, BRIEF2	BRIEF-Preschool, BRIEF, BRIEF2	BRIEF-Preschool, BRIEF	BRIEF-Preschool, BRIEF	BRIEF2	BRIEF-Preschool, BRIEF, BRIEF2	NA
Total N.[a]	187	144	140	71	68	67	53	730
Math N.	185	131	139	70	68	56	53	702
Reading N.	186	118	139	71	64	67	53	698
Writing N.	187	85	136	70	66	66	16	626
Inhibitory control N.	187	144	140	71	68	67	53	730
Flexibility N.	187	143	140	71	42	66	53	702
Emotional control N.	187	143	140	71	68	67	53	729
Working memory N.	187	144	140	71	68	67	53	730
Planning/organization N.	186	141	140	71	68	66	53	725
Overall executive function N.	187	144	140	71	68	67	53	730
Age, median (range), y	13.09 (4-18)	9.76 (4-18)	11.54 (4-18)	10.25 (6-15)	10.21 (6-16)	9.67 (5-17)	10.20 (5-18)	11.01 (4-18)
Sex, N.	187	140	140	70	68	67	53	725
Male, N. (%)	113 (60.4)	73 (52.1)	62 (44.3)	39 (55.7)	36 (52.9)	33 (49.3)	29 (54.7)	385 (53.1)
Female, N. (%)	74 (39.6)	67 (47.9)	78 (55.7)	31 (44.3)	32 (47.1)	34 (50.7)	24 (45.3)	340 (46.9)
Race, N.	187	51	137	0	0	0	48	423
White, N. (%)	147 (78.6)	31 (60.8)	120 (87.6)	0	0	0	32 (66.7)	330 (78.0)
Racial Minorities,[b] N. (%)	40 (21.4)	20 (39.2)	17 (12.4)	0	0	0	16 (33.3)	93 (22.0)[b]
Parent education, N.	182	7	128	69	38	32	0	456
Low,[c] N. (%)	62 (34.1)	0 (0)	60 (46.9)	28 (40.6)	11 (28.9)	14 (43.8)	0	175 (38.4)
High,[c] N. (%)	120 (65.9)	7 (100)	68 (53.1)	41 (59.4)	27 (71.1)	18 (56.3)	0	281 (61.6)
NF1 heritability, N.	170	0	137	71	55	66	48	547
Sporadic, N. (%)	105 (61.8)	0	92 (67.2)	47 (66.2)	33 (60.0)	33 (50.0)	32 (66.7)	342 (62.5)
Familial, N. (%)	65 (38.2)	0	45 (32.8)	24 (33.8)	22 (40.0)	33 (50.0)	16 (33.3)	205 (37.5)

Note. NA = not applicable. N. = valid number of participants. NF1 = neurofibromatosis type 1. WJ = Woodcock-Johnson Tests of Achievement. WIAT = Wechsler Individual Achievement Test. BRIEF = Behavior Rating Inventory of Executive Function. [a]The reported sample sizes are based on available data encompassing both executive function and academic achievement variables. [b]Among racial minorities, there were Black/African American (n = 28, 6.6%), Latino/x (n = 16, 3.8%), Asian (n = 14, 3.3%), Mixed/Biracial (n = 5, 1.2%), and Others (n = 30, 7.1%). [c]Low = high school or lower education. High = some college or higher education.

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Epidemiologic and Survival Analysis of Malignant Peripheral Nerve Sheath Tumors: A SEER-Based Analysis

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Background: Malignant peripheral nerve sheath tumor (MPNST) is a sarcoma characterized by poor prognosis, whereas updated epidemiologic and survival data are lacking. Here, we determined to systematically describe the evolving epidemiology and survival for MPNST.

Methods: MPNST in the Surveillance, Epidemiology, and End Results Program database were included. Annual percentage changes (APCs) of incidence and mortality during 2000- 2019 were estimated to quantify the trends. Standardized incidence ratio (SIR) and standardized mortality ratio (SMR) were calculated to quantify the risk of subsequent primary cancers (SPCs) and death by cause.

Results: Among 2645 patients, the overall incidence decreased from 0.14 in 2000 to 0.13 per 100,000 persons in 2019 (APC, -2.2%), which remained constant among the younger and non-White individuals, and those with distant stage at diagnosis. The mortality spiked from 0.02 in 2000 to 0.09 per 100,000 persons in 2008 and stabilized to 2019 (APC, -1.6%). In addition to the grim prognosis with a 47.3% 5-year survival rate, MPNST survivors ran a 1.6-fold higher risk of developing SPCs, including soft tissue, small intestine, brain cancers. (SIRs, 26.53, 13.21, and 9.17). Excess death risk was observed for congenital anomalies, benign tumor and cardiovascular disease (SMRs, 197.21, 66.61 and 1.38), as well as certain SPCs.

Conclusions: The incidence remained constant among those vulnerable groups, who may suffer from neurofibromatosis type 1. Poor prognosis, elevated risk of subsequent tumors and co-morbidities posed challenge to the management. Findings indicated the importance of long-term surveillance and efforts of disease prevention and health promotion for MPNST.

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The Potential Therapeutic Impact on Diffuse Neurofibroma and Optic Pathway Glioma in Patients with Neurofibromatosis Type 1

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Background: Selumetinib, a selective MEK inhibitor to RAS-MAPK overactivation, has been introduced and approved for treating inoperable symptomatic plexiform neurofibroma in pediatric patients with neurofibromatosis type 1 (NF1). Beyond its primary target, our study aims to assess the therapeutic potential of selumetinib for diffuse neurofibroma and optic pathway glioma in patients with NF1.

Methods: In this phase II open-label study, we enrolled 90 Korean patients (60 children and 30 adults) with NF1 and plexiform neurofibroma between May 2019 and December 2021. Selumetinib was administered orally twice daily at doses of 20 or 25 mg/m^2 or 50 mg. For inclusion in this analysis, patients had to have received selumetinib for at least 2 years. Clinical data were collected, and efficacy was assessed based on magnetic resonance imaging (MRI) findings.

Results: The current study assessed 88 patients (29 adults, 59 children), including 79 with classic NF1 and 9 with mosaic NF1, over a median follow-up of 2.8 years (range 1.6-4.5). Selumetinib demonstrated a reduction of over 20% in the baseline tumor volume of the target plexiform neurofibroma in about 92% of all patients. Additionally, we investigated changes in non-target lesions, diffuse neurofibroma, and optic pathway glioma. Among the patients, 29 (33%) had diffuse neurofibroma, with two excluded from analysis due to their combination with target plexiform neurofibroma. Optic pathway glioma was observed in 4 patients (5%). A significant number of patients with diffuse neurofibroma showed a reduction in volume after selumetinib treatment (Figure 1). Patients with optic pathway glioma exhibited some improvement in thickness and signal intensity on MRI (Figure 2).

Conclusion: Selumetinib appears to hold therapeutic potential for diffuse neurofibroma and optic pathway glioma, along with plexiform neurofibroma. This finding may broaden the potential indications for selumetinib in patients with NF1. Further investigations into the therapeutic potential of selumetinib in these indications are planned.

Figure 1. Diffuse neurofibroma in the right scalp (Patient S17)

101.05 mm — 73.59 mm

Baseline — Selumetinib 2Y
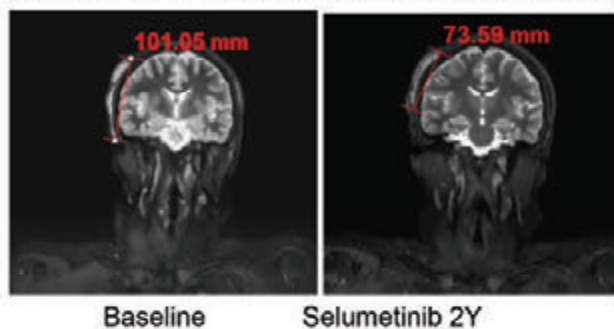
Figure 2. Optic pathway glioma (Patient S11)

Baseline — Selumetinib 2Y
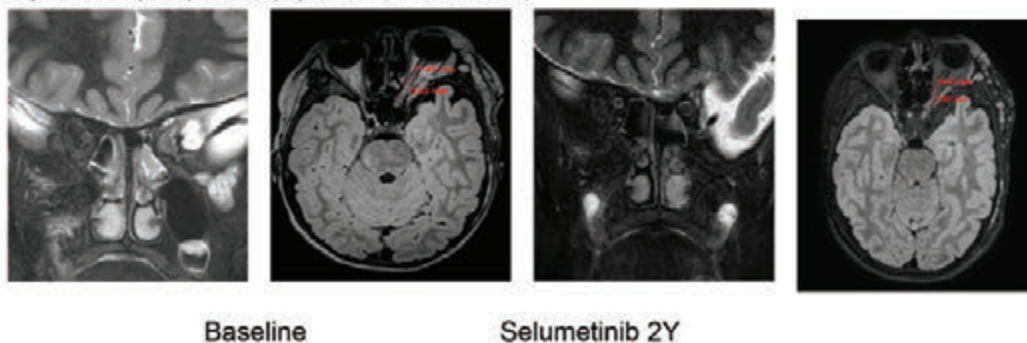
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Evaluation of Symptoms Attributed to Cutaneous Neurofibromas in People with Neurofibromatosis Type 1

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Purpose: This study aims to assess the predominant effects of cutaneous neurofibromas (cNFs) on the quality of life (QoL) in individuals with Neurofibromatosis Type 1 (NF1) through the utilization of the modified cNF-Skindex in a large and diverse cohort.

Background: cNFs are the most common manifestation in people with NF1 with over 98% of affected individuals developing them. Although benign, cNFs frequently have a negative effect on QoL. The validated cNF-Skindex tool specifically evaluates the impact of cNFs on three domains: symptoms, emotions, and functioning. There is a paucity in data of the effects of cNFs in a diverse population of people with NF1.

Methods: Commencing in July 2021, the Neurofibromatosis Therapeutic Acceleration Program initiated a natural history study of cNFs (NCT05581511). The study is recruiting people of all ages with NF1 from across the USA. Participants complete annual visits that include a whole-body 3D photograph and validated patient-reported questionnaires, including the cNF-Skindex. QoL measures are completed by parents of pediatric participants aged 5 years and younger. cNF burden is determined by a clinician and categorized as low (0-10 cNFs), moderate (11-50), or high (>50). Descriptive analysis was employed.

Results: As of March 1, 2024, 357 participants are enrolled (58% female, 42% male) with 347 (97%) evaluable participants completing the cNF-Skindex. cNF burden was classified as: low N=214 (62%), moderate N=58 (17%), high N=75 (21%). Participants representing all skin phototypes were included. 251 (72%) reported concerns attributable to cNFs which resulted in a cNF-Skindex score above 0, with Emotions domain ranked most frequently (N= 234, 67%), followed by Symptoms domain (N=182, 52%) and then Functions (N=179, 52%). Within the Symptoms domain, itching was the predominant symptom (61.4%; mean = 2.7). In the Emotions domain participants were most bothered by the appearance of their cNFs (75.6%; mean = 2.8), and the most affected function was "interactions with others, including family" (51.2%; mean = 2.2).

Conclusion: cNFs have a significant impact on the QoL of people with NF1 affecting all domains of the cNF-Skindex in most participants. The patient-reported data derived from this study offers an in-depth understanding of firsthand symptoms, emotional impact, and functional effects related to cNFs across a large and diverse cohort of participants. This information highlights the need for and importance of further research.

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Cell-Free DNA Targeted Sequencing Reveals Oncogenic Point Mutations and Structural Variants in NF1 Patients with Peripheral Nerve Sheath Tumors

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Purpose: The purpose of this study is to determine if targeted sequencing of NF1 patient plasma-derived cell-free DNA can differentiate patients with Malignant Peripheral Nerve Sheath Tumors (MPNST) from patients with benign Plexiform Neurofibromas (PN).

Methods: Four targeted panels were designed based on prior literature and assessed *in silico* on four publicly available MPNST tumor sequencing datasets totaling 160 samples (Lee, 2014; Cancer Genome Atlas Research Network, 2017; Nacev, 2022; Cortes-Ciriano, 2023). The targeted panel yielding the largest number of mutations overlapping with these datasets was synthesized. Targeted sequencing for single nucleotide variants (SNVs), indels, and structural variants was then performed using this panel applied to plasma cfDNA and peripheral blood germline DNA from 12 MPNST patients and 12 PN patients, and plasma from 12 healthy donors (used for digital error suppression). PACT (A pipeline for Analysis of Circulating Tumor DNA) was used for variant calling and annotation.

Results: *In silico* analysis of the four targeted panels yielded on average 2-3 mutations detected per patient, with the largest panel (287 kilobases) yielding a mean of 3.04 and a median of 3 per patient. Applying this panel to MPNST and PN patient plasma (with peripheral blood germline mutations filtered out) revealed 3.08 and 0.75 coding mutations on average, respectively (P=0.03). Median variant allele frequency (VAF) was 2.54% in MPNST versus 0.66% in PN (P = 0.0011). Using the median VAFs in ROC analysis yielded AUC of 0.74 for discriminating MPNST from PN. Variants detected in MPNST plasma were more enriched for canonical oncogenic mutations (i.e. frameshift *ATRX,* missense *TP53*) compared to those detected in PN (missense *ATM,* missense *NF1*). Among the mutations detected by targeted sequencing in plasma, 68% (25/37) for MPNST and 55% (5/9) for PN were annotated as likely oncogenic by OncoKB. Stringent preliminary structural variant calling resulted in one plexiform patient with indeterminate intronic deletions to MTAP, while structural variants were called in six of twelve MPNST patients including inactivating *CDKN2A* translocations, deletions, and inversions as well as broad chromosome 8q duplication.

Conclusion: Initial results suggest that targeted sequencing of cfDNA can detect likely oncogenic mutations and structural variants specific to MPNST patients that are sufficient to distinguish them from PN patients.

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Updated Finnish NF1 Cohort: Cancer Incidence and Risk for Multiple Cancers

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Purpose: Neurofibromatosis 1 (NF1) is well known for the high cancer risk associated with the syndrome. However, the diagnostic coverage of individuals with mild disease manifestations has increased over the years, and it is unclear whether the previous estimates of cancer incidence and risk are applicable to those diagnosed with NF1 in the present day. We aim to provide updated estimates of cancer incidence in NF1, and to study whether the history of cancer predicts the incidence of new cancers among individuals with NF1.

Methods: The Finnish NF1 cohort has previously covered verified NF1 diagnoses from 1987 to 2011. The cohort has now been extended by searching for NF1-related hospital visits in all Finnish central and university hospitals in 2012-2020. The medical records of each newly identified individual with potential NF1 were reviewed to verify the NF1 diagnosis. Crosslinking of national registers with the NF1 cohort allowed us to study cancers in the NF1 cohort with the follow-up spanning 1987-2020. Cancer incidence was examined by computing standardized incidence ratios (SIRs) relative to the general population cancer incidence rates.

Results: The updated Finnish NF1 cohort encompasses 1,811 individuals with verified NF1 and 30,612 person-years of follow-up. Cancers were observed in 283 individuals with NF1 during the follow-up. The SIR for any first cancer was 4.71 (95% CI 4.18-5.28). Cancers of the brain and central nervous system were diagnosed in 109 individuals with NF1 (SIR 33.1, 95% CI 27.3-39.7), cancers of the peripheral and autonomic nervous system (including malignant peripheral nerve sheath tumors) were observed in 75 individuals (SIR 1,464, 95% CI 1,157-1,821), and breast cancers were diagnosed in 47 individuals with NF1 (SIR 3.30, 95% CI 2.45-4.34). The SIR for a second cancer among individuals with NF1 and a history of cancer was 4.15 (95% CI 3.17-5.33), indicating that those with a history of cancer did not show a significantly different incidence of new cancers compared to individuals with NF1 in overall (P=0.390).

Conclusions: The results increase the accuracy of the previously published estimates of cancer incidence in NF1 and reduce the risk of selection bias arising from hospital-based ascertainment. Having a history of cancer does not seem to be a major predictor of the future risk for cancers in NF1.

Parent-Infant Interaction Qualities in Infants with NF1 and ADHD

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Early parent-child interactions (PCI) promote cognitive, language, and socio-emotional development. However, early features of neurodevelopmental conditions may alter aspects of PCI. While the relation between the parent-child interaction characteristics of infants with an increased likelihood of autism and their autism outcomes in childhood are established in recent studies, research in the context of other emerging neurodiversity conditions, such as NF1 and ADHD, has rarely been studied.

This study aimed to identify whether PCI differs in infants with an increased likelihood of ADHD and infants with NF1 relative to typically developing infants. The sample comprised infants with NF1 (10 months: n = 22; 14 months: n = 26), infants at an increased likelihood of ADHD defined by having a parent or sibling with ADHD (10 months: n=19; 14 months: n=20) and infants with typical development (10 months: n=40; 14 months: 28). PCI data was gathered in a lab setting when either parent played with the infant and recorded for six minutes. These free toy-play video recordings were blind coded using a validated global rating scale (Manchester Assessment of Caregiver-Infant Interaction).

Analysis is ongoing; however, pilot analysis suggests that each group is showing a particular pattern of PCI at both 10 and 14 months, providing a clearer perspective on the parent-infant interactions in infants with NF1 and with an increased likelihood of ADHD. This study's findings could inform early intervention programs and improve parent-child interactions for better outcomes in children with neurodevelopmental conditions.

Surgeries in Individuals with Neurofibromatosis 1 — A Danish Nationwide Cohort Study

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Purpose: Surgical procedures are commonly used in the management of neurofibromatosis 1 (NF1); however, no large studies have yet given a comprehensive description of the surgical procedures performed in individuals with NF1. Thus, we conducted a large population based cohort study to assess surgeries performed and how often they are performed in individuals with NF1.

Methods: The NF1 cohort included 2,452 individuals with NF1, who were alive and living in Denmark on 1 January 1996, from which date we had information on surgical procedures from the Danish National Patient Registry. The individuals with NF1 were matched to randomly selected population comparisons from the Danish Civil Registration System (n=23,987) on sex and birth month and year in a 1:10 ratio. For all study participants, we retrieved information on all surgical procedures in public and private hospitals in Denmark for the period 1996 to 2018 using the Nordic Medico-Statistical Committee Classification of Surgical Procedures (NOMESCO). Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) of surgical procedures were estimated using Cox proportional hazard models. Mean cumulative frequencies (MCFs) were also estimated with time since NF1 diagnosis as the underlying time scale.

Results: We found an increased HR for a surgical procedure of 6.8 (95% CI 5.9−8.0) in the first two months after the NF1 diagnosis, which decreased to 1.5 (95% CI 1.5−1.6) more than seven months after NF1 diagnosis. The HRs were higher in men shortly after NF1 diagnosis (HR 8.2, 95% CI 6.6−10.3) than in women (HR 5.8, 95% CI 4.7−7.1), while a similar HR was seen for both men and women with NF1 more than seven months after the diagnosis of NF1 (HR$_{men}$ 1.5, 95% CI 1.2−1.7; HR$_{women}$ 1.5, 95% CI 1.3−1.7). The mean number of surgical procedures were markedly higher among individuals with NF1 than in the comparisons. Twenty years after NF1 diagnosis, the MCF of surgical procedures were 75 in individuals with NF1, while the mean number of procedures were 20 among the comparisons (Figure 1). Especially women with NF1 had a high number of surgical procedures (Figure 2).

Conclusions: We found that individuals with NF1 have a significantly higher burden of surgical procedures than the general population. Further detailed analyses of specific types of surgeries will be conducted.

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Figure 1. Mean cumulative frequency of surgical procedures for individuals with NF1 and population comparisons by time since NF1 diagnosis/matching date
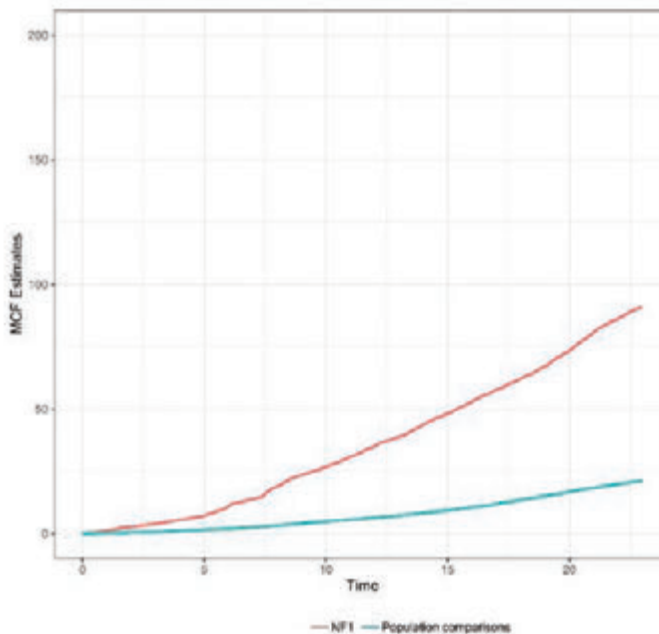
Figure 2. Mean cumulative frequency of surgical procedures for individuals with NF1 and population comparisons stratified by time since NF1 diagnosis/matching date and stratified by sex.
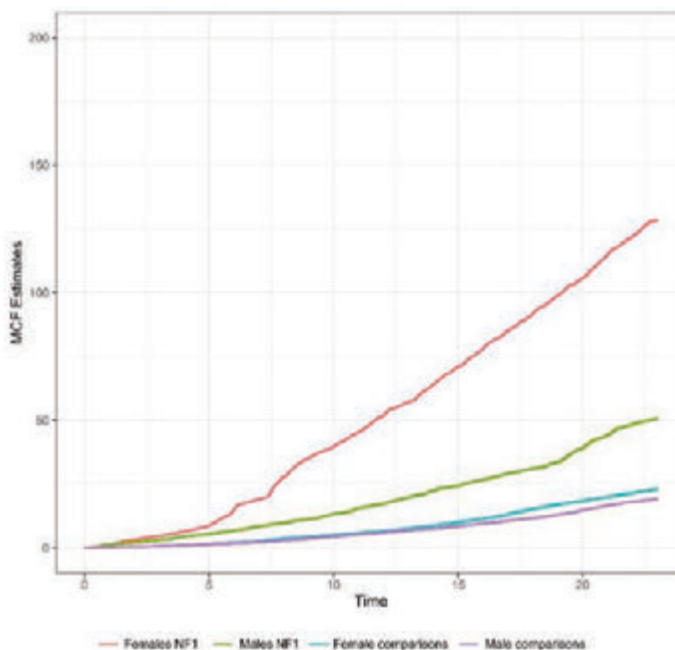
Frequency of Acne and Acne Scars in Patients with Neurofibromatosis 1

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Background: Neurofibromatosis 1 (NF1) is a genetic disorder clinically characterized by cafe-au-lait spots and neurofibromas, but many other comorbidities are also reported. Meanwhile, it is known that certain negative comorbidities, including postoperative hypertrophic scars, are rarely observed in patients with NF1 despite repeated surgery for multiple cutaneous neurofibromas. We have previously demonstrated that patients with NF1 may have specific mechanisms that make them less prone to inflammation serologically. Statistically, patients with NF1 have fewer complications from inflammatory skin diseases such as psoriasis vulgaris and hidradenitis suppurative (M.Koga, et al., J Dermatol. 2014; 41: 885–889., M.Koga, et al., J Dermatol. 2016; 43: 799–803.).

Purpose: This study aimed to reveal the frequency of comedones, inflammatory acne, and acne scars in NF1 patients.

Methods: A case-control study was conducted with 78 patients with NF1 and a randomly selected group of 102 age- and sex-matched non-NF1 controls. The number of each type of lesion on their faces was manually counted and then statistically analyzed.

Results: Multivariate logistic regression analyses adjusted according to sex, age, and the existence of NF1 demonstrated that NF1 was independently associated with inflammatory acne (odds ratio; OR 0.403, 95% Confidence Interval; CI, 0.165~0.981, $p = 0.045$) and acne scars (OR 0.221; 95% CI, 0.089~0.547, $p = 0.001$) although the differences were not significant in comedones.

Conclusions: These findings suggest that NF1 patients form scars less frequently than controls owing to reduced levels of inflammation, possibly due to a loss of function of the *NF1* gene. Recent reports have also indicated adverse effects such as acne-like eruptions with MEK inhibitors approved for plexiform neurofibroma and malignant melanoma. Our new insights, combined with previous research, suggest that the activation of the Ras-MAPK pathway in individuals with NF1 may result in less inflammation of skin lesions.

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Specific for NF1, Based on Social Cognition Deficits, Maladaptive Behaviors as Risk Factors of Difficulties in the Socio-Emotional Functioning

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Purposes: The basic neurocognitive effects of NF1 include impairments in attention and memory, executive functions, language, visuospatial abilities. Among them, social cognition deficits are significant, because cause cognitive disorders and limit in social functioning. Therefore besides specific learning difficulties the main complaints patients with NF1 are problems in social life and lack of participations in the open community activities. Social cognition is defined as cognitive processes related to the perception and understanding of cues in the environment that communicate social and interpersonal information and the ability to use those cues to interpret the thoughts and reactions of others and to modify one's own behaviors to fit the social context. The purpose of the study was to describe cognitive deficits and behavioral phenotype of NF1, especially challenging behaviors and socio-emotional functioning. The second level of analyze was looking for connections between deficits of social cognition and socio-emotional status in NF1. This project is dedicated to clarify the nature of these relations.

Methods: Psychological repeated testing and long-term observation was performed in 100 patients with NF1. Age at psychological diagnosis ranged from 3 to 18 years. Full psychological outcome assessed psychophysical, cognitive, verbal and affective problems associated with childhood manifestations of NF1. The patients were examined using battery of neuropsychological methods, psychological interview and analysis of medical history. For research of social functioning used some subscales of *Vineland Adaptive Behavior Scales* – second edition (VABS-II) and Child Behavior Checklist (CBCL). We assessed also maladaptive behavior in this population as a risk of social rejection, especially ability to controlling anger, tendency to impulsive behavior, temper tantrums, lack of considerations, strange habits, bizarre speech etc. The main point of the assessment was to discover link between social cognition and challenging behaviors.

Results: In the sample of patients with NF1 we observed specific social difficulties associated with emotional disturbances and social rejection. This specific behavioral phenotype and social cognition deficits disturbed learning process and their social life. Patients with NF1 were distinctly ineffective in interpersonal communications and establishing relations in the peer group as well as following the rules of social convention.

Conclusions: The results of our study suggest multiple factors, especially social cognition deficits, are responsible for socio-emotional status of patients with NF1. Draft recommendations for diagnosis of specific emotional problems typical for NF1 behavioral phenotype and program of social development stimulation in NF1 will be presented and discussed.

Clinical Features and Treatment Outcomes of High-Grade Glioma in Children and Adults with Neurofibromatosis Type 1

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Background: The incidence of high-grade glioma (HGG) is elevated in individuals with neurofibromatosis type 1 (NF1) relative to the general population and increases with age. Despite this increased risk, adequately powered studies of clinical features, prevalent treatment approaches, and survival outcomes have not been performed to date, resulting in knowledge gaps potentially limiting improvements in patient care.

Methods: Individuals with NF1 and pathologic and/or molecularly confirmed HGG and high-grade astrocytoma with piloid features at Children's Hospital of Philadelphia, University of Pennsylvania, New York University Langone Health, University of California San Francisco, Washington University/Barnes-Jewish Hospital, Mayo Clinic, and Cincinnati Children's Hospital Medical Center diagnosed between 2005 to 2021 were included. Retrospective clinical, molecular, treatment, and imaging data were integrated and analyzed.

Results: Forty-two subjects met criteria for inclusion, 22 (52%) were male. The median age at diagnosis was 23.8 years and 18 tumors (32%) were located in the cerebellum. The most common diagnoses included glioblastoma multiforme (10), high-grade astrocytoma (8), anaplastic astrocytoma (7), and high-grade astrocytoma with piloid features (5). Five subjects (12%) had cranial radiation exposure preceding the diagnosis of NF1-HGG. Twelve tumors (29%) were considered to have undergone malignant transformation from a pre-existing low-grade lesion. In the 30 cases with molecular testing, the most prevalent genetic variants involved the *NF1, CDKN2A/B, ATRX, TP53,* and *SUZ12* genes. Seven (17%) subjects had a gross total resection of tumor at pathologic diagnosis. Thirty-one individuals (78%) received radiation as part of their frontline therapy and 7 (18%) received a MEK inhibitor as part of initial therapy. Overall survival at 2 years from diagnosis was 52% (95% CI 34,66). There was no difference in overall survival at 2 years by inclusion of radiation therapy as part of frontline therapy (52% with radiation-based regimens vs 50% without radiation).

Conclusion: Neurofibromatosis type 1-associated high grade glioma demonstrate recurrent alterations in the *ATRX* and *CDKN2A/B* genes and individuals with these tumors have unacceptable survival rates despite radiation-inclusive treatment regimens. Collection of data from collaborating institutions is ongoing to expand enrollment numbers and facilitate additional analyses.

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Impact of Neurofibromatosis on Behaviour and Cognition

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Introduction: NF1 is associated with an increased risk for neurodevelopmental disorders such as learning disorders, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD). Reliable estimates of these risks in NF1 are not yet available, due to the scarcity of studies applying standardized assessments in sufficiently large cohorts. We address this issue by conducting a comprehensive assessment of the neuropsychiatric phenotype in children with NF1 and systematically characterizing the prevalence of neurodevelopmental and psychiatric symptoms and diagnoses in children with molecularly confirmed NF1.

Methods: We provided a psychiatric evaluation and a psychoeducational screen for 19 children between 7-12 years of age with NF1 at the Hospital for Sick Children. We quantified the rate of neurodevelopmental and psychiatric pathology both categorically (diagnoses) and on a dimensional level (symptoms). A quantitative level of symptoms was obtained from caregivers for the following questionnaires: Revised Children's Anxiety and Depression Scale (RCADS), Strengths and Weakness of ADHD Symptoms and Normal Behavior Rating Scale (SWAN), and Social Responsiveness Scale (SRS). An intellectual quotient (IQ) level was obtained from the Weschler Abbreviated Scale of Intelligence (WASI). Academic functioning was screened using the Wide Range Achievement Test (WRAT).

Results: Eight children (42%) had ADHD, two children (11%) had an anxiety disorder and two children (11%) had ASD. One child (5%) had a diagnosis of intellectual disability. Three children (16%) had a past diagnosis of a learning disorder. Four children (21%) were referred for a full psychoeducational assessment due to low scores on the WRAT or WASI on the psychoeducational screen.

The mean SWAN inattention symptom score was 1.84 (SD=2.36), and the mean SWAN hyperactive/impulsive symptom score was 2.26 (SD=2.94). The mean RCADS Depression T-score was 51.28 (SD=12.17) and the mean RCADS Anxiety T-score was 48.11 (SD= 11.56). The mean SRS T-score was 56.59 (SD=12.99), and five children (26%) had scores of 60 and above. The mean IQ score was 92.50 (SD=18.98). The mean math computation percentile was 19.88 (SD=22.12), the mean sentence comprehension composite percentile was 33.47 (SD=27.80), and the mean reading composite percentile was 32.90 (SD=26.26).

Conclusions: Our preliminary findings indicate a high prevalence of neurodevelopmental diagnoses in children with NF1, including attention, anxiety, autism, and learning disorders. Our findings also indicate high levels of autism symptomatology. These findings highlight the importance of providing a comprehensive cognitive, developmental, and psychiatric assessment for children with NF1. We plan to continue assessment to reach a larger sample size.

Can Selumetinib Delay or Prevent the Need for Recurrent Surgery in Neurofibromatosis Type 1 (NF1) with a Spinal Phenotype? – A Multi-Disciplinary Approach

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The manifestations of neurofibromatosis type I (NF1) are characterised by constitutive activation of the MAPK pathway, and this plays a central role in the growth of plexiform neurofibromas (PN). Small molecule inhibitors of MEK (mitogen-activated protein kinase, a serine/tyrosine/threonine kinase within the canonical MAPK signaling pathway) (MEKi) have been successfully developed for the treatment of MAPK-driven malignancies, and are now the subject of clinical investigation for the treatment of plexiform neurofibromas. While the activity of MEKi for the treatment of complex PN has been established by a number of prospective trials in paediatric patients, the utility of MEKi in adult patients is less clear. Given the distinct growth dynamics of PNs in adults compared to children, it is important to better define how benefit may be delivered from MEKi therapy.

We present two cases of young adults with a severe spinal phenotype. Both had previously undergone major neurosurgical procedures to relieve cord and nerve root compression related to growing nerve root PN. At the point of further clinical and radiologic progression, multi-disciplinary discussions involving the patients were undertaken. Both were reluctant to undergo further surgery, due to the potential morbidity of further surgery (with risk of spinal destabilization) and interruption to life due to potentially prolonged periods of rehabilitation. A recommendation was made to proceed with MEKi therapy, via an early access programme offered at that time via the manufacturer. The primary aim of this was to reduce threat to function and the clinical/psychological burden further surgery may pose.

The patients (now aged 21 and 29) have been treated with Selumetinib for 16 and 43 months respectively. Both continue to show convincing clinical and radiological evidence of PN response to MEKi therapy. Benefits evident include reduced PN-related pain, visible reduction in size of superficial neurofibromas, generalised small but appreciable reduction in disease burden on whole body imaging, in one case absence of significant neurological worsening, and in the other, marked improvement in neurological assessment. Both have avoided further neurosurgical intervention and NF1-related quality of life has been shown to improve with serial completion of INF1-QoL questionnaires.

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MPNST in Neurofibromatosis Type 1 (NF1) – The Manchester Experience of Survival in the Current Era

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Purpose: The overall lifetime risk of developing a malignant peripheral nerve sheath tumour (MPNST) in individuals with neurofibromatosis type I (NF1) is 8-13%, but those with high internal burden or certain types of *NF1* pathogenic variant are at higher risk. MPNSTs in NF1 are often high grade and associated with a poor prognosis, frequently difficult to diagnose and have limited treatment options. Anatomical location, being within a larger plexiform, and morbidity associated with nerve resection can each hamper or preclude complete surgical excision.

NHS England have commissioned a two centre Highly Specialised Service to provide holistic multi-disciplinary care for complex NF1 since 2009. The primary outcomes being to improve mortality and morbidity of NF1-associated MPNST.

Methods: Evaluation of clinical records of 64 individuals diagnosed with NF1-associated MPNST since 2009.

Results: 30 remain alive. 93% remain alive greater than 1 year following diagnosis. 36% have survived > 5 years since diagnosis and 23% greater than 10 years since diagnosis.

Of the 34 who have died, 50% died less than 1 year following diagnosis of MPNST.

Discussion and conclusion: 47% survival to date for NF1-associated MPNST was observed for patients in this cohort, with high early mortality as has previously been observed. Inoperability at presentation and a lack of effective adjuvant treatments may be implicated in this, but the role of delays in diagnosis is not unclear. Further exploration of this cohort for genotype-phenotype correlation, histological review, and the timeline of interventions for each individual may help us identify prognostic factors and pathways for improving treatment for this challenging problem.

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Impact of Trametinib on the Neuropsychological Profile of NF1 Patients

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Purpose: The use of trametinib in the treatment of pediatric low-grade gliomas (PLGG) and plexiform neurofibroma (PN) is being investigated in an ongoing multicenter phase II trial (NCT03363217). Preliminary data shows potential benefits with significant response in the majority of PLGG and PN and an overall good tolerance. Moreover, possible benefits of MEK inhibitor therapy on cognitive functioning in neurofibromatosis type 1 (NF1) were recently shown which supports the need for further evaluation.

Methods: Thirty-six patients with NF1 (age range 3-19 years) enrolled in the phase II study of trametinib underwent a neurocognitive assessment at inclusion and at completion of the 72-week treatment. Age-appropriate Wechsler Intelligence Scales and the Trail Making Test (for children over 8 years old) were administered at each assessment. Paired t-tests and Reliable Change Index (RCI) analyses were performed to investigate change in neurocognitive outcomes. Regression analyses were used to investigate the contribution of age and baseline score in the prediction of change.

Results: Stable performance on neurocognitive tests was revealed at a group-level using paired t-tests. Clinically significant improvements were however found on specific indexes of the Wechsler intelligence scales and Trail Making Test, using RCI analyses. No significant impact of age on cognitive change was evidenced. Lower initial cognitive performance was associated with increased odds of presenting clinically significant improvements on the neurocognitive outcomes.

Conclusion: These preliminary results show a potential positive effect of trametinib on cognition in patients with NF1. We observed significant improvements in processing speed, visuo-motor and verbal abilities. This study demonstrates the importance of including neuropsychological evaluations into clinical trial when using MEK inhibitors for patients with NF1.

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Effective Connectivity Differences Between Neurofibromatosis Type 1 Patients and Controls During a Working Memory Task

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Evidence demonstrates that approximately Neurofibromatosis Type 1 diagnosis impacts working memory in 70% of patients[1]. This study explored the effects of Neurofibromatosis Type 1 (NF1) diagnosis on effective (directed) connectivity during a working memory task, which involves information storage and manipulation over a short period of time[2]. Neuroimaging data was obtained for 28 NF1 patients and 16 neurotypical controls (age range 11-18 years, 21 males and 23 females). Functional magnetic resonance images were obtained during performance of 0-back and 2- back conditions of verbal N-back task (Figure 1). Dynamic Causal Models[3] of the effects of 2-back task were defined for each participant and the Parametric Empirical Bayes[4] approach was used to compare differences in connections between patients and controls (Figure 2). Very strong evidence (posterior probability >0.99) demonstrated that patients had: i) reduced self-connections of left supplementary motor area and bilateral dorsolateral prefrontal cortex (dlPFC), ii) atypical connectivity between bilateral dlPFC and inferior parietal gyrus (IPG), iii) increased connectivity from left to right dlPFC and from left to right IPG, iv) increased connectivity from bilateral dlPFC and the right IPG to right precuneus (Figure 3).The present study demonstrated that during a working memory task NF1 patients rely on a more rightlateralised endogenous connectivity than controls, particularly connections to right dlPFC, IPG and Precuneus. This suggests that patients are more sensitive to visual characteristics of letters and rely less on their 'inner voice' or articulatory coding of linguistic inputs[5]. Patients have weaker self-connectivity in bilateral dlPFC, suggesting greater inhibition of inputs, which may be related to reduced top-down attentional control frequently reported in this population[6]. Understanding these mechanisms will now motivate development of interventions that target attentional control and verbal rehearsal skills in this patient population Understanding these mechanisms will now motivate development of interventions that target attentional control and verbal rehearsal skills in this patient population. We also found that the patients had weaker connectivity from right IPG to right dlPFC, which typically supports performance under heavy working memory load[7]. This suggests that patients are more sensitive to the effects of working memory load than controls due to differences in interregional brain connectivity rather than region's hypoactivation[8]. This mechanism will be important to consider in further development of pharmacological and non-invasive brain stimulation treatments.

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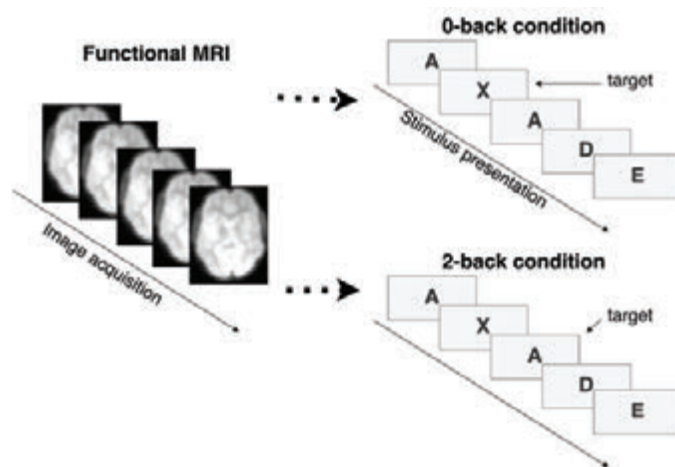
Figure 1 A schematic of fMRI N-back task acquisition. Participants were presented with a series of letters displayed on a screen. During the 0-back condition, the participants responded to target letter 'X'. During the 2-back condition, the participants responded when the current letter matched one presented 2 screens before.
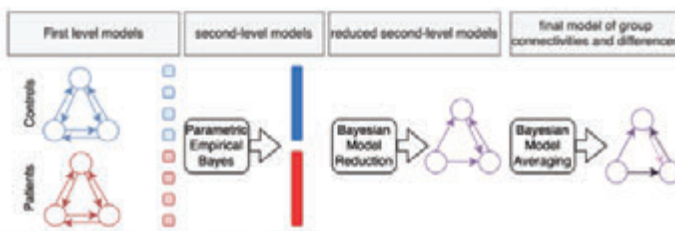
Figure 2 A schematic of first and second level analysis.
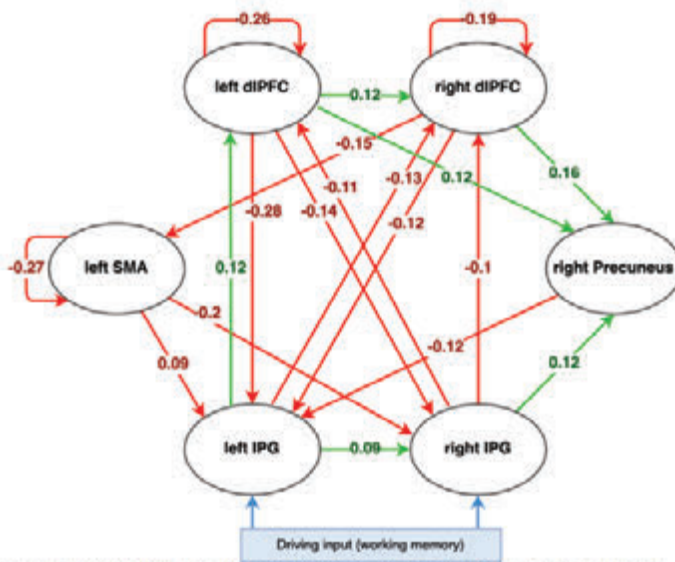
Figure 3 Results of DCM PEB analysis comparing effective connectivity during 2-back task. Positive values (green) reflect stronger connectivity in patients than controls, negative values (red) reflect weaker connectivity in patients.

Age Trends of Internalizing and Externalizing Problems in Children and Adolescents with Neurofibromatosis Type 1: Integrative Analysis of Data from Six Institutions

Dan Liu, PhD, *Florida State University, USA*

Purpose: This study sought to explore how internalizing and externalizing problems in children and adolescents with neurofibromatosis type 1 (NF1) vary across ages and how the age trends differ across demographic and NF1-related disease factors.

Methods: This study used individual-level data of 1,089 children and adolescents with NF1 (aged 3-18 years) that were combined from six institutions (see Table 1) using integrative data analysis. Internalizing problems (anxious/depressed, withdrawn/depressed, somatic complaints, and total internalizing problems) and externalizing problems (rule-breaking behaviors, aggressive behaviors, and total externalizing problems) were measured with the parent-rated Child Behavior Checklist (CBCL). Time-varying effect modeling (TVEM) was used to delineate the age trends of internalizing and externalizing problems for the total sample and for subgroups based on child sex and whether NF1 was inherited (familial) or sporadic. TVEM is a non-paramatric technique that allows for the exploration of time-varying associations without imposing constraints on the shapes of intercepts or slopes. In this study, age was treated as the time variable, and each scale of internalizing and externalizing problems as the age-varying outcomes.

Results: Levels of internalizing and externalizing problems (total and subscale scores) in children and adolescents with NF1 were generally higher than the normative means across ages 3-18. While internalizing problems (total and subscale scores) showed an increasing trend across ages (e.g., Figure 1), externalizing problems (total and subscale scores) showed a decreasing trend across ages (e.g, Figure 2). Based on results from subgroup analyses, these age trends did not differ significantly between males and females, or between individuals with familial NF1 and those with sporadic NF1.

Conclusions: With a large sample, this study revealed strong evidence for elevated internalizing and externalizing problems in children and adolescents with NF1 across 3-18 years of age, as compared with normative means. The findings also indicate that children with NF1 may face higher risks for increased internalizing problems over time, while the risks for externalizing problems decrease, possibly due to brain maturity. These age trends of internalizing and externalizing problems were consistent between males and females and between children with different origins of NF1. The following additional analyses will be conducted, and results presented at the conference: How age trends of internalizing and externalizing problems vary by parental education levels, whether children had plexiform neurofibromas, the severity of NF1, and the visibility of tumors. Together, the findings will illustrate how internalizing and externalizing problems may change across developmental stages and how demographic and NF1-disease related factors are linked to the changes. The findings can inform intervention and prevention programs tailored to individuals' developmental periods and demographic and disease-related characteristics.

Figure 1. Age Trends of Anxious/Depressed Problems
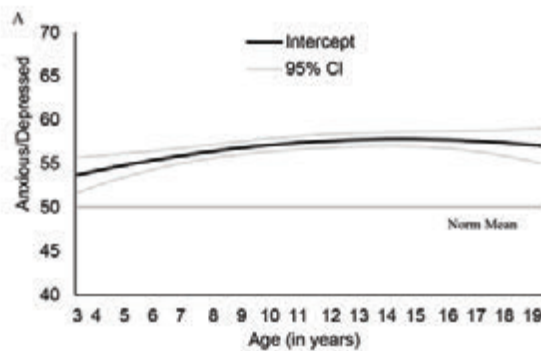
Note. Significant deviations from the normative group are indicated by 95% confidence intervals (CIs) that do not include the normative mean of 50. Significant age differences are indicated by non-overlapping 95% CIs between specific age points.

Figure 2. Age Trends of Externalizing Problems
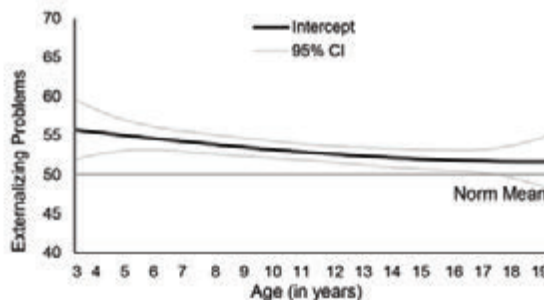
Note. Significant deviations from the normative group are indicated by 95% confidence intervals (CIs) that do not include the normative mean of 50. Significant age differences are indicated by non-overlapping 95% CIs between specific age points.

Table 1. Characteristics of Data for Each Site and the Combined Sample

Characteristic	Children's National Hospital, USA	Children's Hospital Colorado, USA	Murdoch Children's Research Institute, Australia	Children's Hospital at Westmead, Australia	University of California, Los Angeles, USA	University of Texas, MD Anderson Cancer Center, USA	Combined
Recruitment population	Convenience sample recruited through patients seen for clinical evaluation in a pre-clinical appointment survey from a pediatric neuropsychology clinic	Convenience sample recruited through patients seen for outpatient neuropsychological evaluation	Convenience sample recruited on a sequential basis from a multidisciplinary NF1 Clinic	Convenience sample recruited on a sequential basis from a neurogenetics clinic	Referrals from NF1 clinics across Southern California and lab website	Clinical referrals through the NF1 clinic	NA
Measure of Behavioral Problems	CBCL/1.5-5, CBCL/6-18	CBCL/1.5-5, CBCL/6-18	CBCL/6-18	CBCL/1.5-5, CBCL/6-18	CBCL/6-18	CBCL/4-18, CBCL/1.5-5, CBCL/6-18	NA
Total N.	214	32	72	51	83	637	1089
Anxious/Depressed N.	214	32	72	51	83	635	1087
Withdrawn/Depressed N.	189	25	72	51	83	611	1031
Somatic Complaints N.	213	32	71	51	83	635	1085
Internalizing Problems N.	187	32	72	50	83	632	1056
Rule-breaking Behaviors N.	189	25	72	50	83	610	1029
Aggressive Behaviors N.	213	25	72	51	83	635	1079
Externalizing Problems N.	187	32	72	50	83	635	1059
Age, median (range), y	9.58 (3-18)	10.56 (3-18)	10.13 (6-15)	9.83 (3-16)	12.92 (7-18)	11.10 (3-18)	10.83 (3-18)
Sex, N.	210	32	71	51	83	637	1084
Male, N. (%)	114 (54.3)	16 (50.0)	41 (57.7)	24 (47.1)	66 (79.5)	352 (55.3)	581 (53.6)
Female, N. (%)	96 (45.7)	16 (50.0)	30 (42.3)	27 (52.9)	17 (20.5)	285 (44.7)	503 (46.4)
Race, N.	110	32	0	0	83	559	784
White, N. (%)	56 (50.9)	27 (84.4)	0	0	34 (41.0)	386 (69.1)	535 (68.2)
Racial minorities, N. (%)	54 (49.1)	5 (16.6)	0	0	49 (59.0)	173 (30.9)	249 (31.8)[a]
Parent education, N.	7	21	70	31	78	0	207
Low,[b] N. (%)	0 (0.00)	9 (42.9)	29 (41.4)	9 (29.0)	12 (15.4)	0	59 (28.5)
High,[b] N. (%)	7 (100.00)	12 (57.1)	41 (58.6)	22 (71.0)	66 (84.6)	0	148 (71.5)
NF1 heritability, N.	0	30	72	46	0	610	758
Sporadic, N. (%)	0	14 (46.7)	48 (66.7)	27 (58.7)	0	367 (60.2)	456 (60.2)
Familial, N. (%)	0	16 (53.3)	24 (33.3)	19 (41.3)	0	243 (39.8)	302 (39.8)
Plexiform neurofibromas, N.	0	31	51	12	0	637	731
No, N. (%)	0	29 (93.5)	39 (76.5)	9 (75.0)	0	430 (67.5)	507 (69.4)
Yes, N. (%)	0	2 (6.5)	12 (23.5)	3 (25.0)	0	207 (32.5)	224 (30.6)

Note. NA = not applicable. N. = valid number of participants. NF1 = neurofibromatosis type 1. CBCL = Child Behavior Checklist. [a]Among racial minority groups, there were Black/African American (*n* = 112, 14.3%), Latino/x (*n* = 16, 2.0%), Asian (*n* = 14, 1.8%), Native American (Pacific Islander/American Indian) (*n* = 8, 1.0%), mixed/biracial (*n* = 12, 1.5%), and others (*n* = 87, 11.1%). [b]Low = high school or lower education. High = some college or higher education.

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Spinal Cord UBOs in NF1 Pediatric Patients: Single Institution Experience

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Introduction: Neurofibromatosis type 1 (NF1) is a neurocutaneous disease that shows specific clinical characteristics and imaging. Being a predisposition syndrome to diferent tpes of tumors, neurofibromas and glioma of the optic pathway being the most common lesions. It is essential to know the typical, non-oncological lesions that can be present and diagnosed through neuroimaging, so that they are not missdiagnosed and treated incorrectly. Within these lesion we foun UBOs (Unidentified bright objects), located on white matter areas in the parenchyma, but little taken into account at spinal cord level.

Aims: To describe the prevalence of UBOs located in the spinal cord, in patients with NF1 during childhood, based on data obtained at our institution.

Materials and methods: Retrospective, observational and descriptive study of patients between 2 and 21 years old with NF1 diagnosis, evaluated during 2009 to 2023 time period, who had whole brain and spinal cord MRI (n=32). The presence of UBOs was evaluated. We refer to spinal UBO as single or multiple lesions located in the cord, hyperintense on T2/STIR sequences and isointense on T1, without gadolinium enhancement or mass effect.

Results: Male patient 58.3%, with an average age of 13.1 years. Twenty seven patients (79%) had brain UBOs and 9 (26.4%) Spinal cord UBOs. Of the latter, 44.4% presented a single spinal cord lesion, the rest had more than one. The most comon location was the subaxial cervical (C3-T1) area, followed by the cervical superior (C1-C2) area, dorsal with 2 lesions and conus medullaris in only one case. In all of them, hypersignal was found in T2 sequence and isointense signal in T1; no case showed enhancement with intravenous contrast or mass effect. There was coexistence of brain UBOs in all our patients. None of the patients presented clinical manifestations associated with these lesions.

Conclusion: There is little literature regarding the existence of spinal UBOs. It is important to recognize the existence and diagnostic imaging characteristics of these in order to avoid diagnostic and therapeutic errors regarding the presence of spinal cord lesion in NF1 patients.

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Risk of Myocardial Infarctions in Individuals with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) may be associated with comorbidities not yet recognized. Case reports of myocardial infarctions related to coronary artery aneurysms and pheochromocytomas have been reported in NF1, while very few epidemiologic data on the incidence of heart attack in NF1 has been available.

Methods: To investigate the incidence of myocardial infarctions among individuals with NF1, we utilized a comprehensive Finnish cohort comprising 1,811 NF1 patients, drawing on nationwide data. For comparison, we selected a control group at a ratio of 10 persons for each patient, matching them by sex, date of birth, and municipality. We tracked the occurrence of myocardial infarctions using data from the Finnish Care Register for Health Care, which is overseen by the Finnish Institute for Health and Welfare, and the Causes of Death Register maintained by Statistics Finland. The Finnish Care Register for Health Care compiles detailed records of inpatient care and hospital-based outpatient visits. We specifically searched the registers for the ICD-10 codes I21 and I22, along with the ICD-9 code 410, to identify individuals with myocardial infarction.

Results: We observed 42 individuals with myocardial infarction and NF1, leading to a hazard ratio (HR) of 1.4 (95% CI 1.0-1.9) when compared with the matched control group. Six individuals with NF1 died of myocardial infarction with no prior hospital visit related to myocardial infarction. The mean age at the time of the first diagnosis for a myocardial infarction among patients with NF1 was 66.2 years (SD 12.8), while in controls the mean age was 69.7 years (SD 12.5). Among the NF1 patients who experienced a myocardial infarction, 19 were women, with a HR of 1.6 (95% CI 1.0-2.6), and 23 were men, with a HR of 1.2 (95% CI 0.8-1.9). Diagnoses preceding the myocardial infarction in NF1 patients included chronic ischemic heart disease (38%), angina pectoris (19%), disorders of lipoprotein metabolism (19%), heart failure (14%), cerebral infarction (14%), and essential hypertension (29%), yet the proportions did not differ from the controls with myocardial infarction.

Conclusions: Our preliminary findings suggest that at least women with NF1 may be at an elevated risk for myocardial infarction, and the underlying causes of myocardial infarctions in NF1 require further investigation. These results highlight the importance of customized follow-up care for patients with NF1 to better address and mitigate their specific health risks.

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Leveraging Patient Voice and AI Tools in Cross-Country Mapping of Quality of Life and Unmet Needs in NF1

Marina Lutoshkina, *Regional Experts Department, Semantic Hub, Lausanne, Switzerland*

Purpose: Disease burden for patients with neurofibromatosis type 1 (NF1) and their caregivers remains largely unexplored. The aim of this research was to highlight common trends and identify distinctive patient needs in multiple countries. The research scope included impact on quality of life (QoL), fears, unmet needs, and barriers to treatment.

Methods: We used a proprietary artificial intelligence (AI)-based engine for automated understanding and processing of natural language. The technology can analyse large volumes of unstructured data and extract meaningful information to capture the "patient voice". Analysed sources included online patient communities and blogs on social media, as well as health-related question and answer portals. The population analysed included patients with NF1 and their caregivers (Table 1).

Results: Interesting differences were observed among countries in the impact of NF1 on QoL of patients and caregivers (Figure 1). In Mexico, the most prevalent aspect was physical pain (43% of mentions), while in South Korea and Argentina, patients rarely discussed pain. Psycho-emotional problems (35%) were most prevalent in Argentina, while less frequently mentioned in Romania (6%).

Informational unmet needs that patients and caregivers sought to address in online groups differed among countries. Questions about symptoms and their progression were frequently mentioned in Sweden (33%) and Malaysia (25%), while recommendations on expert healthcare professionals (HCPs) or clinics were sought in South Korea (23%), Romania (21.5%) and Argentina (18%).

Access to treatment was a major issue, but barriers associated with flaws in the healthcare systems were also frequently mentioned (Figure 2). Lack of awareness among HCPs about NF1 and lack of available information about NF1 were cited in 27% and 23% of the messages in Sweden and in Mexico, respectively.

Fear of disease progression was prevalent in most countries (22-58% of cases). In Romania caregivers had more concerns regarding their children (37%), such as fear of bullying at school, fear of poor academic performance, but also fear of inheritance (17%). In 4 out of 6 countries, up to 17% of cases mentioned a fear of treatment and procedures (Figure 3).

Conclusions: The use and value of AI technologies is expanding in healthcare. This study showcases the problems, fears, and challenges that patients with NF1 and their caregivers face worldwide. These tools provide an opportunity to improve the patient journey to ensure better and timely care in each country.

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Reference:
1. Evans DG, et al. *Am J Med Genet A.* 2010;152A(2):327-332.

Region	Europe		Asia		Latin America	
	Sweden	Romania	South Korea	Malaysia	Argentina	Mexico
Number of posts	5,667	4,762	65,741	3,092	6,394	5,168
Number of patients with NF1*	435	357	5,474	63	1,196	813
Share of digitally active patients**	12.4%	5.6%	31.6%	0.6%	7.6%	1.9%
Average number of posts per patient	13.03	13.34	12.01	49.08	5.35	6.36

Table 1. Statistics for 6 countries. *Number of patients/caregivers who posted about their NF1 journey. **Percentage of patients who go online to discuss their problems and seek alternate opinions (calculated based on the reported NF1 prevalence of 1/3,000[1]). Data collection occurred between 2017-2022 for Sweden, 2016-2022 for Romania and Argentina, 2016-2021 for South Korea and Mexico, and 2010-2023 for Malaysia.

Figure 1. Quality of life. The number of unique mentions (n) on this topic for each country is indicated in the legend. Psycho-emotional problems includes emotional burden, lack of support, depression and anxiety. School and daycare issues include cognitive issues in children, home schooling, nutrition, routine issues, problems with dressing and mobility. Appearance (incl. clothes) includes public perception, difficulties selecting clothes (comfort vs the desire to hide visual manifestations of the disease). Work-related issues include inability to find a job or being forced to quit a job because of NF1.
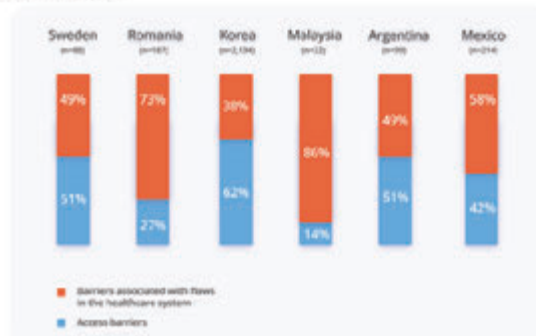
Figure 2. Barriers for treatment and diagnostics. The number of unique mentions (n) on this topic for each country is indicated in the column. Barriers associated with flaws in the healthcare system include lack of specialists, experts, clinics working with NF1, lack of information about NF1. Access barriers include financial burden such as high prices and insurance issues.
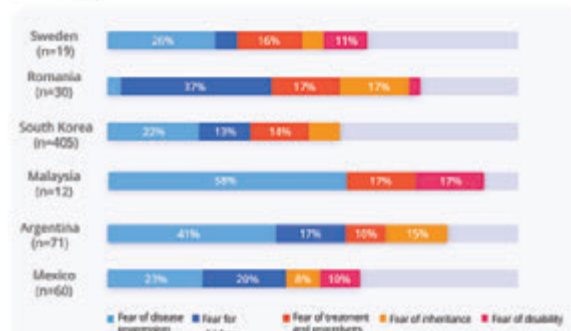
Figure 3. Selected fears and concerns. The number of unique mentions (n) on this topic for each country is indicated in the column.

Pulmonary Valve Stenosis and Missense Variants: Confirmation of a Known Genotype-Phenotype Correlation on a Large Single Center Pediatric Cohort

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Purpose: Ras-MAPK pathway functioning is known to be involved in cardiac development, in particular for endocardial-mesenchymal transition and proliferation[1]. Pulmonary Valve Stenosis is one of the most common Congenital Heart Defects (CHDs) affecting 2-4.7% of NF1 patients, and seems to be associated with non-truncating variants, missense (M) and in-frame indel (I), especially among NF1-Noonan Syndrome (NSNF) patients[2-3].

Methods: We enrolled 440 NF1 pediatric patients (0-18 years of age) with heterozygous *NF1* pathogenic or likely pathogenic variants. Clinical data were extracted retrospectively from the diagnostic records and included in an anonymized file together with the genetic information on NF1 variants. Variants were classified as non-truncating: M and I and truncating: frameshift (F), nonsense (N), silent (S), splicing (L). All patients in our center were evaluated with Echocardiogram to exclude CHDs. The $\chi 2$ test and Fisher's exact probability test were used to compare the frequencies of the independent variables between the NF1 population with PVS and the unaffected group. Odds ratios (ORs) and their 95% confidence intervals (CIs) were reported. p-values of <0.05 were considered statistically significant.

Results: When classifying patients for variants we detected 87 patients with M, 125 with F, 143 with N, 5 with S, 62 with L and 18 with I. Fifteen patients (3.4%) with PVS were detected. Among them 8 (53.3%) with M variants and 1 (6.7%) with I variants. Four patients (26.7%) with F, 1 (6.7%) with N, 1 (6.7%) with L and 0 (0) with S variants. Nine (60%) of patients showed non-truncating variants. M variants showed a strong correlation (p-value <0.001) with an OR of 5.00 (CI 1.76-14.21) showing an implication in PVS development. N variants were rarely associated with PVS with an OR of 0.14 (CI 0.01-1.09) with a p-value of 0.03. As reported in previous literature patients with PVS and NFNS in our cohort showed other features typical of NS[4].

Conclusions: In our cohort more than a half of patients with PVS harbored a M variant. PVS reached a statistically significant association with M variants with an OR of 5.00 (CI 1.76-14.21) showing a causative role, while N variants seems to be rarely associated with PVS showing an OR of 0.14 (CI 0.01-1.09). NFNS phenotype was common among patients with PVS and M variants. Cardiological evaluation with Echocardiogram should be considered for patients harboring non-truncating variants (especially with M variants), due to the high prevalence of this CHDs.

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Tolerability of Long-Term Therapy with Selumetinib in Patients with Neurofibromatosis

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Selumetinib is an innovative drug to control the growth of plexiform neurofibromas. Patients are required to continue taking the medication for an extended period, potentially lifelong. Nevertheless, the potential long-term adverse effects of this prolonged therapy have received inadequate research attention. The drug was prescribed to 104 patients, of whom 55 were under dynamic observation from April 2021 to October 2023. The most common adverse events reported in our patients were skin rash (acneiform/maculopapular or eczematous rash), dry skin, discoloration and hair loss, paronychia, and asymptomatic elevation of CPK levels. Patients with acne were treated with topical corticosteroids with a positive effect. In 6 (11%) patients, grade 3 adverse events were observed, necessitating temporary discontinuation of the medication and subsequent administration of the drug at a lower dosage. The remaining patients experienced grade 1-2 adverse events. One of the cases recorded an previously undescribed complication known as acute hemorrhagic neuroretinopathy in the left eye. This patient was receiving selumetinib for a plexiform neurofibroma in the parotid gland. The tumor visibly decreased while on the drug, but brain stem glioma progressed slowly. Considering the positive effects of the drug, a decision was made to continue administering it at a reduced dosage once the signs of hemorrhage regressed.

Therefore, for the majority of cases, patients undergoing long-term therapy with selumetinib experienced no significant safety concerns. However, additional research is necessary to ascertain the potential for prolonged utilization of this medication.

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Expanding Resources Available to the NF1 Research Community Through the Johns Hopkins NF1 Biospecimen Repository

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Background: Neurofibromatosis type 1 is a prevalent inherited neurogenetic condition that predisposes to the development of cutaneous neurofibromas (cNF), plexiform neurofibromas (pNF), atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP), and malignant peripheral nerve sheath tumors (MPNST). In order to overcome long-standing barriers in NF1 research including access to primary human tissues, cell-based and animal models, the Johns Hopkins NF1 Biospecimen Repository was established in 2016. It continues to thrive as a valuable resource to NF1 investigators and has expanded the scope of available resources.

Purpose: In response to the growing needs of the NF1 research community, the biobank continues to bank tissues, and has also expanded scope with the addition of 1) single-cell suspensions, 2) tissue microarrays (TMA), and 3) genetically diverse cell lines and patient derived xenografts (PDX).

Methods: The biorepository includes clinically and genomically-annotated samples from patients with NF1. Samples include blood fractions, frozen tumor tissues, paraffin embedded tissue, single-cell suspensions, TMA, MPNST cell lines, and PDX. Several novel patient-derived MPNST cell lines have been authenticated and expand the genetic diversity of available cell-based models. In these new cell lines, proliferation was monitored using the IncuCyte live cell imaging, to characterize sensitivity to pharmacologic MEK and SHP2 inhibition. Banked specimens have been genomically characterized using whole exome sequencing (WES) and RNAseq and these data are available through the NF Data Portal. Our database includes NF1-associated clinical symptoms, tumor characteristics, and outcomes data, which are available to researchers upon review of a scientific request and IRB approval.

Results: Since its inception, over 350 unique samples have been banked from 183 unique patients, including pNF (n=89), MPNST (n=62), cNF (n=103), blood fractions, and xenograft (n=5) specimens. RNAseq (n=73) and WES data (n=114) are available through the NF Data Portal. Three novel MPNST cell lines were generated and characterized using MEK and SHP2 inhibitors. A new TMA of cNF, pNF, MPNST, and control tissues has been validated using several key biomarkers. To date, 70 research requests from outside institutions have been granted access to specimens for research interests.

Conclusions: The Johns Hopkins NF1 Biospecimen Repository represents a high-quality, clinically and genomically characterized resource for ongoing scientific efforts in the NF1 research community. To respond to the needs of the scientific community, we have added single-cell suspensions, TMA blocks, and novel MPNST cell lines to our repertoire of resources available for sharing.

Difficulties in the Differential Diagnosis of Nodular Lesions in NF1 Patients

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Nodular lesions (NL) in patients with NF1 are at significant risk for malignant transformation, therefore it's crucial to detect the malignancy at an early stage in order to implement radical surgery, which is the only curative treatment currently available. Diagnostic methods, such as FDG-PET/CT and MRI with apparent diffusion coefficient (ADC) mapping are of great help in this task. However due to various SUVmax cut-off values and potential increased FDG uptake in NL (especially in intermediate tumor, like atypical neurofibroma), sometimes it can be challenging to differentiate benign lesion from malignant. ADC mapping reported to offer higher sensitivity. Here we present two clinical cases of NF1 pediatric patients with NL showing similar clinical behaviour and diagnostic details, but different histology.

1st case: 17yo patient presented with a stable pelvic lesion during 1 year of follow-up with a sudden onset of pain in the lower extremity, 14% enlargement on MRI during 3 months of follow-up and significant ADC restriction along with increased metabolic activity (SUVmax=8) on PET/CT. Diagnostic findings suggested MPNST. An extended surgery was performed, including resection of the roots of the sacral plexus, which led to temporary neurological deficiency. Histological examination confirmed MPNST (grade1, FNCLCC) with negative margins. Up to this moment the patient is alive with 2 years of follow-up.

(Fig.1 ADC mapping on the top left image with red arrow indicates the lesion with low ADC. Other three images demonstrate FDG-PET/CT with increased metabolic activity and the location of the lesion)

2nd case: 15yo patient developed a NL on the right neck within a year, following with rapid growth in the next 6 months. MRI revealed bilateral neck lesions. The largest right-side lesion demonstrated high ADC along with moderately increased metabolic activity on PET/CT (SUVmax=5.7-6.7). Surgery was performed - removal of left and right neck lesions with microsurgical neurolysis of the accessory and vagus nerves, venolysis of the internal jugular vein. Histological examination revealed diffuse neurofibromas.

(Fig.2 FDG-PET/CT on the top images, demonstrating the increased metabolic activity of the lesion. ADC mapping on the bottom right image with blue arrow indicating the lesion with high ADC. MRI image with red arrow demonstrates the location of the lesion)

In the 1st case both diagnostic techniques suspected malignancy confirmed by histology and the extended surgery was reasonable in this case. In the 2nd case ADC mapping showed higher specificity suggesting a benign etiology of the lesion. Nevertheless, extended surgery was carried out, although was associated with high surgical risks due to the tumor location.

It's essential to detect malignant transformation at an early stage in NF1 patients with NL, meanwhile it's important to avoid excessively invasive treatment. ADC mapping reported to be more specific than FDG-PET/CT in this modality. Innovative tools are awaited, however it's essential to run multicenter studies in order to better comprehend the nature of NL.
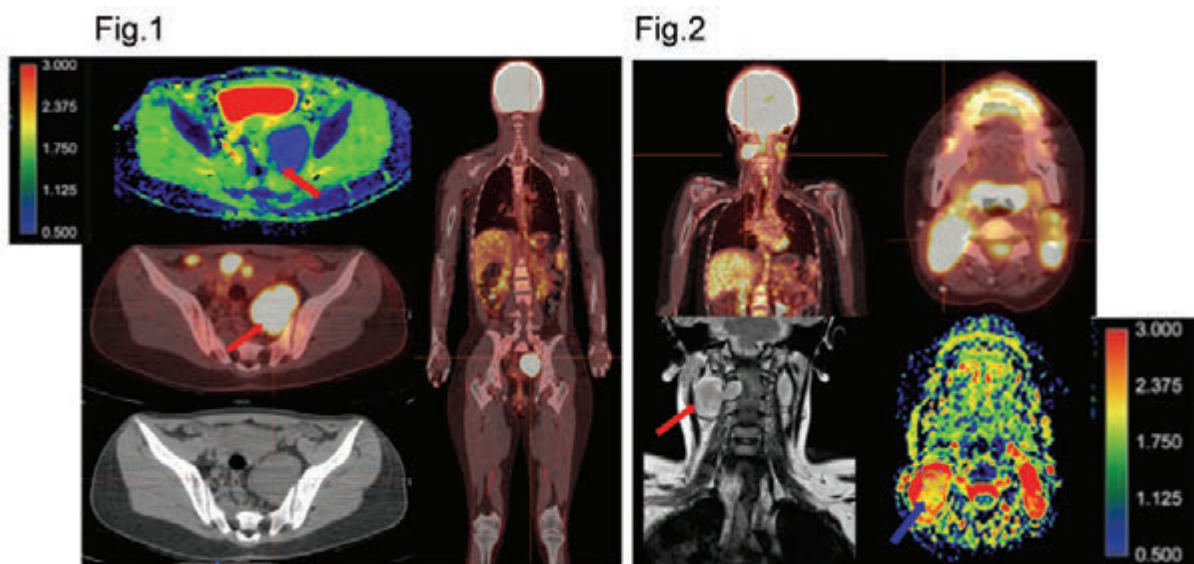
Providing Higher-Quality Care to People with NF1 Who Do Not Attend NF Clinics: Qualitative Interviews with U.S. NF1 Patients, Parents, and Primary Care Providers

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Purpose: The majority of NF1 patients in the United States (U.S.) do not attend specialized NF clinics; this population is less likely to receive NF1-related health screenings recommended by U.S. medical societies. We explored facilitators and barriers to educating NF1 families about NF1 care recommendations and engaging primary care providers (PCPs) to perform recommended NF1 screenings for children and adults who do not have an NF specialist.

Methods: We conducted virtual qualitative interviews with adults with NF1, parents of children with NF1, and PCPs who care for individuals with NF1 from across the U.S. Interviews solicited feedback on a planned educational intervention to improve patient/parents' understanding of recommended NF1 health screenings and PCPs' provision of these screenings during annual wellness visits. Using rapid qualitative analysis, participant input was summarized according to pre-identified domains of interest and aggregated into common themes.

Results: We interviewed 30 NF1 patients/parents who spoke English or Spanish from nineteen U.S. states and 20 PCPs from two U.S. states (Table 1). Patients/parents discussed information they wanted to receive about NF1 care; ways to make this information trustworthy, easy to understand, and visually appealing; and ways to mitigate feeling scared or overwhelmed by this information. Potential barriers to patients/parents using this information included difficulty with online access, lack of trust in their PCP, or not seeing value in preventative care visits for NF1. To overcome these barriers, PCPs and patients/parents recommended having a community health worker or other trusted party available to answer questions about NF1 care, fill out online forms, and help individuals prepare for PCP appointments. PCPs preferred receiving information on NF1-related health screenings tailored to their patient, presented in an easily digestible format, integrated into the electronic medical record, and explaining the urgency of follow-up testing/referrals for abnormal results. Potential barriers to PCPs using this information including time-constrained appointments; unclear division of labor with specialists; and unfamiliarity with how to perform/interpret selected screenings.

Conclusion: U.S. NF1 families and their PCPs largely welcome efforts to improve primary care-based health screenings for NF1. Interviews revealed several ways to make information on NF1-related health screenings understandable and reassuring for patients/parents, as well as quickly digestible and useful for PCPs. Participant feedback will be used to refine educational materials for patients, parents, and PCPs for use in a decentralized randomized clinical trial to improve care for U.S. patients who don't attend NF clinics (NCT06262113).

Table 1. Participant Demographics

	Adults with NF1 (n=16)[1]	Parents of Child with NF1 (n=19)[1]	Primary Care Providers (n=20)
Age (median, range)	40 (19-64 years)	Self: 37 (26-59 years) Child: 8 (0-18 years)	43 (33-61 years)
Female Gender (n, %)	11, 69%	Self: 19, 95% Child: 7, 37%	13, 65%
Race/Ethnicity[2]			
White	6, 38%	6, 32%	15, 75%
Black	3, 19%	2, 11%	2, 10%
Hispanic	7, 44%	10, 53%	2, 10%
Asian	2, 13%	3, 16%	3, 15%
Other	1, 6%	0, 0%	0, 0%
Language Interview was Conducted In			
English	13, 81%	12, 63%	20, 100%
Spanish	3, 19%	7, 37%	0, 0%
Had not been to an NF Clinic within past 3 years[3]	16, 100%	14, 74%	N/A
Number of NF1 Patients (median, range)	N/A	N/A	2 (1-10)
Practice Type: n, %	N/A	N/A	
Academic Medical Center			4 (20%)
Academic Satellite Sites			5 (25%)
Community Health Center			7 (35%)
Private Practice			4 (20%)

Notes: [1]Five interviewees were both an adult with NF1 and a parent of a child with NF1 and so are counted in the totals for both groups. [2]Individuals could select more than one race/ethnicity, so totals do not sum to 100%. [3]To increase recruitment of Spanish-speaking participants, prior attendance at an NF clinic was allowed for Spanish-speakers only.

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Surveillance and Treatment of NF1 in Outpatient Clinic for Hereditary Bone and Soft Tissue Tumors

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Purpose: Patients of NF1 suffer from various malignancy such as malignant peripheral nerve sheath tumor (MPNST) and breast cancer. The patients frequently have metastases at their presentation of malignancy. However, the evidence-based recommendation of surveillance has not been established. We established the outpatient clinic of hereditary bone and soft tissue tumors in September 2021. We provided genetic counseling and genetic testing for patients and their families and performed multidisciplinary surveillance for early diagnosis and treatment of malignancy.

Methods: From September 2021 to January 2024, we provided genetic counseling to 80 patients of NF1. Surveillance with annual whole-body MRI was performed in 55 adult patients. We examined the rate of malignancy among these patients.

Results: Two patients developed new malignant tumors and one patient developed recurrence of MPNST after the treatment. The one patient had general fatigue and anemia was detected during a medical examination. The patient visited a nearby doctor, and a thorough examination revealed the leukemia. The patient was referred to a specialist hospital and underwent chemotherapy. The other patient had large neurofibroma in the popliteal region at the first presentation. The patient received Surveillance with annual whole-body MRI. The tumor become larger and the pain got worse. Open biopsy revealed MPNST. PET/CT revealed no metastases. The patient underwent chemotherapy and surgical resection has been planned.

Conclusions: Counseling and surveillance by whole-body MRI may be effective for early diagnosis and early treatment of malignant tumors in patient with NF1. Longer-term follow-up is required to assess the effectiveness and frequency of surveillance.

Establishment of a Pilot Program for Transitioning NF1 Patients from Pediatric to Adult Care

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Purpose: Neurofibromatosis type 1 (NF1) is a complex, multi-system disorder. A variety of potential complications can develop over the life span, often requiring coordinated multi-disciplinary care by clinicians with experience and knowledge of comprehensive NF1 care. Children's hospitals have long been the center for coordinated care of children and adolescents with complex genetic disorders. Transitioning to adult care can be challenging, and collaboration of pediatric and adult providers would be essential to a successful transition process. The CHLA pediatric and the UCLA adult NF clinics describe their collaboration in a pilot project for transitioning patients.

Methods: In October 2022, the CHLA pediatric NF clinic and the UCLA adult NF clinic established a pilot to transition young adults to UCLA for ongoing care. NF clinic nurse coordinators were put in place in both institutions to oversee the process. NF1 patients 18 years and older were identified at CHLA and comprehensive medical needs were assessed. Patients were then referred through the Center for Healthy Adolescent Transition (CHAT) to coordinate insurance authorization and medical records transfer. A warm transfer between clinic coordinators then occurred prior to a new patient being seen at UCLA. At UCLA, we reviewed our records and referrals of adult patients with NF1 to determine barriers for transitions and most common concerns.

Results: A framework was developed for transitioning patients between the two institutions. Since October 2022, 11 patients were referred and successfully transitioned from a pediatric center. The median age at transition was 26. Upon transfer, a care plan according to published NF1 care guidelines and necessary subspecialist referrals were established. As expected, the most common barriers include aligning insurance, reluctance of families to transition, youth being unprepared to manage their own health care and geographic challenges. In total, 80 new adult patients with NF1 established care at UCLA. The most common concerns for patients in the UCLA adult NF clinic include pain, psychosocial and cognitive issues, and disease surveillance.

Conclusions: This pilot program highlights the importance of aiding young adult patients with NF1 in the process of moving from a child/family-centered model of health care to an adult/patient-centered model. Clinic coordinators have played a critical role in the success of this project. We anticipate an ongoing relationship between the two institutions with the goal of providing the best quality of life for this patient population across the lifetime.

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Malignant Peripheral Nerve Sheath Tumors, a Malignant Transformation From a Plexiform Neurofibroma in a Pediatric Patient; A Case Report

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Malignant peripheral nerve sheath tumors (MPNST) is a rare, aggressive soft tissue sarcoma of neural origin posing tremendous challenges to effectively treat. MPNST can arise spontaneously or from plexiform neurofibroma (PN), a benign tumor associated with neurofibromatosis type 1 (NF-1). Radical surgical resection in early disease is the standard of care for MPNST with adjuvant therapy when resection cannot be accomplished. Without radical resection, prognosis is generally poor due to the low response rate to other treatment modalities. We present a 5 year-old-male with NF-1 and extensive plexiform neurofibroma treated with selumetinib who developed malignant transformation to MPNST.

He was diagnosed with an extensive plexiform neurofibroma of lower abdomen, pelvis and upper bilateral lower extremities on selumetinib, MEK1/2 inhibitor, since January 2021. On March 2023, scan report no new lesions or tumor growth. Three months later, he presented to the emergency room with abdominal distention of 4 weeks of evolution, pain, weight loss, unable to walk. Laboratory evaluation revealed hemoglobin 8.9gm/dL; abdomopelvic CT scan and MRI demonstrated a large, bulky, lobulated with central necrotic component measuring 14 x 12 x 10 cm. A biopsy of the tumor confirmed the diagnosis of MPNST. Selumetinib was discontinued. Due to the size of tumor, neoadjuvant chemotherapy with ifosfamide and doxorubicin was initiated. Despite 2 cycles of chemotherapy, the tumor did not respond so radiation therapy was offered with some decreased in size with improvement of symptoms associated with the large compressive abdominal mass. Subsequently he was started on pazopanib, a growth factor receptor inhibitor associated with angiogenesis and tumor cell proliferation to try to decrease tumor progression.

MPNST could be difficult to manage due to the aggressive behavior and poor response to standard therapy, limiting its management and outcome. Transformation of PN into MSPNST in children while on selumetinib has not been reported in the literature. MPNST transformation should be suspected in a rapidly growing PN, even while treated with MEK inhibitor.

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Establishment of Comprehensive Multidisciplinary Medical Care Facilities for NF1 Patients and Their Families in Japan (NF1-JNET)

Yoshihiro Nishida, MD, PhD, *Department of Rehabilitation Medicine, Nagoya University Hospital; President, The Japanese Society of Recklinghausen Disease*

Purpose: In Japan, there were very few facilities capable of providing comprehensive care by multidisciplinary team (MDT) for neurofibromatosis type 1 (NF1) patients and their families. Since 2014, the presenter's facility, Nagoya University Hospital, has been providing multidisciplinary medical treatment to NF1 patients and their families, and reporting on the results[1]. Following the Nagoya University method and the nationwide comprehensive care for NF of the The NF Clinic Network (NFCN) established by the Children's Tumor Foundation (CTF), the Japanese Society of Recklinghausen Disease (JSRD) decided to expand the MDT treatment system for NF1 patients throughout Japan.

Methods: The committee urged members of JSRD to establish MDT treatment systems for NF1 patients and families at their facilities. Facilities that have established a medical treatment system based on MDT could apply to the JSRD, and if the conditions are met, the facilities will be approved by the JSRD committee. Approval requirements are shown in Table 1.

Results: As of February 25, 2024, there are 7 facilities certified by JSRD that can perform MDT for NF1 (NF1 Japan Clinic Network: NF1-JNET) (Figure 1). Currently, facilities in the northern and southern regions are preparing to apply.

Conclusions: In Japan, MDT treatment for NF1 patients and their families had been facility-dependent, and the quality of the treatment had not been guaranteed. Information regarding MDT treatment for NF1 was not disseminated nationwide. Like the NFCN established by CTF, efforts are being made in Japan to increase the number of facilities that can perform MDT for NF1 and improve the quality of medical treatment. It will be necessary to further improve the level of medical care through conferences held by all MDT-approved facilities.

Table 1. Certification requirements for facilities where MDT can provide NF1 treatment.

Extensive knowledge and experience regarding NF1 treatment
NF1 medical treatment system based on multidisciplinary collaboration
Both pediatric and adult NF1 treatment system
Abundant number of NF1 patients (annual)
Active research activities related to NF1: Papers and conference presentations
Includes members of the Japanese Society of Recklinghausen Disease

Fig.1. NF1-JNET

● Approved facilities
- Niigata Univ.
- Keio Univ.
- Tokyo Medical Univ.
- Shinshu Univ.
- Nagoya Univ.
- Okayama Univ.
- Tottori Univ.

Directors of The Japanese Society of Recklinghausen Disease: Hideyuki Saya, Toyoko Ishiji, Shinichi Imafuku, Arihito Ota, Hiroyuki Kawashima, Kazuo Kishi, Kenjiro Kosaki, Takaya Goto, Akihiro Sakurai, Toshiyuki Seto, Toshiki Takenouchi, Katsumi Tanito, Toshiro Nishida, Muneaki Matsuo, Yuichi Yoshida

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Observational Study of Selumetinib in Chinese Pediatric Patients with NF1-PN

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Aim: Here we present a study design, the rationale of the study is to expand understanding of disease characteristics and treatment pattern of Chinese pediatric patients with neurofibromatosis type 1 (NF1) -related plexiform neurofibromas (PN) and to evaluate effectiveness and safety of selumetinib for these patients in a real-word setting.

Material and Method: This is a prospective, multicenter, observational study that will be conducted at approximately 12 centers in China. Approximately 80-100 eligible pediatric patients with NF1 aged 3-16 years who have symptomatic, inoperable PN and intend to use selumetinib will be enrolled in the study. The decision to treat with selumetinib will be made by the treating physician per standard of care prior to participant enrollment in this sub-study. Patients will receive $25mg/m^2$ selumetinib orally twice daily until disease progression, unacceptable toxicity, risk, no longer benefit from treatment under the judgement of physicians, or reaching the end of 24-month follow-up period. Data will be collected from medical records, laboratory reports, imaging results, and patient-reported outcomes via patient/caregiver's self-report forms at each clinic visit. All data will be entered at the site directly into an electronic data capture system by trained study staff. All statistical analyses will be performed using SAS Version 9.4 (or later) statistical software.

Results: The primary objectives are to describe NF1-PN patient demographics and disease characteristics, treatment profile, effectiveness including qualitative assessment of disease status by physicians and NF1 disease status, and safety outcomes of selumetinib. The secondary objectives are to assess tumor activity of NF1-PN upon discontinuation of selumetinib, to describe the course of patients' disease and treatment, caregiver- or patient-reported treatment adherence and whether patients treated with selumetinib have a clinically meaningful decrease in pain intensity .

Conclusions: The data obtained from this study will contribute to a better understanding of the effectiveness and safety of selumetinib in Chinese NF1-PN patients and provide insights on disease characteristics and treatment patterns in this population.

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High Resolution Nerve Ultrasound in Neurofibromatosis Type 1

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Background: High-resolution nerve ultrasound (HRUS) can be used to visualize peripheral nerve sheath tumors (PNST) in neurofibromatosis type 1 (NF1) patients.[1] The aim of this study is to describe HRUS abnormalities in NF1 patients, with or without symptoms related to the peripheral nervous system (PNS), and relate them to nerve function by nerve conduction study (NCS). In addition, we explore the potential value of HRUS in clinical practice as a screening tool for PNST.

Methods: Sixty patients with a clinical diagnosis of NF1 were invited for a visit including clinical examination, NCS and HRUS. Patients were split into two groups: with PNS related symptoms (PNS group) and without PNS related symptoms (non-PNS group). We performed a one-sided NCS protocol of the median, ulnar and radial nerve (motor and sensory), peroneal and tibial nerve (motor) and sural nerve (sensory). HRUS was used to visualize the brachial plexus, median-, radial-, ulnar-, peroneal-, tibial-, and sural nerves two-sided. We measured the cross-sectional area (CSA) of the nerve at predefined anatomical sites and additionally at sites of focal nerve enlargement. The pattern of HRUS findings was categorized per patient as: 1) normal, 2) nerve enlargement(s) with normal nerve morphology, 3) focal PNST and 4) continuous PNST.

Results: Thirty-seven (62%) patients had PNS-related symptoms. HRUS was abnormal in 52 (87%) patients (PNS n=34, 92% vs non-PNS n=18, 78%; p=0.240). Figure 1, panel A shows a normal segment of the median nerve at 1/3 of the left forearm (CSA 5mm^2), panel B shows a PNST at the same location (CSA 71 mm^2, 789% of the upper limit of normal). The most common HRUS pattern was continuous PNST (n=26, 43%), which was found to be a very distinct phenotype compared to other HRUS categories. The distribution of HRUS patterns was similar between the groups. NCS was abnormal in 20 (33%) patients, more often in the PNS group (PNS n=17, 46% vs non-PNS n=3, 13%; p=0.011). NCS was normal in 65% of the patients with HRUS abnormalities.

Conclusions: PNSTs are commonly seen with HRUS in NF1 patients, often without symptoms or abnormalities on NCS. HRUS can potentially be useful in screening NF1 patients. A clear distinction could be made between patients with continuous PNST and other HRUS patterns. Patients with continuous PNST pattern are likely to have a higher risk of developing malignant PNST and should therefore be closely monitored.

Figures
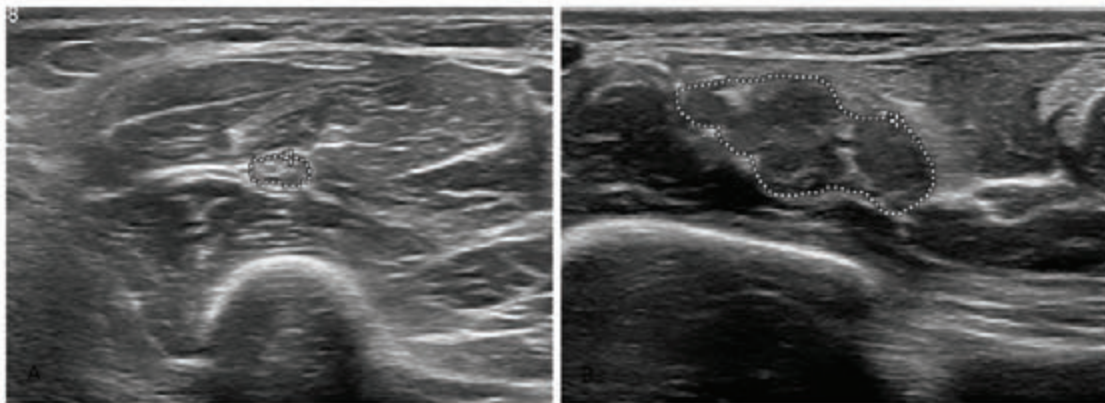
Figure 1: Median nerve CSA at 1/3 of the left forearm in two patients

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Efficacy and Tolerability of Radiation, Temozolomide Plus MEK Inhibitors for Newly Diagnosed High Grade Gliomas in People with NF1

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Background: People with neurofibromatosis type 1 (NF1) carry an increased risk of developing gliomas, including low- and high-grade gliomas (HGGs). MEK inhibitors have emerged as a treatment option for NF1 patients with low-grade gliomas such as optic pathway glioma. However, no studies to date have defined a treatment regimen for NF1-associated HGGs. In patients with sporadic glioblastoma, standard treatment includes daily temozolomide with involved field radiation therapy (IFRT), followed by six monthly cycles of adjuvant temozolomide. There is little published data on the tolerability and efficacy of adding MEK inhibition to standard treatment in people with NF1 and HGG. In a retrospective study of 45 people with NF1 and gliomas, no complete responses to treatment were reported *(Romo et al., Neuro-Oncology, 2023).* The purpose of this study was to review our institutional experience in managing patients with NF1-as-sociated HGGs with combination radiation therapy, temozolomide, and MEK inhibitor.

Methods: We retrospectively identified 3 patients with NF1-associated HGGs who were treated with 3 or 6 weeks of concurrent temozolomide (75mg/m2/day) and IFRT, followed by six cycles of monthly temozolomide along with a daily MEK inhibitor. We evaluated treatment response using Response Assessment in Neuro-Oncology (RANO) criteria for HGG, toxicity using Common Terminology Criteria for Adverse Events (CTCAE v5.0), and duration of treatment as a measure of tolerability of this regimen.

Results: Patient demographics and tumor characteristics are shown in table 1. Patients 1 and 3 underwent biopsy of the enhancing tumor while biopsy was deferred in patient 2 due to tumor location in the pons. Patient 1 received trametinib, and patients 2 and 3 received selumetinib (table 1). The most common adverse events during the concurrent treatment phase were fatigue, nausea, and myelosuppression (all grade 1), similar to the side effect profile seen in patients with sporadic HGGs. Side effects seen during the adjuvant phase were acneiform rash, myelosuppression, and retinopathy (table 1). Two patients had a complete radiographic response (CR) after 11 and 19 months of treatment. Patient 3 had a partial radiographic response (PR) after 13 months of treatment. No patients discontinued treatment because of adverse events, and two patients remain on treatment, 16 and 20 months after treatment initiation. One patient stopped treatment at the time of tumor progression, 24 months from the time of diagnosis. All patients remain alive.

Conclusions: MEK inhibitor therapy added to adjuvant temozolomide following chemoradiation in NF1-associated HGGs was well tolerated, associated with partial and complete radiographic responses not typically seen in sporadic HGGs, and led to sustained disease control. Larger systematic studies are needed to confirm the efficacy of this regimen for NF1-associated HGGs.

Table 1. Patient demographics, tumor characteristics, and treatment.

	Patient 1	Patient 2	Patient 3
Age, sex at diagnosis	24 years, female	31 years, female	56 years, female
Karnofsky Performance Score (KPS) at diagnosis	100	90	50
Pathology	NF1 associated glioblastoma, Grade IV Ki-67 50% ATRX retained P53 strongly positive IDH negative MGMT negative	No tissue collected	NF-1 associated glioma with pilocytic features and high grade features, not elsewhere classified Ki-67 4.1% ATRX retained p53 positive CDKN2A loss IDH negative MGMT negative
Initial chemoradiation	6 weeks of concurrent RT (60Gy) + TMZ 75mg/m2/day	6 weeks of concurrent RT (55.8 Gy) + TMZ 75mg/m2/day	3 weeks of concurrent RT (40 Gy) + TMZ 75mg/m2/day
Adjuvant temozolomide (1 month after radiation)	TMZ 150-200mg/m2/day on days 1-5 of 28 day cycle x 6 cycles	TMZ 75-200mg/m2/day on days 1-5 of 28 day cycle x 6 cycles	TMZ 150-200mg/m2day on days 1-5 of 28 day cycle x 6 cycles
Adjuvant MEK inhibitor	trametinib 2mg daily x 21 months	selumetinib 25-50mg BID on days 6-28 when given with temozolomide then daily x 14+ months	selumetinib 25-50mg BID on days 6-28 when given with temozolomide then daily x 17+ months
Adverse events	Grade 3 acneiform rash	Grade 4 neutropenia during chemoradiation Grade 1 selumetinib-related retinopathy Grade 1 acneiform rash	Grade 3 thrombocytopenia during cycle 3 of adjuvant treatment Grade 2 paronychia Grade 1 acneiform rash
Best radiographic response	CR	CR	PR
Duration of response	4 months	6+ months	6+ months
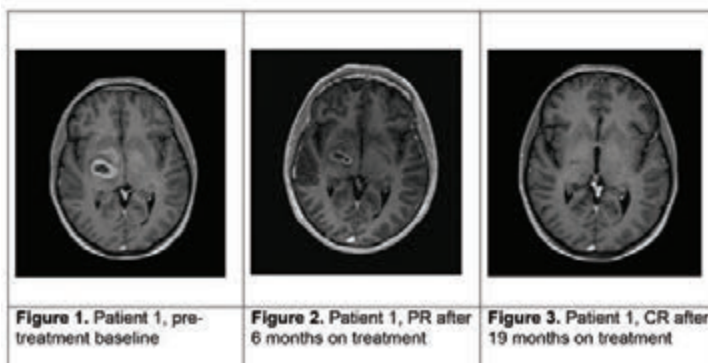
Figure 1. Patient 1, pre-treatment baseline

Figure 2. Patient 1, PR after 6 months on treatment

Figure 3. Patient 1, CR after 19 months on treatment

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Genotype-Phenotype Correlations For Missense Variants in the NF1 Gene: The French Experience for Previously Described Associations and Identification of a New Correlation

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Background: Neurofibromatosis type 1 (NF1) is a clinically heterogeneous genetic disorder with an autosomal dominant inheritance resulting from loss-of-function variants in the *NF1* gene. More than 2,200 distinct pathogenic variants are identified in the Leiden Open Variant Database, distributed along the *NF1* coding sequence. The disorder is clinically highly heterogeneous, even within a family. Only a few genotype-phenotype correlations have been published for some missense variants in *NF1* in international cohorts. We describe our experience of these variants in the French cohort.

Methods: Patients were included and phenotypically described by referent clinicians using a standardized questionnaire between 2005 and 2020. We compared the main clinical features of those patients with those of a reference NF1 cohort.

Results: Clinical data were recorded from patients with missense variants modifying neurofibromin at the following codons: Met1149, Arg1809, Arg1276, Lys1423 and codons 844 to 848 (2, 21, 23, 31, and 25 patients respectively). None of the patients with missense variants at Met1149 or Arg1809 had neurofibromas. Cutaneous neurofibromas were less frequent in patients with missense variants at Arg1276 compared to the reference cohort (46% *vs* 91%), whereas spinal neurofibromas were more frequent (50% *vs* 1.7%). Patients with mutations at codons Arg1276, Lys1423 or 844 to 848 developed plexiform neurofibromas more frequently (60%, 63% and 43% *vs* 18.5%, respectively). Skeletal abnormalities were more frequently associated with mutations at Lys1423 and at codons 844-848 (80 and 61% *vs* 15.2%). Contrary to what was described in a previous study, cutaneous neurofibromas appeared less frequent and optic pathway gliomas more frequent in patients with missense variants at codons 844-848. We describe for the first time the association between a moderate form of NF1 and missense variants affecting neurofibromin at position Arg1204: none of the 10 patients developed neurofibromas.

Conclusion: The description of the French cohort is in line with previously reported associations in international cohorts. Some discrepancies were observed for missense variants affecting codons 844 to 848 of neurofibromin, suggesting that individual correlations at the level of each amino acid might be more relevant in this region of the protein. We describe a new genotype-phenotype correlation, paving the way for future studies of NF1 pathogenic variants.

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Validation of Three Previously Reported Genotype-Phenotype Correlations in a Single Center Large Pediatric Cohort

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Purpose: The spread of new genetic testing techniques such as Next Generation Sequencing (NGS) gave a renewed impulse in genotype-phenotype correlation studies on large cohort of NF1 patients[1]. Until now 8 different genotype-phenotype correlations (one with NF1 microdeletion and 7 with different SNV) were reported in literature[1, 2, 3, 4]. We focused on three different variants in-frame indel M992del and missense variants affecting codon R1809 with a milder oncological phenotype and missense K1423E with higher-than-expected frequencies of plexiform neurofibromas.

Methods: We enrolled 440 NF1 pediatric patients (0-18 years of age) with heterozygous *NF1* pathogenic or likely pathogenic variants. Clinical data were extracted retrospectively from the diagnostic records and included in an anonymized file together with the genetic information on NF1 variants. Patients with variants with a known genotype-phenotype correlation were included in this study and their phenotype reported and correlated with patients previously reported.

Results: We reported 11 patients with M992del variant with a mild phenotype, characterized by cutaneous signs (100% of patients with CALMs and 27.2% with freckling) accompanied by frequent and usually more severe neurological manifestations (18.1% of patients with Developmental delay and 36.3% with specific learning disabilities) and complete absence of oncological complications (both OPG and PN). We also described 4 patients with K1423E variant with a higher prevalence of PN (50% of patients), as reported in previous literature. Six patients with different variants affecting codon R1809 were reported in our cohort (R1809S, R1809C in three different patients, R1809P, R1809L), showing a milder phenotype with cutaneous findings (100% of patients with CALMs and 50% with freckling) and no oncological complications (both for OPGs and PNs), as reported in literature.

Conclusions: Clinical features of patients with variants M992del, K1423E and codon R1809 associated with known genotype-phenotype correlations in our cohort were consistent with those previously described in literature, reinforcing these important findings on a large pediatric cohort.

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P3, N2, and ERN Differences on a Go/NoGo Task between Children with Neurofibromatosis Type 1 and Children with Idiopathic Attention-Deficit/Hyperactivity Disorder

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Purpose: Currently, the literature concerning the electroencephalography (EEG) profile of individuals with NF1 is limited in that the samples rely on large age ranges, often lack control groups, and no studies to date focus on school-age children with NF1. The goal of the present study was to compare attention-mediated event related potentials (ERPs) between children with NF1 and children with idiopathic ADHD: N2, P3, and Error-Related Negativity (ERN).

Methods: School-age (7-11 year old) children with NF1 ($n=11$) and children with ADHD ($n=5$) completed a Go/NoGo task while undergoing EEG to ascertain the amplitude and latencies of the ERPs of interest, N2, P3, and ERN. The Go/Nogo task was comprised of 4 blocks of 70 trials (280 trials; 70% "go"; "30% "nogo"). Electrode sites were selected based on prior NF1 literature for replication purposes: Fz, Cz were used for N2, Pz was used for P3, and Fz, Cz were used for ERN.

Results: There were no significant differences between the groups in the number of errors made or in reaction time on the Go/Nogo task. A significant difference in P3 amplitude was identified between children with NF1 and children with ADHD, $t(14)=-2.810$, $p=.014$. Children with NF1 exhibited reduced amplitudes compared to children with ADHD. Significant group differences in N2 latency were measured at Fz ($t(5.11)=2.92$, $p=.032$) and Cz ($t(14)=-3.48$, $p=.004$). For both Fz and Cz, children with NF1 had delayed latencies. A significant difference in ERN amplitude was identified between children with NF1 and children with ADHD measured at Fz ($t(14)=-2.28$, $p=.039$). Children with NF1 had enhanced ERN amplitudes compared to children with ADHD.

Conclusions: School-age children with NF1 exhibited reduced P3 amplitude, enhanced ERN amplitude, and delayed N2 latency on a Go/Nogo task in comparison to same-age peers with ADHD. These findings may suggest that these ERP indices may differentiate children with NF1 from children with idiopathic ADHD, especially given that there were no significant differences behaviorally between the two groups. The groups may be exhibiting different underlying information processing strategies or mechanisms to complete the same attentional processing tasks.

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Magnetic Resonance Imaging Harmonization Improves Detection of NF1-OPG Vision Loss

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Purpose: Advanced analytics of brain MRI scans that utilize machine learning techniques and radiomic features are impacted by the heterogeneity of both acquisition protocols and MRI platform manufacturer. Since large NF1 studies require multicenter collaboration, we propose a deep-learning method to harmonize multicenter MRIs to ensure accuracy. In this study, we performed radiomic analysis of the anterior visual pathway (AVP) in children with optic pathway gliomas associated with neurofibromatosis type 1 (NF1-OPG) acquired at two different institutions. Our goal is to determine if image harmonization improves our ability to detect vision loss.

Methods: T1-weighted clinical MRI scans (N = 135) of children with NF1-OPG from Children's National Hospital (CNH, N=60, General Electric platform) and Children's Hospital of Philadelphia (CHOP, N=75, Siemens platform). Neuro-ophthalmic evaluations identified vision loss (> = 0.2 LogMAR decline) in 28 children from CNH and 24 from CHOP. Our approach involved training a branched neural network using images from both institutions, simultaneously addressing variations in imaging protocols and harmonizing them to match a chosen protocol (e.g., CHOP's protocol). The harmonization network was trained with an unsupervised loss function to preserve patient anatomy and harmonize image intensities. Experts annotated the AVP to establish volumetric ground truth for NF1-OPG. We harmonized all MRIs to a standard fixed protocol. Then, we used brain volume, tumor location, child age, and 1,172 radiomic features of the AVP to predict vision loss with support vector machines. We conducted a comparative analysis of predictions based on both harmonized and unharmonized images to evaluate the effectiveness of our harmonization approach.

Results: Detection of vision loss for unharmonized images yielded balanced accuracy, sensitivity, specificity, and AUROC of 0.846, 0.647, 0.943, and 0.821, respectively. Gray-level run length entropy of the AVP emerged as a significant risk factor. For harmonized images, these metrics improved to 0.885, 0.706, 0.971, and 0.891, respectively, with gray-level non-uniformity of the AVP identified as the significant risk factor.

Conclusion: The deep learning method enables the harmonization of images from different MRI protocols, thereby improving our ability to detect patient vision loss due to NF1-OPGs.

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Natural History and Treatment Response of Disseminated Pediatric Low-Grade Gliomas (DLGGs) in Patients with NF-1; An International Multicenter Retrospective Review

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Purpose: To understand the natural history, treatment response, and report neuro-endocrine morbidity in DLGGs that arise in patients with NF-1.

Methods: Multicenter retrospective patient cohort of over 200 patients with DLGGs collected from 30 institutes. DLGG was defined as Low grade glioma with secondary metastasis or dissemination, either at presentation or later during the course of the disease. Out of over 200 patients 9 were reported to have NF-1.

Results: Out of nine patients with DLGG, 7 had Optic pathway gliomas (OPG) with secondary lesion(s). Amongst other 2/7, One patient had a brain stem tumor and one patient had multifocal disease with largest tumor being in cerebellum. None of the patients with OPG underwent biopsy at initial diagnosis. The 2 non-OPG patients underwent debulking, and later both classified as Pilocytic astrocytoma (PA). Two patients with OPG underwent biopsy after progression on chemotherapy. There was one death in the cohort. Two patients with OPGs type DLGGs continue to be observed and have never received any therapy. The overall progression free survival (PFS) of DLGG in NF-1 cohort was longer in comparison to non-NF population, 3.75y v 1.52y (p: 0.21). Average PFS of Vinblastine was 5 years. Average PFS for Carboplatin based therapy was 3.05 years. Three patients underwent targeted therapy (MEK inhibitors and Sirolimus) after other interventions 3 remain stable on last follow up. Other therapies were also used. Treatment details in table1. Endocrine and vision impairment was seen in about 50% patients.

Conclusions: Optic pathway gliomas were the most common tumors to have secondary dissemination in NF-1 cohort, most of them had secondary lesions at diagnosis. Though tissue was obtained only in 4 patients, no RAF aberration was seen unlike non-NF pool which showed RAF and FGFR alteration in 73 % population. Patients with non OPGs were biopsied and had features of PAs which is similar to the non-NF cohort. Vinblastine based chemotherapy may have shown better PFS when compared to the Carboplatin based therapy, but factors such as age of onset and limited sample size need to be considered. Patients with NF-1 tend to have longer progression free survival. And the PFS on targeted therapies need to be studied more. Three patients continue to be on targeted therapy without progression.

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Semiautomated Artificial Intelligence Based (AI) 3D Segmentation vs Manual Linear Measurements of Plexiform Neurofibromas in Neurofibromatosis Type 1 Patients

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Purpose: This study's purpose is to evaluate the feasibility of using semi-automated AI-based volumetric segmentation in children with neurofibromatosis type 1 and plexiform neurofibromas compared to traditional manual linear methods.

Methods: Three patients with neurofibromatosis type 1 and plexiform neurofibromas were selected as a part of retrospective cohort. Volumetric analysis of a target plexiform neurofibroma was done in each patient at two separate time points, one before treatment and one during MEK inhibitor therapy, using Sectra PACS (Sectra AB, Linköping, Sweden). An AI based segmentation tool in the Sectra multiplanar reformation viewer was used to segment lesions and generate volumes for each plexiform neurofibroma. Axial and coronal fat suppressed 2D sequences (T2 STIR or DIXON) were used for segmentation and the volume was calculated by the Sectra software. Linear measurements were also performed on the same lesions in the axial, coronal and sagittal planes, the technique used in routine clinical practice. The segmented volumes and volumes derived from simple three plane measurements were compared and correlated with subjective clinical findings.

Results:

	Patient 1	Patient 2	Patient 3
Sectra segmentation volume (cc³)	Pre: 92.4 cc³ On Tx: 69.5 cc³	Pre: 16.5 cc³ On Tx: 16.2 cc³	Pre: 35.4 cc³ On Tx: 25.6 cc³
3 plane measurements (AP x TRV x SAG) (cc3)	Pre: 296.7 cc³ On Tx: 198.7 cc³	Pre: 26.3 cc³ On Tx: 25.9 cc³	Pre: 66.5 cc³ On Tx 46.8 cc³
Time for Sectra segmentation (minutes)	Pre: 5 min On Tx: 3 min	Pre: 3 min On Tx: 2 min	Pre: 3 min On Tx: 1 min
Time for manual 3-plane measurements (minutes)	Pre: 4 min On Tx: 3 min	Pre: 3 min On Tx: 3 min	Pre: 3 min On Tx: 1 min
% change in volume Sectra	-24.8%	-1.8%	-27.7%
% change in volume manual 3-plane	-33.0%	-1.5%	-29.5%

Conclusions: Obtaining an accurate measurement of plexiform neurofibromas on MRI is a challenge due to their complex trans-spatial nature. Radiologists standardly measure neurofibromas in 3 planes; however, these measurements often fail to capture the complex shape of the lesions and can grossly overestimate the true volume. AI-based semiautomated methods can measure the volume of these complex masses more accurately without adding significant analysis time. Here, we showed that the Sectra semi-automated segmentation tool more accurately determined the volume measurement in plexiform neurofibromas than linear measurements, most likely due to better margin detection. Manual 3-plane measurements overestimated the volume of the plexiform neurofibromas because they do not consider the complex geometry of the tumors. The percentage change in the volumes between time points was comparable. This correlated to subjective clinical exam findings. Time to determine the volume by manual measurement and Sectra were comparable.

Our results demonstrate that semi-automated volumetry of plexiform neurofibromas in NF1 is clinically feasible and may reflect clinically relevant lesional volume changes more accurately than standard 3-plane measurements. Additionally, 3D segmentation is feasible using imaging not specifically acquired for volumetric analysis. While segmentation errors do occur, additional training of the AI models to segment complex plexiform neurofibromas will enhance the clinical utility of these tools and improve patient care.

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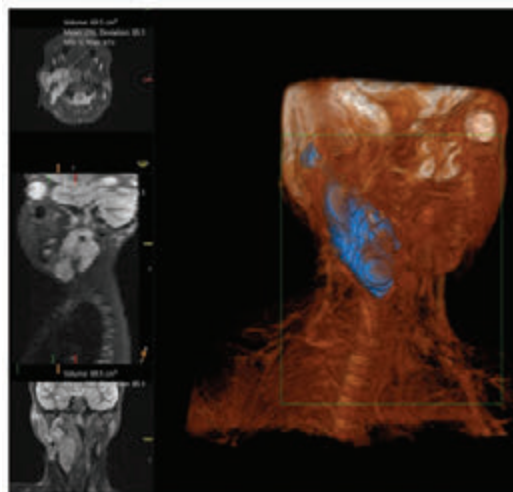
Figure 1: Semi-automated segmentation of the right submandibular plexiform neurofibroma in patient 1 using the Sectra 3D volumetric tool.

Figure 2: 2D orthogonal measurement of right submandibular neurofibroma from patient 1.

Assessing Socially Oriented Attention in Young Children with Neurofibromatosis Type 1: An Eye-Tracking Study

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Purpose: Social attention is a multifaceted set of processes including orienting to socially relevant stimuli such as faces and eyes, and the ability to coordinate the focus of attention with that of another person (i.e., joint attention). Soon after birth infants preferentially orient toward social stimuli in the environment and joint attention typically emerges around 6-12 months of age. The objective of the present study was to employ eye-tracking technology to compare attentional patterns towards socially relevant stimuli among young children with neurofibromatosis type 1 (NF1), children with non-syndromic autism, and typically developing (TD) controls.

Methods: This study is a prospective cross-sectional examination involving children with NF1, children with autism, and typically developing controls aged between 2-5 years. The research utilized eye-tracking technology to assay various aspects of participant eye gaze during the observation of videos containing both social and non-social information, or towards objects that were either gazed-at (congruent object) or not gazed-at (incongruent object) by an unfamiliar adult (joint attention).

Results: To date, data from 25 children with NF1 (mean age 54.1 months, $SD = 8.9$), 25 children with non-syndromic autism (mean age 49.9 months, SD = 9.7), and 20 TD controls (mean age 51.2 months, SD = 12.2) have been collected and analyzed. After adjusting for differences in cognitive ability, both children with NF1 and non-syndromic autism showed a lack of attentional bias towards social stimuli (both, $p > .05$), an opposing pattern to that seen in TD controls ($p < .001$). In the NF1 group, elevated ADHD traits and poorer sustained attention were associated with less attention to non-social stimuli and reduced attentional bias towards social stimuli, respectively.

Conclusions: We have previously shown that school-aged children with NF1 spend less time attending to faces when presented in social scenes relative to TD controls. The current findings extend this observation providing evidence that the reduced bias towards attending to socially rich information is also present in young children with NF1. Ongoing analyses are being conducted to determine whether these differences in NF1 are primarily social in nature, or influenced, at least in part, by individual variations in attentional control. These findings could have important implications for early interventions aimed at improving social attention and communication skills in young children with NF1.

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Exploration of Imaging and Molecular Markers in Germline and Somatic Neurofibromatosis Type 1 *(NF1)* Driven Glioma

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Background: Germline *NF1* alterations result in increased incidence of brain tumors. The natural history of *NF1* glioma is not well understood, and standard therapeutic options are poorly tolerated. The clinical and molecular prognostic markers applied to sporadic gliomas do not appear to be appropriate for *NF1* associated glioma complicating treatment planning.

Purpose: Patient driven data collection is needed in order to collate information from NF centers worldwide. An integrated patient facing web portal for collection of clinical, pathologic and radiologic information on patients with *NF1* associated glioma will enable aggregation of key data to better understand clinical course and treatment options for this rare form of glioma. By assessing this data compared to data from people with sporadic gliomas with and without NF1 alterations, the study aims to determine whether there are radiologic and molecular features in germline and sporadic *NF1* driven glioma that may serve as accurate prognostic biomarkers.

Methods: To date, we have enrolled 10 out of 50 planned prospective and 30 retrospective participants with germline NF1 and glioma, 40 participants with sporadic glioma and intratumoral NF1 mutation, 40 participants with sporadic glioma and intratumoral EGFR mutation at MSKCC, JHU, and other institutions diagnosed between 2000 and 2024. Retrospective and prospective clinical, molecular, imaging data are being integrated and analyzed. Results: 120 participants have been included, and clinical data for 40 germline and 80 sporadic participants has been collected (Table 1). The majority of patients with germline NF1 glioma have midline tumors (58-60%) and biopsy only (73%), compared to sporadic GBM which are mostly located in the frontal lobes (40-43%) and amenable to resection (53-55%). Approximately 1,000 MRI scans have been uploaded to XNAT. We have created 40 flip books and 6 volumetric curves, using auto-segmentation and co-registration (Figure 1). Molecular profile for 80 sporadic participants has been conducted and intratumoral NF1 and intratumoral EGFR mutations are mutually exclusive (Figure 2).

Conclusions: *NF1* driven gliomas have a poor prognosis compared to sporadic GBM, possibly attributed to midline location and lack of surgical access. MRI neuroimaging can be quantified by tools such as flip books and volumetric curves. Borrowing these radiology techniques from sporadic GBM may highlight dynamic growth rates and texture in *NF1* glioma, providing a valuable prognostic indicator. Molecular characterization is underway, in order to determine whether germline *NF1* glioma and *NF1* silenced sporadic glioma have interrelated molecular features that may enable shared therapeutic opportunities to inform future clinical trials. We will identify a comprehensive panel of imaging and molecular biomarkers for somatic and germline *NF1* gliomas. Continued recruitment and analysis is ongoing.

Table 1. Demographics

	Germline NF1 glioma				Sporadic GBM			
	Retrospective		Prospective		intatumoral NF1		intratumoral EGFR	
	N (26)	%	N(5)	%	N (40)	%	N(40)	%
Gender								
Male	15	58%	5	100%	22	55%	23	58%
Female	11	42%	0	0%	18	45%	17	43%
Median age at diagnosis	32		25		62		66	
Tumor location								
Midline	15	58%	3	60%	2	5%	0	0%
Frontal lobe	6	23%	1	20%	17	43%	16	40%
Temporal Lobe	3	12%			12	30%	15	38%
Parietal Lobe	0	0%			8	20%	7	18%
Occipital Lobe	0	0%			1	3%	1	3%
Cerebellum	1	4%	1	20%	0	0%	0	0%
Spinal Cord	1	4%			0	0%	0	0%
Histologic diagnosis								
Glioblastoma	7				40		40	
High Grade Astrocytoma	4							
Diffuse Astrocytoma	9							
Pilocytic Astrocytoma	4		1					
Low-grade astrocytoma NOS	0		4					
Diffuse midline glioma	2							
Extent of Resection								
Biopsy	19		N/A		4	10%	4	10%
GTR			N/A		22	55%	21	53%
STR	7		N/A		13	33%	14	35%
Median KPS at diagnosis	80		90		80		80	
First Line Tx								
RT and TMZ	20		2		35		33	
selumetinib			2		0		0	
Other	6		1		5		7	
Alive at 2 years								
Yes	12		5		4	12%	9	26%
No	14				30	88%	26	74%
Lost to follow-up					6		5	

Figure 1. Example of a volumetric curve for patient with germline *NF1* glioma
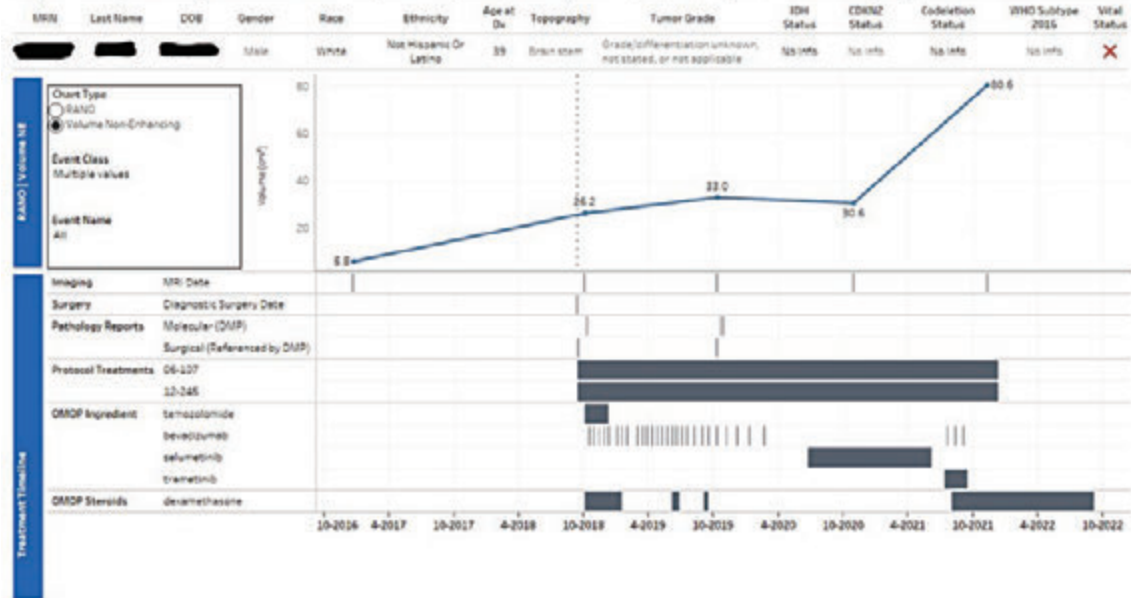
Figure 2. Oncoprint
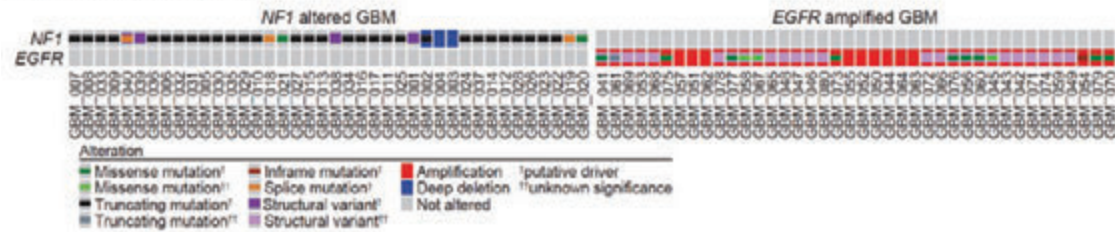
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Results of a Prospective, Direct Comparison of Non-Invasive Treatments of Cutaneous Neurofibromas (cNFs)

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Purpose: We previously compared the safety, tolerability, and efficacy of four minimally invasive cNF treatments in adults with Neurofibromatosis Type 1 (NF1); with the goal of improving tolerability and efficacy, we initiated a second phase of the trial in which we treated larger tumors and more tumors per participant.

Methods: We compared the safety, tolerability, and tumor response (volume/height via 3D imaging and clinical improvement via physician assessment) of three different treatment modalities in 11 adults with an adequate number of 2-8mm cNFs (minimum of 6 cNFs per modality, no upper limit of tumors). These modalities included a double pulse of 755nm alexandrite laser with suction (8mm spot size, 60-100J/cm2 fluence, 3ms pulse duration, DCD 40/20), a double pulse of 1064nm Nd:Yag laser with suction (8mm spot size, 75-120J/cm2 fluence, 3ms pulse duration, DCD 40/20), and intratumoral injection of 10mg/mL deoxycholic acid (Kybella[R]) at a volume approximately equal to that of the tumor. For each subject, one treatment area no greater than 600 cm^2 was selected per modality, with a complementary area serving as the untreated control. The treatment areas were then randomized to treatment versus control (no treatment). Topical anesthetic (5% lidocaine/prilocaine) was applied for 40 minutes before treatment. At baseline, 3, and 6 months, the pain score (0-10), tumor height/volume (via 3D Cherry Imaging[R]), clinical tumor clearance, pigmentation and scarring were assessed.

Results: Of the 11 participants enrolled (average age of 49; 11/11 female), 8 have completed the 6-month assessment. A total of 328 cNFs were treated. All modalities reduced some cNFs by 6 months post-treatment, with large variation between tumors and between participants (Table 1). Mild hyperpigmentation was seen in three subjects (following treatment with Kybella or alexandrite) at the 3-month assessment and had resolved in 2 of the 3 by 6 months. Mild to moderate hypopigmentation was seen in two subjects treated with alexandrite laser and while markedly improved was not completely resolved by 6 months. Alexandrite laser and deoxycholate injection were only mildly painful (average pain scores of 2.8 and 2.5, respectively), but deoxycholate injection resulted in tumor reduction more reliably. Moderate pain limited the Nd:Yag laser treatment dose (average pain score of 4.5), and this treatment produced no apparent tumor reduction in most participants. No cNF growth stimulation or recurrence from these treatments has been noted.

Conclusions: All three modalities were safe and demonstrated at least some degree of efficacy.

Table 1. Changes in height and volume across modalities.

	Deoxycholate injection (median % change from baseline [Q1-Q3])	Alexandrite laser (median % change from baseline [Q1-Q3])	Nd:Yag laser (median % change from baseline [Q1-Q3])
Height (3 months)	Treated: -16.7 (-43.1-0.0) Control: 0.0 (-20.0-18.2)	Treated: -7.1 (-25.0-12.5) Control: +4.5 (-6.0-13.0)	Treated: -5.0 (-15.2-20.0) Control: 0.0 (-12.7-19.1)
Height (6 months)	Treated: -27.8 (-50.0- -9.1) Control: +17.0 (-2.8-40.0)	Treated: 0.0 (-23.8-25.0) Control: +18.8 (0.0-38.9)	Treated: +11.1 (-18.2-50.0) Control: +21.1 (2.1-42.9)
Volume (3 months)	Treated: -21.2 (-35.8-16.8) Control: +3.5 (-6.3-23.0)	Treated: -8.8 (-22.6- -1.5) Control: +6.2 (-6.6-16.8)	Treated: -6.2 (-23.1-10.3) Control: -5.3 (-13.9-10.7)
Volume (6 months)	Treated: -32.1 (-48.8- -6.6) Control: +22.3 (1.9-39.6)	Treated: -10.6 (-23.1-20.1) Control: +22.0 (1.9-32.7)	Treated: -1.1 (-19.6-23.2) Control: +18.2 (-2.6-31.2)

Q1: First Quartile
Q3: Third Quartile

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The Elusive Genotype-Phenotype Correlation in Neurofibromatosis Type 1: A Single Center Experience

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Purpose: Neurofibromatosis type 1 (NF1) is one of the most common Autosomal Dominant genetic disorders, caused by mutations in the *NF1* gene. It is a multisystemic, cancer predisposition syndrome, which manifests in childhood and adolescence and presents usually in a highly variable clinical presentation even in individuals with the same *NF1* variant[1]. The genotype-phenotype correlation in NF1 patients is still elusive, and to date more than 3000 different variants have been identified[2]. It is of utmost importance to have a better understanding of the effect of *NF1* variants (ex. pathogenic, likely pathogenic, VUS, etc.) to be able to establish the reliable correlation between the genotype and phenotype in NF1[3].

Methods: A retrospective chart review study included all NF clinic patients (children and adults) between January 2020 and February 2024. We are submitting the study for the Institutional Review Board (IRB) evaluation. Data were recorded by child neurologist with expertise in NF and related specialties within our Multidisciplinary group. A sign was considered not present if no information was provided in the patient's chart and/or magnetic resonance imaging (MRI) and/or ophthalmologic evaluation was not performed.

Statistical analyses include descriptive statistics and data appropriate for continuous and discrete variables.

Results: A total of 201 NF1 patients by clinical criteria were reviewed. Of those, about one third underwent genetic testing for *NF1* and *SPRED1.* Among the mutations, we found: nonsense variants, missense variants, deletions and intronic mutations as the most common mutations. We also found two patients who fulfill clinical criteria for NF1, but all genetic tests were negative for *NF1* and *SPRED1.*

Conclusions: This study will expand the spectrum of mutations in the NF1 gene and the associated phenotype. We also highlight the importance of the positive effect on diagnosis and genetic counselling in patients referred for concerns of NF1. Of note, clinical evaluation is still very important for patient diagnosis, not relying solely on genetic results. We discuss the complexity of the field, and how to improve and contribute to genetic counseling, risk stratification, clinical management, differential diagnosis, personalized medicine and outcomes for the NF1 population.

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MEK Inhibitor Treatment in Plexiform Neurofibromas in NF1: Approach and Experiences in The Netherlands

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Introduction: Plexiform neurofibromas (PN) in children and teenagers with NF1 cause serious morbidity, varying from functional deficit, threatening vital organs, pain to change of appearance. Currently, oral treatment with a MEK inhibitor becomes more widely available as part of the treatment for NF1-PN, but broad expertise on treatment goals, outcome measures, adverse events and longer-term effects is lacking. Therefore, we aim to describe our treatment protocol and current expertise gained in our center.

Case Description: In the Erasmus MC – Sophia's Children's hospital, Rotterdam, the Netherlands, we have access to MEK inhibitor treatment by compassionate use since three years. Our center is the national NF1 expertise center, leading the national NF1 network in the Netherlands encompassing 12 centers. In the network, we developed a treatment protocol, with start and stop criteria and outcome assessments. We defined an indication committee for selection of patients to be treated, including a neurosurgeon, neurologist, pediatrician, advanced nurse practitioner, radiologist, and pediatric oncologist.

Outcome / Treatment: We have treated 8 patients so far with a median age of 12.6 years (range: 8.1 years – 19.7 years) , of which 62.5% is male. Goal of treatment was volume reduction in 4 patients (median age 11.3 years, range 8.1 – 16.7 years) and pain in 4 patients (median age 14.9 years, range 9.3 – 18.7 years). Median treatment time was 0.7 years (range: 0.2 - 2.3). Earliest pain reduction was seen in four weeks after start. Earliest volume reduction was observed after nine months after treatment. Most frequent side effects reported are skin infections (7 out of 8, 88%), a.o. acneiform eruptions, paronychia, eczema. We neither observed cardiac nor opthalmological adverse events and no abnormal laboratorium values were seen. In one patients treatment was stopped due to skin side effects after 9 months before evaluation of treatment effect.

Discussion: In our small sample, we observed a trend for pain as indication for treatment in older children with NF1-PN versus growth in younger children. Treatment effects on pain are earlier reported than on tumor size. We hope our results contribute to experience in MEKi treatment, defining treatment indication and timing, outcome measures and follow-up protocols for adverse events. We encourage our international partners for a standardized follow-up in order to harmonize treatment results.

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The Effect of Selumetinib Treatment Interruptions on Plexiform Neurofibroma Growth

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Background: The MEK inhibitor selumetinib is FDA approved for symptomatic, inoperable plexiform neurofibromas (PN) in pediatric patients with Neurofibromatosis type 1 (NF1) based on evidence of partial tumor response and clinical benefit. However, some patients experience toxicity or other events that required treatment interruptions or have discontinued treatment for various reasons. The impact of suspending or terminating selumetinib treatment on the residual PN has not been systematically studied. This analysis investigates the effect of (1) prolonged drug holds and (2) treatment discontinuation on PN growth in patients with NF1-PN.

Methods: All participants of the pediatric phase 1/2 selumetinib study (NCT01362803) and adult phase 2 selumetinib study (NCT02407405) were included in this analysis. Electronic health records and research databases were utilized for retrospective review. We collected age and PN volume determined by MRI analysis at the start and end date of prolonged drug holds (defined as ≥28 days), end of treatment and first off study follow up. We calculated percentage volume change between events and performed univariate analyses with linear regressions, unpaired t-test, and descriptive statistics using Prism10.

Results: 132 participants (pts) (99 pediatric, 33 adult) with median age of 12.9 years (range, 2.9 to 59.4) were enrolled. We identified 71 pts with 113 drug hold events for treatment interruption analysis and 37 pts for off treatment analysis (16 patients were included in both). The median duration of prolonged drug holds was 47 days (range, 28 to 399). The median change in PN volume following drug hold was 6.7% (range, -15.5% to 108.2%). Longer duration of drug hold resulted in greater increase in PN volume ($p<0.0001$). The staging MRI following the drug hold was performed a median of 73 days after restarting selumetinib therapy (range -35 to 293). Pts who were back on treatment after the drug hold for a longer duration before the next re-staging MRI showed less regrowth ($p<0.0001$). The age at time of the drug hold was not significantly correlated with the degree of PN regrowth observed ($p=0.4566$). However, slightly more regrowth was observed in patients <18 years (mean 22.1%) compared to ≥18 years (mean 11.5%) after stopping treatment ($p=0.0222$). In the <18 age group, 8 of 16 patients (50%) had ≥ 20% PN volume increase on consecutive MRI, while in the ≥ 18 age group only 4 out of 21 patients (19%) had ≥ 20% PN volume increase. In pts who discontinued treatment, the median change in PN volume was 13.0% (range, -7.2% to 140%) at the next consecutive MRI performed after a median of 167 days (range, 32 to 1947). Pts who enrolled on study with growing PN had more regrowth following drug hold ($p=0.0006$) and treatment discontinuation ($p=0.0044$) compared to pts with stable PN. Tumor response status immediately prior to the drug hold or discontinuation had no impact on the tendency to regrow ($p=0.9104$; $p=0.4007$ respectively). The median duration of treatment before drug hold was 2.8 years (range, 0.1 to 9.2), and before treatment discontinuation was 2.2 years (range, 0.3 to 6.6). Longer treatment duration did not result in reduced tendency for regrowth following drug holds ($p= 0.2176$) or discontinuation ($p=0.2097$).

Conclusions: Our analysis indicates that in most patients undergoing treatment with selumetinib for NF1-PN, the interruption of discontinuation of treatment results in some degree of PN regrowth. The median volume increase after treatment interruption was 6.7% and 13.0% after stopping treatment, that is still considered stable disease (<20% volume increase). Patients who started treatment with growing PN had more regrowth when treatment was held or stopped. After stopping treatment, fewer patients ≥18 years had progressive disease (≥ 20%) compared to those <18 (19 vs. 50%). Analyses of the longitudinal PN growth trajectory following treatment discontinuation are ongoing and will provide insights into determining the optimal treatment duration with selumetinib. Additional statistical and multi-variate analyses are ongoing.

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Survey of an Urban NF Clinic Hispanic Population to Improve NF Education and Outreach

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Background: The CHLA NF Clinic serves a large inner city Hispanic population. It is recognized that patients and families may have a more positive experience in clinic and be better informed if information is provided in their native language. The goals of this study were to better understand how to best provide our Hispanic population with NF education as well as opportunities to participate in the broader NF community.

Methods: A questionnaire with 23 multiple choice and open-ended questions was developed and translated into Spanish to query our Hispanic population's home language fluency, internet access, preferred means of receiving NF educational materials and engagement in the local and national NF communities. Participating Hispanic families were prospectively recruited through our NF clinic with surveys being completed on site at the time of the office visit.

Results: Fifteen families (8 females, 7 males) have been recruited to date. Patient ages ranged from 1 to 27.9 years (mean 12.3 years) at the time of enrollment. 13.3% of households speak only Spanish at home with the majority being bilingual. 69% of families prefer office visits to be conducted in Spanish and 93% agree that they are receiving adequate interpreting services in our clinic. The majority prefer a Spanish interpreter in person for clinic visits. All families have internet access and only one parent has no email address. 53% of families prefer to receive brochures in Spanish provided in clinic but 35% have interest in receiving information by internet link. All families but two have received NF information in Spanish from our clinic. 86% have interest in attending an educational symposium in Spanish either in person or virtually. 93% of families would be interested in watching educational videos in Spanish. 78.5% are aware of the CTF NF Registry. 71% have a good understanding of their child's NF diagnosis and 78.5% have done research online. Only one family has attended our local annual CTF Shine A Light walk.

Conclusions: The majority of our Hispanic NF clinic population come from bilingual households. There is appreciation for receiving information in clinic through a live Spanish interpreter and with written brochures in Spanish. All families have internet and most have email access. There is strong interest in learning through videos presented in Spanish. There are many opportunities to increase our Hispanic population's NF educational experiences and engagement in NF community events.

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Clinical Characteristics of Seizures and Course of Epilepsy in Children with Neurofibromatosis Type 1 – A Tertiary Center Experience

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Purpose: The purpose of the study is to evaluate frequency, clinical characteristics of seizures, and course of epilepsy in children with neurofibromatosis type 1 (NF1)

Methods: The retrospective study included all patients with NF1 treated at Institute in period from 2013 to 2023, who experienced et least one seizure. Clinical, electroencephalographic and neuroimaging evaluation were performed in all cases. Diagnosis of epilepsy was established in the children with, at least two unprovoked seizures. The type of the seizure was defining according ILAE classification (2017). A new definition for status epilepticus (SE) was used (Trinka et al, 2015). Antiseizure medication (ASM) was recommended in cases with recurrent unprovoked seizures. The further parameters are analyzed: sex, age at the time of the first seizure, type and duration of the seizures, provocation, rescue and ASMs, response to the treatment and outcome (good or poor epilepsy control).

Results: The study included 100 children with NF1. The seizures were experienced by 17 children (17%), 9 males, and 8 females. The mean age of the first seizures was 59.23 ± 47.3 (range 2 to 166) months. Eight children had generalized onset first seizures: generalized tonic-clonic (5), atonic (2), and epileptic spasms (1), while seven children had focal onset seizures. In five children, the first seizures were provoked: by fever (2), acute enterocolitis (2), and acute systemic infection (1). Eight children experienced SE. The first seizure was stopped by rectal administration of diazepam in nine cases, by midazolam in two, and phenobarbital in two patients, while in four cases the seizures stopped spontaneously. In 13 children, ASMs was recommended, and the mean number of ASMs was two (range 1-9). At the end of follow up (mean duration 63 months), good seizure control was achieved in all patients except one girl with initial epileptic spasms followed by focal epilepsy, resistant to ASMs.

Conclusion: In our cohort, the seizures were experienced by respectable percentage (17%) of children with NF1. The careful inspection of the skin is essential in all children with seizures, even in those with provoked seizures. The recommendation of rescue medication and education of the parents are very important since nearly half of patients were suffering status epilepticus. The outcome of epilepsy is favorable in all cases except one girl with initial epileptic spasms. The further investigation might contribute to better understanding of epileptogenesis and impact of new agents to clinical course to epilepsy in NF1.

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Cutaneous Toxicities of Mitogen-Activated Protein Kinase Inhibitors in Children and Young Adults with Neurofibromatosis-1

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Purpose: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder which commonly causes neoplasms leading to disfigurement or dysfunction. Mitogen-activated protein kinase inhibitors (MEKi) are generally well-tolerated treatments which target neural tumor progression in patients with NF1. However, cutaneous adverse events (CAEs) are common and may hinder patients' abilities to remain on treatment, particularly in children. We aim to characterize CAEs secondary to MEKi treatment in pediatric and young adult patients with NF1.

Methods: We reviewed institutional medical records of patients under 30 years with a diagnosis of "NF1," "NF2," or "other neurofibromatoses" on MEKi therapy between January 1, 2019 and June 1, 2022. We recorded the time-to-onset, type, and distribution of CAEs, non-cutaneous adverse events (AEs), AE management, and tumor response.

Results: The median age of 40 patients with NF1 was 14 years old. Tumor types included low-grade gliomas (51%) and plexiform neurofibromas (38%). MEKi used included selumetinib (69%), trametinib (25%), and mirdametinib (6%) (Table 1). A total of 74 CAEs occurred, with 28 cases of acneiform rash (38%) (Figure 1). Twenty-three out of the 28 cases of acneiform eruption were in post-pubertal patients (82.1%). Other common CAEs were paronychia, seborrheic dermatitis, eczema, xerosis, and oral mucositis. The most common treatments included oral antibiotics and topical corticosteroids. Most patients had clinical (stable or improved) tumor response (71%) while 29% had tumor progression while on a MEKi (Table 2). There was no significant association between CAE presence and tumor response (p=0.39).

Conclusions: MEK inhibitors have been demonstrated as a valuable treatment, but CAEs complicate the ability to stay on the treatment. Most patients in our cohort developed a CAE, demonstrating its prevalence in this population. Improvement in characterization of MEKi toxicities and their management is important to develop treatment guidelines for pediatric and young adult patients with NF1 on MEKi therapy.

Table 1.

Individual-level variables

Age (years), Median (Range)	14 (1-35)
Race, N (%)	
White/Caucasian	27 (67.5)
Black/African American	5 (12.5)
Other	4 (10)
Asian	3 (7.5)
Unknown	1 (2.5)
Hispanic	0 (0)
Sex, N (%)	
Male	25 (62.5)
Female	15 (37.5)
Primary tumor type, N (%)	
Plexiform neurofibroma	26 (65)
Glioma	12 (30)
MPNST[a]	1 (2.5)
Glioblastoma multiforme	1 (2.5)
MEKi[b] drug name,[c] N (%)	
Selumetinib	32 (69.6)
Trametinib	12 (26.1)
Mirdametinib	2 (4.3)
Duration of MEKi treatment (days), Median (Range)	395.5 (16-1334)

[a]Malignant peripheral nerve sheath tumor, [b]Mitogen-activated protein kinase inhibitor; [c]Patients treated with multiple drugs are represented more than once

Table 2. Adverse Event Description and Management

	N (%)	CTCAE[b] Grade 1 (N)	CTCAE Grade 2 (N)	CTCAE Grade 3 (N)
Cutaneous adverse events (CAEs)[a]	74 (100)	41	26	7
Acneiform rash	28 (38)	11	13	4
Paronychia	9 (12)	2	6	1
Seborrheic dermatitis	6 (8)	5	1	0
Eczema	6 (8)	4	2	0
Xerosis	6 (8)	6	0	0
Oral mucositis	5 (7)	3	1	1
Uncharacterized rash	5 (7)	3	2	0
Pruritus	4 (5)	4	0	0
Alopecia	2 (3)	2	0	0
Cellulitis	2 (3)	1	0	1
Onychomycosis	1 (1)	0	1	0
Cutaneous adverse event incidence, N (%)	35 (87.5)			
Total CTCAE Grade 1/2/3, N (%)	41 (55)/ 26 (35)/7 (9)			
AE[c] time-to-onset (days), Median (Range)	20 (5-713)			
Duration of AE (days), Median (Range)	252 (0-2293)			
Tumor response, N (%)				
Stable	18 (45)			
Improved	14 (35)			
Progression	8 (20)			
Management of CAE, N (%)				
Oral antibiotic	12 (35)			
Topical corticosteroid	18 (53)			
Oral retinoid	4 (12)			
Discontinuation due to CAEs, N (%)	5 (12.5)			
Time to next treatment (days), Median (Range)	463 (54-2323)			

[a]CAEs with incidence of N1 and grade 1 were excluded from the table (e.g., hyperpigmentation, etc.); [b]Common Terminology Criteria for Adverse Events; [c]Adverse events

Fig. 1

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Left Ventricular Ejection Fraction Changes Over Time in Children and Adults with Neurofibromatosis Type 1 (NF1) on Clinical Trials with Selumetinib for Inoperable Plexiform Neurofibromas

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Purpose: Decreased left ventricular ejection fraction (LVEF) is a known potential side effect of treatment with mitogen activating protein kinase kinase inhibitors (MEKi), such as selumetinib. Treatment for NF1 related plexiform neurofibromas (PN) often requires prolonged treatment with MEKi, and the long-term impact on LVEF is not known. Here we describe changes in LVEF in children and adults with NF1 and inoperable PN treated with selumetinib on clinical trials.

Methods: We conducted a retrospective analysis of all LVEF from echocardiograms and the incidence of "ejection fraction decreased" adverse events (AEs) in patients receiving selumetinib on the clinical trials of selumetinib for children (NCT01362803) and adults (NCT02407405) from 9/11/11-1/23/24. Age at enrollment for the pediatric and adult trials were <18 and ≥18 years, respectively. Sex, age at enrollment, grade of LVEF related AE (grade 2: 10-19% drop from baseline LVEF, grade 3: ≥ 20% drop from baseline LVEF as defined by CTCAE criteria), date of registration on study, date of onset of LVEF related AE, date of resolution, action taken for AE (none, dose interrupted, interrupted and reduced), treatment for AE (if applicable), date of echocardiogram, and LVEF from echocardiogram report were extracted from the study databases.

Results: A total of 132 participants (pts) (99 pediatric, 33 adult) were included in the analysis, with a median age of 13 years (range: 3.0-60.2 years) at study entry, of which 75.0 %(n=99) were males. The total duration of follow-up was 5.1 years (IQR: 2.4-7.0 years) for the pediatric study and 3.0 years (IQR: 1.9-4.7 years) for the adult study. Overall, LVEF related AEs were reported in 25.7% (26 pediatric and 8 adults) pts, of whom 6.1% (n=8) had more than 1 LVEF related AE. 26.2% of children (26/99) and 24.2% of adults (8/33) experienced at least one LVEF related AE. LVEF AEs (n=46) reported were grade 2 in 97.8% (n=45) and grade 3 in 2.2% (n=1). All LVEF related AEs were asymptomatic, and action taken for these AEs (n=46) included dose reduction only in 2.2% (n=1), regimen interruption only in 2.2% (n=1), regimen interruption and dose reduction in 13.0% (n=6), and no action needed in 82.6% (n=38). Mean baseline LVEF was 69.4% (SD±3.7%) and mean LVEF at last echo was 61.4% (SD±4.0%). The median time to first LVEF related AE after starting selumetinib was 2.6 years (IQR: 0.9-3.7 years) and the median time to resolution of the AE was 1.6 months (IQR:1.2-4.1). Overall, 8.3% (n=11) of pts had a >10% reduction in LVEF from baseline to a LVEF <53%.

Conclusions: While we observed decrease in LVEF in pts treated with selumetinib on clinical trials, all were asymptomatic and most had normalization of LVEF without the need to hold or discontinue therapy. Close monitoring of LVEF during these events for recovery or worsening is recommended, as drug interruptions/reductions may be required and initiation of cardioprotective medications may be necessary with cardiology guidance. The median time to first LVEF related AE was >2 years from start of treatment, and rates were similar in the pediatric and adult populations, indicating that long-term monitoring while on treatment is likely needed.

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MRI-Based Long-Term Evaluation on the Development and Growth Prediction of Peripheral Nerve Sheath Tumors in NF1 in Children

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Background and Purpose: A subset of patients with neurofibromatosis type 1 (NF1) exhibit plexiform neurofibromas (PNF) and a large tumor burden is a predictor for the development of malignant peripheral nerve sheath tumors (MPNST). Whole body magnetic resonance imaging (MRI) is the imaging method of choice for evaluation of PNF. According to the ERN GENTURIS tumor surveillance guidelines a whole-body MRI is recommended for all NF1 patients during transition from adolescence to adulthood. In the absence of internal PNF usually no further monitoring using whole-body MRI is recommended. However, it remains unclear whether pediatric patients who display no tumor burden on initial MRI scans can develop PNF over time. Therefore, we aimed to retrospectively review whole-body MRI scans of pediatric patients with NF1 without initial tumor burden and aimed to compare these with long-term follow-up scans for presence of newly identifiable PNF.

Methods: We retrospectively reviewed whole-body MRI scans of 17 children (twelve male) diagnosed with NF1 (median age: 9 [IQR 6.1 - 11.9] years) who displayed no PNF on initial MRI scans. Follow-up MRI scans with a follow-up interval of at least six years (median follow-up interval: 9.5 [IQR 6.1- 12.9] years) were reviewed by two radiologists for the development of new PNF over time in consensus.

Results: In two out of 17 children without initial tumor burden new PNF were identified in follow-up examinations. One of these two patients developed two larger PNF of 4.5 cm on the right upper arm and of 2.5 cm on the left thoracic wall around the age of twelve. The second child developed multiple small PNF along all larger peripheral nerves. In addition, 15 of the children without initial tumor burden did not develop any distinct tumors for at least six years.

Conclusion: Our results indicate that PNF can be newly detected in pediatric patients over time, even if no PNF were detectable on initial MRI scans. This finding strengthens the argument for repeated whole-body MRI scans over time in pediatric NF1 patients to identify growth of PNF at an early stage and assess potential malignant transformation. One possible reason for the new detection of PNF in follow-up studies could be that the tumors can develop over the course of a lifetime, but further research is needed this regard.

Figure 1: Exemplary images of coronal T2-weighted, fat saturated (SPAIR) magnetic resonance imaging sequences of two patients acquired at 1.5 or 3T:

Patient 1 (a,b), female patient 10 years of age, who displayed no tumor burden neither at first scan in 2009 (a) nor at the follow-up examination in 2017 (b).

Patient 2 (c,d), male patient 10 years of age, who displayed no tumor burden at first scan in 2007 (c). In the follow-up examination in 2021 (d), two larger distinct nodular lesions were detected in the right upper arm (arrow) and the left thoracic wall (arrowhead).

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Characterization of NF1 Loss in IDH-WT GBM Using Next Generation Sequencing and Immunohistochemistry

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Purpose: Functional loss of NF1 has been associated with sensitivity to MEK inhibition in low-grade and some high-grade gliomas. Partial sensitivity in sporadic glioblastoma with NF1 loss may be a harbinger of exploitable vulnerabilities. Detection of NF1 loss, however, is fraught given the large size of the gene, challenges with complete coverage and variant calling upon sequencing, and mechanisms of protein regulation that result in early degradation in the absence of genomic alterations. Here, we seek to perform a composite analysis for NF1 loss, accounting for genomic alterations and protein expression via immunohistochemistry. We also characterize the landscape of *NF1* alterations in *IDH*-wildtype glioblastoma.

Methods: We assembled a single-institution, retrospective cohort of 542 *IDH*-wildtype glioblastoma and used next generation sequencing to investigate the prevalence and characteristics of detected *NF1* alterations. Germline testing was not routinely obtained. Sixty-nine glioblastoma were used build a tissue microarray (TMA): 44 *NF1*-wildtype and 25 *NF1*-mutant (one patient had NF-1). We performed NF1 immunohistochemistry using two NF1 antibodies (NFC, Sigma-Aldrich; and iNF-07E, Infixion) scored by three independent pathologists, and correlated results with immunohistochemical, genomic, and clinical features.

Results: In our retrospective cohort, we identified 88 (16%) *IDH*-wildtype GBM with *NF1* alterations. *NF1* alterations were found to be mutually exclusive with *EGFR* alterations ($Log_2(OR)=-2.3$, p-adj<0.001), but tended to co-occur with *PIK3R1* alterations ($Log_2(OR)=-1.6$, p-adj$=0.3$). Of the 69 tumors in the TMA, 14 had genomic loss-of-function alterations in *NF1;* among these, 12 (86%) demonstrated loss of NF1 immunostaining by NFC antibody, while 10 (67%) demonstrated loss of NF1 immunostaining by iNF-07E. Among *NF1*-wildtype gliomas in the TMA, NF1 immunostaining by NFC antibody was lost in 11 (26%) and 4 (10%) by iNF-07E, potentially reflecting differential protein regulation. Across all TMA tumors, loss of NFC immunostaining was mutually exclusive with *EGFR* alterations (p$=0.034$) but co-occurred with alterations in *TSC2* (p$=0.02$) and *PIK3R1* (p$=0.049$), consistent with our larger patient dataset. Loss of NFC immunostaining was associated with decreased median overall survival (7.3 vs 14.4 months, p$=0.027$). Cox proportional hazards model correcting for prognostic variables in this subset revealed HR 5.4 (95%CI 1.94-15, p$=0.001$) associated with loss of NF1 expression. When stratified by iNF-07E IHC, median overall survival did not differ between groups.

Conclusions: NF1 immunostaining may serve as a valuable extension to next generation sequencing for defining NF1 status in *IDH*-wildtype glioblastoma.

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Race and Ethnicity Differences in Treatment-Limiting Toxicities in Patients Treated with MEK Inhibitors

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Background: MEK inhibitor (MEKi) therapies are used to treat pediatric low grade glioma (pLGG) and plexiform neurofibroma (PN) in patients with Neurofibromatosis Type 1 (NF1). The toxicity profile reported in clinical trials have been favorable, but samples have been demographically homogeneous. In this study, we evaluate differences in treatment-limiting toxicities by race and ethnicity.

Methods: This is a single-institution, retrospective study of patients treated with MEKi either on a clinical research study or off-study for pLGG or PN from 2014 to 2023. Patient data was abstracted from clinical records. Treatment-limiting toxicity was defined as temporary hold, dose reduction, or discontinuation due to toxicity.

Results: Fifty-eight evaluable patients received MEKi for PN (N=40) or pLGG (N=18; 6 NF1, 12 sporadic), 5 of whom received multiple agents. Selumetinib was used in 44 cases, trametinib in 16 cases, and binimetinib in 3 cases. Median age was 11.0 years (IQR 7.7-14.5) and 37 (64%) were male. Twenty-nine (50%) patients identified as White, 15 (26%) as Black, 6 (10%) as Asian, 4 (7%) as Hispanic, and 4 (7%) as other/declined. Treatment-limiting toxicity occurred in 27 (47%) patients. Each demographic group had a similar rate of treatment-limiting toxicities; 52% (n=15) of White, 40% (n=6) of Black, 66% (n=4) of Asian patients, and 50% (n=2) of Hispanic patients. The most prevalent treatment-limiting toxicity was skin-related (N=8; 5 White, 1 Black, 2 Asian) and included rash, paronychia, and impetigo. Other recurring treatment-limiting toxicities were gastrointestinal (N=6, 5 White, 1 Black), peripheral edema (N=4, 1 White, 1 Black, 1 Asian, 1 Hispanic), cardiac (N=2, 1 White, 1 Black), and weight gain or loss (N=4, 2 White, 2 Asian).

Conclusions: The overall frequency of treatment-limiting toxicity was similar across race and ethnicity. Dermatologic and gastrointestinal toxicity were more commonly seen in White patients relative to other adverse events. Findings should be interpreted with caution due to the small sample size of non-White patients, but support future efforts to understand demographic differences in MEKi toxicity.

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Treatment Patterns and Healthcare Resource Utilization of Pediatric Patients with Neurofibromatosis Type 1 and Unresectable Plexiform Neurofibroma in Canada

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Background: Neurofibromatosis type 1 (NF1), a rare genetic disease, often appears in early childhood. Plexiform neurofibromas (PNs) are benign, often painful neural tumors that develop in ~30-50% of patients with NF1. The burden of NF1 and PNs in Canada is understudied, limiting the ability to measure therapeutic value of new treatments. This study describes treatment patterns and healthcare resource utilization (HCRU) of pediatric patients with NF1 and ≥1 unresectable PN in Canada prior to selumetinib approval.

Methods: This retrospective chart review extracted data across 3 Canadian multispecialty healthcare sites. Pediatric patients aged ≤18 years were included if diagnosed with NF1 from 1 January 2000 through 30 June 2020, had ≥1 unresectable PN, were followed for ≥12 months, and had ≥3 visits to a specialty clinic before 30 June 2021. All analyses were descriptive.

Results: This study included 53 patients, with 35 (66.0%) reporting PN symptoms. The median age of first unresectable PN diagnosis was 5.0 years. Median follow up was 99.0 months. All patients had ≥1 NF1-related manifestation. Twenty-two patients (41.5%) reported only symptomatic unresectable PN, 18 (34.0%) reported only asymptomatic unresectable PN, and 13 (24.5%) reported both. Twenty-eight patients (52.8%) received treatment for unresectable PN(s). Among patients receiving treatment (n=28), 13 patients (52%) with symptomatic and 1 patient (33.3%) with only asymptomatic PNs underwent debulking or partial resection; 7 of these patients (all with symptomatic PNs, 53%) reported ≥1 complication. Only patients with symptomatic PNs received surgeries other than debulking or partial resection (n=13, 52%). More patients with symptomatic PNs received chemotherapy (16, 64%) and radiotherapy (2, 8%) than patients with asymptomatic PNs (1 and 0 patients, respectively). Pharmacotherapy (e.g., kinase inhibitors, pain medications) was received by 84% of patients with symptomatic and 66.7% with only asymptomatic PNs who received any treatment. PN(s) progressed for 20 patients (71.4%) during or after treatment. All patients (n=53) received outpatient specialist care, mostly ophthalmologists, orthopedic surgeons, and endocrinologists. More patients with symptomatic PNs visited the emergency department (54.3%) and were hospitalized (60%) than patients with only asymptomatic PNs (50% and 22.2%, respectively). Five patients, all with symptomatic PN, had ≥1 malignant PNs.

Conclusions: Patients with NF1 and unresectable PN(s) face substantial monitoring and treatment burdens, resulting in high HCRU. Specific unmet needs for these patients included the high rate of supportive care treatment and economic burden. This is one of the first studies to characterize burden of NF1 and PNs in Canada.

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The Significance of Incidental Findings Beyond Tumors in Children and Young Adults with Neurofibromatosis Type 1 Who Undergo Whole-Body Magnetic Resonance Imaging

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Purpose: Characterize non-tumor findings (NTFs) of whole-body magnetic resonance imaging (WB-MRI) in a cohort of children and young adults with neurofibromatosis type 1 (NF1) to guide pre-imaging patient counseling.

Methods: We performed a prospective study of individuals with confirmed NF1 under 25 years of age who underwent WB-MRI as part of a longitudinal imaging and plexiform neurofibroma biomarker study at Ann & Robert H. Lurie Children's Hospital of Chicago in collaboration with the National Institutes of Health from 2022 to 2023. NFTs were interpreted as findings not related to the purpose of the imaging. Clinical significance was stratified using published criteria into three categories: low significance, potentially significant and significant. Change of management was defined as new referrals, imaging and/or therapy/intervention. NFTs were also categorized based on anatomical location and known NF1 relationship.

Results: 45 out of 65 (69.2%) individuals who underwent WB-MRI were noted to have at least one NTF. For these 45 individuals, the mean age was 16 years (range: 9-23 years) and 25 (55.6%) were female sex assigned at birth. A total of 120 NTFs were identified with an average of 2.7 NTFs per individual (range: 1-11). Of these 120 NFTs, 69.2% were low clinical significance, 15% were potentially significant and 15.8% were significant. Of the changes in management derived from significant and potentially significant NTFs, additional imaging was most common (15.3%), followed by clinical follow-up (8.1%) and new referrals (6.5%). No therapy/interventions were performed in this cohort for the NTFs. The most common anatomical distribution for the NTFs was musculoskeletal (47.5%), primarily spinal curvature deformities. This was followed by central nervous system (CNS) (18.3%), primarily spinal focal areas of signal intensity (FASIs). To date, follow-up imaging for the latter has been performed in two individuals, showing stable lesions. The majority of NTFs were considered not NF1-related (61.7%). Of the 41 NF1-related NTFs, most were musculoskeletal (56.1%). 4.1% of the NTFs had uncertain relation to NF1 based on current literature. We also identified a novel bone dysplasia in the sacroiliac joint in two individuals which may be a unique finding in individuals with NF1.

Conclusions: In a population under 25 years of age, our findings suggest that most NTFs are musculoskeletal, have no or low clinical significance and the majority are not NF1 related. This information should be discussed with individuals undergoing WB-MRI screening for plexiform neurofibromas and associated malignancies.

Growth Modulation for Tibial Bowing in Neurofibromatosis Type 1

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Skeletal findings in neurofibromatosis type 1 (NF1) such as long bone dysplasia can cause significant morbidity. The tibia is the most affected long bone with anterolateral bowing and cortical thickening/medullary canal narrowing as the first presentation frequently leading to fracture and non-union (i.e. pseudarthrosis). The etiology is not fully understood and therapies are lacking. Conditional *Nf1* mouse models with complete inactivation of *Nf1* in osteoprogenitor cells result in some skeletal phenotypes, and we have shown in humans that *NF1* loss of heterozygosity is found in the hyperproliferative tissue between the fractured bone segments (although not in DNA extracted from the cortical bone segments).

Not all NF1 individuals with tibial bowing progress to fracture/pseudarthrosis. Hypotheses for progression to pseudarthrosis include contribution of biomechanical forces on an abnormal bony matrix. We previously performed assessment of bone quality in NF1 individuals with dysplastic tibial bowing that had not yet fractured (n=21) using a Sunlight Omnisense 7000P scanner to measure the speed of sound (m/s) at the mid-shaft compared to their unaffected tibia. Results showed that the speed of sound z-score mean difference was -2.4 when comparing the bowed vs. unaffected tibia. Individuals younger than 8 years had lower mean difference speed of sound z-scores (-2.7 vs. -1.4) suggesting the importance of delaying fracture. We have also previously obtained surgical samples of discarded affected tibia, and histomorphometry confirm an abnormal bone quality. The additive impact of mechanical forces on this abnormal bony matrix to disease progression is likely.

Most surgical interventions occur post-fracture. However, pre-fracture interventions could potentially lead to better outcomes. Some suggest that surgical growth modulation to guide growth of the distal tibial physis prior to fracture could decrease mechanical forces on the apex of the bowing and reduce cortical hyperostosis and fracture risk. We present results of 6 individuals who underwent growth modulation with anterior-lateral distal tibial tension band plate prior to fracture. All individuals had improvement or no worsening of angulation of the tibial bowing. None have sustained a tibial fracture post intervention to date.

We hypothesize that improving alignment with growth modulation can result in decreased strain on a compromised osseous matrix to help prevent/delay fracture and improve weight bearing periods. Although the bone quality is still abnormal and future therapies to proactively improve bone quality of the affected tibia would be beneficial, guided growth modulation of the distal tibial physis appears to improve tibial angulation.

cfDNA End Motif Deconvolution Identifies Atypical Neurofibroma

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Purpose: Early detection of neurofibromatosis type 1 (NF1) associated peripheral nerve sheath tumors (PNST) could inform clinical decision-making, potentially averting deadly outcomes. Approximately 50% of patients with NF1 develop benign plexiform neurofibromas (PNs) with a subset of PN evolving into pre-malignant atypical neurofibromas (ANs) and, ultimately, malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs account for the majority of NF1-associated mortality, however differentiating AN from PN and MPNST remains clinically challenging as a result of insensitive clinical exams, overlapping findings on imaging, and tissue heterogeneity and sampling biases on biopsy. Biopsy also carries the risk of peripheral nerve injury, further complicating the diagnostic workup. We hypothesized that patterns in cfDNA fragment end motifs could distinguish pre-malignant AN from non-malignant PN and from malignant MPNST.

Methods: Blood plasma was collected from patients with PN (n = 69), AN (n = 35), MPNST (n = 60), and healthy controls (n = 21). Plasma cfDNA was isolated and whole genome sequencing (WGS) was performed to a target depth of 6x. cfDNA WGS reads were profiled for fragment 5'-end 4-nucleotide patterns (end motifs). End motifs were deconvoluted by non-negative matrix factorization (NMF) into distinct cleavage patterns (F-profiles). The ability of F-profiles to differentiate tumor types was evaluated in one-versus-one (OVO) comparisons and benchmarked against other cfDNA methods, copy number alteration (CNA) and bin-wise fragment length ratios.

Results: cfDNA fragment end motifs have been shown to reflect cfDNA processing and are altered in malignancy. Here, we show plasma end motifs also distinguish among pre-malignant states in patients with NF1. Motif diversity score (MDS), an aggregate measure of motif diversity, tended to be higher in patients with MPNST, but could not differentiate between clinical states (Fig. 1). Deconvoluted by NMF, motifs contributed non-randomly to F-profiles, with some motif contributions consistent with specific DNase activity (Fig. 2). Several individual end motif F-profiles were able to differentiate clinical states (Fig. 3). Incorporated together in a logistic regression model, F-profiles differentiated clinical states with receiver-operator characteristic areas under the curve (ROC AUCs) between 0.62 and 0.94. F-profiles performed better than CNA or bin-wise fragmentomics when differentiating AN from healthy controls (AUC 0.81) and from PN (AUC 0.83).

Conclusions: This study demonstrates that the spectrum of benign, pre-malignant and malignant peripheral nerve sheath tumors have distinct, disease state-specific cfDNA end motif signatures. Resolved as F-profiles, these signatures can differentiate between pre-malignant states, potentially facilitating accurate early detection.

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Figures:

Figure 1: Distribution of motif diversity scores (MDSs) in each clinical state. No clinical states were statistically different by MDS (Kruskal Wallis H-statistic p-value > 0.05).
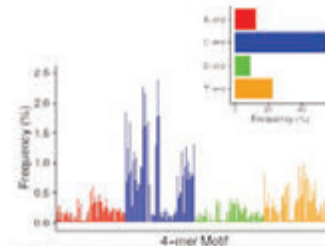
Figure 2: Percent contribution of 4-mer end motifs in F-profile 6. Cytosine end enrichment in F-profile 6 as CCNN is consistent with preferential cleavage by DNase1L3.
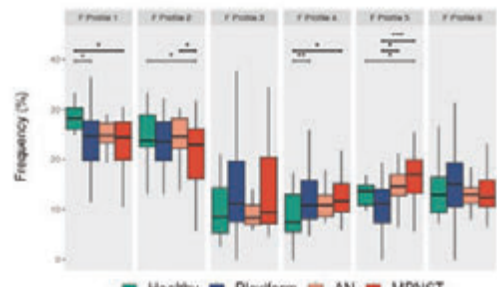
Figure 3: Percent contribution of NMF-deconvolved end motif profiles (F-profiles) in each plasma cfDNA sample by clinical state (p-values by Tukey post-hoc test after Bonferroni-corrected ANOVA).

Identifying At-Risk Groups for Non-Transition Among NF1 Patients Moving to Adult Healthcare

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The transition from pediatric to adult healthcare, known as Healthcare Transition (HCT), marks a crucial phase in the lives of adolescents and young adults (AYA). It signifies the commencement of assuming control over charge of their own treatment management and the coordination of their medical care.

Adolescents with chronic conditions, including neurofibromatosis type 1 (NF1), demonstrate significantly less readiness for HCT compared to their peers without such conditions, leading to disrupted continuity of care, poorer health outcomes and increased healthcare costs.

The SMART model, an extended socioecological framework for AYA transition readiness, pinpoints 7 alterable constructs posited to shape the preparedness for transition: knowledge, skills/self-efficacy, relationships/communication, psychosocial/emotions, developmental maturity, beliefs/expectations and goals/motivation.

These constructs can be further segregated into those intrinsic to the patient and their family, as well as those inherent to both patient and healthcare provider. Additionally, barriers linked to the healthcare provider and the system at large, such as the lack of coordinated services and insurance coverage, play a pivotal role.

Evaluating these factors within our population is important because it enables the proper execution and effectiveness of interventions for transition of care. Particularly, because these elements can differ among organizations and be influenced by transition preparation interventions.

Currently, we are enlisting individuals aged 14 to 22 years with NF1 who are under the care of our CTF-sponsored NF Clinic. During this presentation we compare the readiness and needs across a diverse population to identify those at higher risk for non-transition into adult care.

Participants will complete the validated Self-Management and Transition to Adulthood with Rx=Treatment (STARx), the NF1-specific version of the Transition Readiness Assessment Questionnaire (NF-TRAQ), and the Brief Health Literacy Screening Tool (BRIEF) to measure health literacy (HL). Patient demographic and clinical information is collected from medical records. The main outcomes include the STARx and NF-TRAQ scores, with analyses comparing primary language, literacy levels, and clinical data. Results are pending.

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Management of Communication Disorders and Swallowing Concerns in Patients with Neurofibromatosis Type 1: A Rapid Review

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Purpose: To integrate available data on intervention for disorders related to communication and swallowing for individuals with Neurofibromatosis Type 1 (NF1).

Methods: The databases PubMed and PsycINFO were searched to identify case reports, case series, randomized control trials, or waitlist control trials published between 2014 and 2024 in English using the following search strategy: (Neurofibromatosis type 1) AND (speech OR language OR voice OR swallowing OR dysphagia OR cognitive communication) AND (intervention OR management).

Results: A total of 13 articles were retrieved and reviewed; 5 provided evidence for intervention methods or tools seeking to target communication or swallowing concerns for individuals with NF1.

Conclusions: Social language and phonological processing were highlighted as areas for intervention for individuals with NF1 with deficits in these domains. Remote microphone listening devices were shown to be helpful in improving classroom listening for school-aged children with NF1. Evidence was provided in support of reading intervention utilizing a multisensory approach for children with learning disabilities and NF1. Additional research into MEK inhibitors that utilize an alternative to a tablet formulation for individuals with NF1 was recommended. Significant gaps remain in the literature concerning evidence-based interventions targeting communication disorders and swallowing concerns for individuals with NF1.

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Phase II Trial of the MEK 1/2 Inhibitor Selumetinib in Adults with Neurofibromatosis Type 1 (NF1) and Inoperable, Symptomatic or Progressive Plexiform Neurofibromas (PN) – Clinical Responses and Pharmacodynamics

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Introduction: Selumetinib is a MEK inhibitor (MEKi) approved in the US for the treatment of children aged ≥2 years with NF1 and symptomatic, inoperable PN based on durable PN shrinkage and clinical benefit seen in the SPRINT study[1]. We present here the results of an open-label phase 2 study of selumetinib in adults with inoperable, symptomatic or progressive NF1 PNs (NCT02407405).

Methods: This study was a single-site phase 2 trial with a Simon 2-stage design. Eligible participants were people with NF1 ≥18 years old with inoperable/symptomatic/ progressive PN. Selumetinib (50 mg by mouth twice daily) given continuously (1 cycle=28 days). Biopsy of PN performed at baseline and on-treatment (pre cycle 2 or 3). The effect of selumetinib on target inhibition in PN biopsies was assessed with a validated multiplex immunoassay, kinome analysis using multiplexed kinase inhibitor bead (MIB), and RNA sequencing. PN response definitions were: partial response (PR) volume decrease ≥ 20% from baseline; progressive disease (PD) volume increase ≥20% from baseline, and objective response rate (ORR) included PR confirmed on the next consecutive restaging evaluation. Changes in pain were evaluated by patient reported outcomes including pain intensity (Numeric Rating Scale-11 (NRS-11)) and pain interference (Pain Interference Index (PII)). Adverse events (AEs) were graded with CTCAEv5.0.

Results: As of April 2022, the study had completed enrollment with 33 participants (pts) (median age 35.6, range 18.3-60.2, 11 female), with an ORR of 63.6% (21/33 pts), no PD. Median best response was -23.6% (range: -48.1% to 5.5%) at a median of 28 treatment cycles (range: 1-78). From baseline to cycle 12, tumor pain intensity and pain interference improved (p=0.002 for both, n=28). Paired pre- and on-treatment PN biopsies were obtained with minimal complications from 24 pts (73%). Phosphorylation ratios of ERK1/2 decreased significantly on treatment (median change ERK1: -64.6%, ERK2: -57.7%, p≤0.001 for both) without compensatory phosphorylation of AKT1/2/3. Kinome analysis showed a significant (log2 fold) decrease in MEK2 MIB binding, which correlated with PN volume change after cycle 4. RNAseq analyses showed downregulation of RAS pathway genes, as well as a contraction of macrophages and an increase in fibroblasts in the treated PN. Fourteen pts (42%) required dose reduction for AEs, and 2 discontinued treatment due to toxicity (elevated ALT/AST). AEs were all reversible and similar to those in the pediatric trial, with acneiform rash (92%), asymptomatic increase in creatine kinase (87%) and alanine aminotransferase (55%), dry skin (70%), pruritis (61%), and limb edema (55%) being the most common.

Conclusions: Selumetinib for adults with NF1-PN resulted in a similar ORR (63.6%) to children in the SPRINT study (68%) with associated improvement in pain and no PD. AE profile was also similar to the pediatric study. Correlative studies on paired PN biopsies were feasible and confirmed target inhibition and provide insights into the mechanism of PN response to MEKi. A Phase 3 trial of selumetinib for adults with NF1-PN (KOMET, NCT04924608) is ongoing.

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Multiple Sclerosis and Neurofibromatosis Type 1: Case Series and Systematic Literature Review

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Purpose: Neurofibromatosis type 1 (NF1) is an autosomal dominant, neurocutaneous disorder with an estimated incidence of 1 in 3000 people. Multiple sclerosis (MS) is an autoimmune, demyelinating disorder with a prevalence of 309.2 per 100,000 adults in the United States. There has been documentation of co-occurrence of NF and MS preexisting in the literature. The rates of occurrence are higher than expected by chance, suggesting that an underlying predisposition may exist. We aimed to systematically review the literature to better understand the concurrence and contribute four additional patients to the existing literature.

Methods: This study is a retrospective case series and a systematic review. Pubmed, Embase, Cochrane, Scopus, and Web of Science were queried for full articles in English reporting on cases of concurrent NF1 and MS between 1990-2023. Search terms included neurofibromatosis 1, NF1, and multiple sclerosis, MS, neuromyelitis optica, MOG, and MOGAD, among others.

Results: Our novel case series includes 4 female patients, ages of 39-64 years old. All patients were diagnosed with NF1 in childhood, three in infancy. Two patients had familial NF1, two were de novo cases. All patients first became symptomatic from MS in their 30s (median age: 38.5 years). All had a Karnofsky Performance Score (KPS) of 90% at their last office visit. Three were prescribed disease modifying therapy (DMT). The DMTs used were glatiramer acetate, dimethyl fumarate, ocrelizumab, and Siponimod.

The systematic review revealed 34 reported cases of patients with both NF1 and MS in the literature across 19 papers. Twenty patients were female and 14 were male. Thirty-two patients were first diagnosed with NF1. One patient had a preexisting diagnosis of MS. One patient was diagnosed with NF1 and MS simultaneously. Ten patients had primary progressive MS, 7 had relapse-remitting MS, 5 had secondary progressive MS, and 12 did not have a specific MS sub-diagnosis. Thirteen had a positive family history of NF1, though six did not have data about NF1 family history. Thirty-three had at least one reported brain lesion and 18 had at least one reported spine lesion.

Conclusions: Although rare, NF1 and MS can occur within the same patient. Previous literature has been inconsistent in the information reported about the patients with these concurrent diagnoses. Consistent reporting of data will help characterize the dual disease processes. Additional research is required to better understand the concurrent diseases including whether there may exist a predisposition.

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Level of Physical Activity (PA) and Factors Related to PA Engagement in Youth with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PNs): An Exploratory Study

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Background: NF1 is associated with lower motor proficiency, problems with coordination, and decreased strength. Children with NF1 and PNs also may experience numbness and pain; thus, engaging in physical activity (PA) may be difficult. The CDC recommends 60 minutes of moderate-to-vigorous PA (MVPA) daily for children. This sub-study explored the degree to which youth with NF1 and PNs engaged in MVPA as well as youth and parent beliefs, perceived barriers, and family support regarding PA engagement.

Methods: Youth with NF1 and PNs, ages 6-17 years, who enrolled in a natural history study (NCT00924196) and their parents were eligible for this sub-study. Youth completed neurobehavioral assessments that included parent ratings of disease severity, and parent and self-report measures of behavioral and social-emotional functioning and quality of life (QOL). Surveys (adapted from Sallis' questionnaires[1]) that assessed estimated MVPA over the past week and factors associated with engagement in PA were mailed to 60 families; an additional 8 families were approached at the NIH clinic. Completed surveys within one year of a neurobehavioral assessment were included. Descriptive statistics, non-parametric statistical tests and regression were used, and the alpha was set at $p < 0.05$.

Results: Thirty-two families returned valid surveys (57% response rate) and all 8 families approached in person participated. The final sample consisted of 35 parent/child dyads and 5 parent surveys only (mean age of child=12.6 years, range 6-18; White=78%). Sixty-one percent of parents rated their child's NF1 disease severity as moderate/severe. The average number of days per week youth engaged in 60 minutes of MVPA was 1.9 per parent report and 1.8 per child report (SD=2 for both). Parents reported that the top 3 reasons why their child did not engage in PA were pain (32%), fatigue (32%), and physical difficulty for the child (30%); youth endorsed fatigue (51%), mood (43%), and pain (40%). Total weekly minutes of MVPA was higher in children with mild versus moderate/severe NF1 severity ($p < 0.05$). Parents' and children's beliefs regarding the ability of people with NF1 to engage in PA, as well as youths' perceived family support, were positively correlated with MVPA engagement over the past week ($p < 0.05$ for all), but only child beliefs about abilities and family support predicted PA engagement ($p < 0.05$ and $p < 0.001$, respectively). In terms of QOL, fewer days engaged in 60 minutes of MVPA per parent report was associated with greater child reported pain interference ($p < 0.05$), sense of inadequacy ($p < 0.05$), and low self-reliance ($p < 0.05$).

Conclusions: The level of MVPA in this cohort is substantially below the CDC guideline of 60 minutes per day. NF1 severity, pain, fatigue, mood, and physical difficulty performing PA are among the top reasons for low PA engagement. Results suggest that more PA engagement is associated with less pain interference and a higher sense of self-agency, suggesting that interventions targeting these constructs would be worthwhile. As PA engagement was related to beliefs about exercise and perceived family support, educating families about children's PA capabilities and potential benefits of PA may help to encourage children to engage in PA and promote a healthy lifestyle.

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Challenges Associated with Urogenital Plexiform Neurofibromas in Neurofibromatosis Type 1 (NF1)

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Purpose: Plexiform neurofibromas (PN) in neurofibromatosis type 1 (NF1) pose a substantial challenge, particularly in young children, given their high growth rates and risk to progress to malignancy. These tumors can manifest in any peripheral nerve throughout the body, leading to pain, functional impairment and disfigurement, significantly impacting the overall disease burden. While diffuse neurofibromas affecting the urogenital tract are rare, their management presents unique challenges. Surgical interventions often fall short in achieving complete tumor excision, carry the risk of postoperative deficits and fail to prevent tumor regrowth or malignancy. In this context, MEK inhibitors emerge as a promising therapeutic option for treating plexiform neurofibromas.

Methods and Results: In this report, we present two cases: a 3-year-old and a 15-year-old boy with NF1, both exhibiting large plexiform neurofibroma masses affecting the prostate, bladder, lower ureters and male genital organs. These tumors were incidentally discovered during abdominal ultrasound and routine whole-body MRI screenings, following established guidelines. Despite the absence of subjective complaints or overt symptoms such as functional impairment, dysuria or infection, the 3-year-old boy developed minimal bladder dysfunction with urinary retention during follow-up, coupled with a 23.8% increase in tumor volume over one year, so that MEK inhibitor therapy with selumetinib was initiated. In the adolescent case, a 52.7% growth over three years was observed, with MRI findings suggesting an atypical neurofibromatous neoplasm with uncertain biological potential (ANNUBP). A multidisciplinary team recommended surgical resection.

Conclusion: Our case reports underline the need for a non-surgical therapy for inoperable PN which now is available since the approval of the MEK inhibitor selumetinib. While selumetinib is currently only indicated for symptomatic, inoperable PN the course of our adolescent patient suggests that excessive tumor growth could have been prevented by early treatment. Therefore presymptomatic selumetinib treatment might be advisable if continued tumor growth indicates that the occurrence of symptoms will only be a matter of time.

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Developing a European Community of Practice for Specialist Nurses in Neurofibromatosis Type 1: Experiences from Clinical Nurse Specialists and Advanced Nurse Practitioners in the Netherlands and the United Kingdom

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Purpose: *Neurofibromatosis type 1* (NF1) is a complex disease that requires a multidisciplinary approach. The role of the 'specialist NF nurse' in care for people with NF1 is yet unexplored, and undoubtedly varies throughout Europe. The natural disease course of NF1 is uncertain and unpredictable in when and which disease-related manifestations will occur during life. Precisely because of this disease unpredictability, a better knowledge and understanding of the role of the specialist NF nurse in NF1 care is pivotal. Therefore, we joined forces to create a *community of practice* to encourage collaboration among specialist NF nurses working in Neurofibromatosis Centers in Europe.

Methods: A qualitative approach based on in-person and digital conversations between specialist NF nurses (i.e. clinical nurse specialists and advanced nurse practitioners) in the Netherlands and the United Kingdom was carried out between September 2022 - February 2024.

Results: Unmet needs and current gaps in nursing collaboration were explored. The specialist NF nurse has a valuable contribution and can be of greater value integrated within multidisciplinary care (i.e. as a coordinating clinician). Although the interpretation (and naming) of the role of specialist NF nurse varies between the Netherlands and the United Kingdom, and presumably also within other European countries, the aim to improve healthcare delivery and strengthening care provided by physicians are equal. But, a lack of clarity exists between the (visible) impact and number of specialist NF nurses working in other Neurofibromatosis Centers in Europe. There is a need for a collaborative space for sharing experiences and best practices. Creating knowledge is evident for improving care provided by a clinical NF nurse. By further exploring the scope of the nursing profession collaboratively, we can strengthen our impact in care for people with NF1.

Future plans: There are no initiatives for international collaborations between specialist NF nurses within Neurofibromatosis Centers in Europe yet. An opportunity for collaboration is a community of practice (CoP). A CoP is an organized group of people with a similar expertise to enable dialogues. Every specialist NF nurse within Europe can voluntarily join this group. Further content needs to be explored collaboratively, preferably by a joint meeting at the Global Neurofibromatosis Conference 2024 for introduction.

Key message for this abstract: As specialist NF nurse pioneers, we believe that collaborations at the level of the nursing professions will be valuable in NF1. This is a unique opportunity that we believe will be the beginning of a fruitful collaboration among specialist NF nurses in Europe.

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Sexuality in Adults with Neurofibromatosis Type 1 : A Cross-Sectional Study

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Purpose: Limited information exists regarding the impact of neurofibromatosis type 1 (NF1) on patients sexuality, despite its significance for quality of life. We therefore explored how adult patients with NF1 experienced their sexuality.

Methods: A cross-sectional single-center study was conducted among adult patients with NF1. An online questionnaire included a socio-demographic and a sexual-specific section. Sex life was ranked on a self-report scale ranging from 0 (very unhappy) up to 10 (very happy). Male sexual function was measured by the International Index of Erectile Function (IIEF). Female sexual function was measured by the Female Sexual Function Index (FSFI) and the Female Sexual Distress Scale-Revised (FSDS-R). Experienced sex life was analyzed by means and standard deviations (SD). To identify the correlation between sexual functioning and the various domains of the respective questionnaires, the Pearson correlation was used. Differences between variables were analyzed using parametric tests.

Results: Out of 568 patients, 294 respondents (52%) started the questionnaire, with 202/294 (69%) completing it. The mean age of respondents was 37 years (SD 14.0) and 63% were female. Self-reported sex life was rated a 4.9 out of 10 (SD 3.1) for the total group. About 48% scored their sex life a 5 or below out of 10. Men scored their sex life a 5.0 out of 10 (SD 2.8). Sixty-seven percent of men reported erectile dysfunction on the IIEF, correlating with lower sex life ratings (4.0 vs. 7.2, p<.001). Women scored 4.9 out of 10 (SD 3.3) for their sex life with 64% reporting sexual dysfunction on the FSFI, associated with lower sex life ratings (3.8 vs. 7.5, p<.001). Women with sexual dysfunction scored lower on all FSFI scales (p<.001).

Conclusion: Half the respondents scored their sex life as inadequate, with about two-thirds experiencing a sexual dysfunction. This research seems important to better address sexuality in a clinical setting. Qualitative research is needed to uncover disease-specific barriers and unmet needs in sexuality among adult patients with NF1.

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Functional and RNA-Based Assays Improve Variant Classification and Diagnostics for Individuals with Neurofibromatosis Type 1 and Related Disorders

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Purpose: Obtaining a clinical and molecular diagnosis in neurofibromatosis and related disorders can be challenging due to the phenotypic diversity, the size and complexity of the affected genetic loci and uncertainty over the effects of some variants on pre-mRNA splicing and/or protein expression and function. To help identify pathogenic variants in individuals with neurofibromatosis, schwannomatosis or Noonan syndrome, we analyzed DNA and RNA from peripheral blood and/or affected tissue samples, performed *in vitro* functional experiments and applied bioinformatic approaches.

Methods: DNA was isolated directly from peripheral blood and cultured skin fibroblasts using standard procedures. DNA was isolated from formalin-fixed sections of tissue samples after enrichment for the areas containing affected cells. RNA was isolated either from PAX blood tubes or cultured skin fibroblasts using standard procedures. RT-PCR and transcriptome analysis was performed as described previously [Douben *et al.,* doi: 10.1002/humu.24487]. Exon trap experiments to investigate the effects of identified variants on pre-mRNA splicing, and *in vitro* functional assessment of the effect of *NF1* variants on the RAS GTPase activating protein (GAP) activity of neurofibromin, the neurofibromin-SPRED1 interaction and neurofibromin stability were performed as described previously [Douben *et al.,* doi: 10.1155/2023/9628049].

Results: We will present an update of our functional data, including the classification of new *NF1* variants and a comparison of our *in vitro* results with different predictive algorithms, including SpliceAI and AlphaMissense. In addition, we will highlight recent developments in our functional approaches, including the expression and functional characterization of full-length neurofibromin missense variants.

Conclusions: Multidisciplinary approaches, combining bioinformatics and *in vitro* functional analysis, as well as in-depth analysis of patient material, contributes to improved molecular diagnostics for individuals with neurofibromatosis and related disorders.

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Neurofibromatosis Type 1 Patient Registry in the Russian Federation: A Step Towards Comprehensive Care

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Neurofibromatosis type 1 can be considered as one of the most prevalent genetic disorders. The prevalence of neurofibromatosis is approximately 1:3000 newborns, with no gender or ethnic predisposition. The disorder arises from pathogenic variants in the *NF1* gene, which lead to a disruption in the synthesis of neurofibromin — a protein that regulates the crucial PI3K/AKT/mTOR pathway. Due to the multisystem involvement, a multidisciplinary approach is essential for the management of neurofibromatosis, involving the collaboration of physicians from various specialties. Additionally, dynamic monitoring and the availability of advanced medical care for these patients, sometimes necessitating the engagement of multiple specialized centers, are of utmost importance.

For the first time, data on the national register of patients with neurofibromatosis type 1 created in Russia is presented. This represents a unique experience given the vast size of the Russian Federation, its population size, multicultural composition, and uneven distribution of medical centers across the country.

In our registry (by 31.12.2023), 3004 patients from 82 different regions of the Russian Federation have been registered (out of 85 regions by date of 31.12.2021). The gender distribution is: males - 49.87%, females - 50.13%. The average age is 15.3 years, with the youngest patient being 3 months old and the oldest 77.9 years. DNA-diagnostics were performed for 82.46% of the patients, with an effectiveness reaching 58%. Among the confirmed cases, SNPs are accounted for 91.66% of cases, while large CNVs accounted for 8.33%. No "hot spots" were found that are characteristic of any ethnic group. In 39.1% of cases, the pathogenic genetic variant was inherited from one of the parents, while in 60.1% arose de novo.

The main clinical manifestations and their frequency are presented in Table1:

Feature	%of Persons
Café au lait macules	96,91%
Intertrigenous freckling	49,39%
Cutaneous neurofibromas	42,64%
Speech delay	24,64%
Plexiform neurofibroma(s)	20,1%
Optic glioma	12,69%
Lisch nodules	8,14%
Intellectual disability	6,76%
Scoliosis	5,56%
Long bone dysplasia	5,1%
ADHD	4,99%
Seizures	3,22%
ASD	1,77%

Register data allows us to understand the problems facing Russian healthcare system. Obvious problems in monitoring our patients are the underrepresentation of ophthalmic examinations and the relatively small percentage of adult patients. An intriguing conclusion is the high frequency of provided DNA-tests, but the efficiency is clearly lower than the worldwide. These data will be analyzed somewhat more fully, and the results of incorporating RNA-analysis into the diagnostic protocol of our patient's protocol will also be presented.

Unstuck and On Target: A Feasibility and Acceptability Study in Children with Neurofibromatosis Type 1

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Purpose: The primary aims of this study were to examine the feasibility and acceptability of the Unstuck and On Target (UOT) executive function intervention in school-aged children with Neurofibromatosis Type 1 (NF1). Exploratory aims were to examine cognitive and psychosocial outcomes following intervention.

Methods: Participants were recruited from our Neurofibromatosis (NF) Clinic with a confirmed NF1 diagnosis, aged 8-14 years, English dominant, with IQ>70, and no sensory or behavioral impairments that would interfere with participation. Qualified participants engaged in 20 weekly group UOT sessions. Demographics, medical history, parent rating of intervention expectations, and neurocognitive assessments were completed prior to treatment and at end of treatment. We hypothesized that the intervention would be feasible (>85% attendance, dropout rates) and acceptable to participants and families. We also explored the individual change from pre- to post-treatment in participants' executive function. We provide descriptive statistics to report on feasibility, pre-intervention expectations, and post-intervention acceptability/satisfaction. Reliable change methodology was employed to analyze pre- to post-intervention executive functioning.

Results: Four participants were enrolled on trial (3 male, ages 9-12). Attendance was 100% with no attrition over 20 sessions (6 months). Primary challenges caregivers hoped the intervention might address including flexibility, working memory, and social-emotional functioning. Post-treatment, parents rated the intervention as moderately to very beneficial (ratings 4-5/5). Parent meetings with the group leader were rated as very beneficial (5/5), but homework activities were rated as less helpful (2-3/5). Parents indicated that participants learned a great deal during intervention (4-5/5) and were able to apply the skills in daily life modestly well (3-4/5). All parents indicated improvements in flexibility and planning abilities resulting from the intervention. Exploratory data examining pre- to post- intervention executive function will also be presented.

Conclusions: The UOT group executive function intervention was found to be feasible and acceptable to the participants and their families and was rated as beneficial to the functioning of the participants by parents. Parent support and group interaction for parents and participants were seen as highly beneficial. Challenges primarily surrounded home practice. This first study of UOT in children with NF1 suggests that this is a feasible and beneficial treatment option targeting the significant executive dysfunction experienced by many children with NF1.

A Retrospective Study of 121 Patients with Plexiform Neurofibroma of Head and Neck Undergoing Surgical Treatment

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Objective: The purpose of this study was to provide a basis for further consensus formation by analyzing the clinical manifestations, surgical conditions, tumor recurrence, post-operation satisfaction, and changes in quality of life of patients undergoing plexiform neurofibroma (PNF) surgery in head and neck.

Methods: Through medical record review and telephone follow-up, a retrospective analysis was conducted on neurofibromatosis type 1 (NF1) patients admitted for surgical treatment for PNF patient in head and neck from May 2012 to July 2022 in Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Based on the data about changes in quality of life before and after surgery and long-term surgical satisfaction, patients were divided into surgical benefit and non-benefit groups. Binary and multivariate logistic regression analysis were used to analyze the clinical characteristics of patients with long-term surgical benefit.

Results: Totally 512 patients with head and neck PNF were admitted for surgery with complete medical records. 121 patients were identified as NF1 related PNF with effective follow-up was obtained. There were 70 males and 51 females, aged (25.60 ± 12.85) years old, ranging from 7 to 63 years old, with 41 patients who were $\leq$ 18 years old and 80 patients over 18 years old. The incidence of postoperative complications was 6.05%(13/215). The follow-up period after last operation was (51.41 ± 27.66) months, and 42.15%(51/121) of patients reported postoperative tumor recurrence. 76.03%(92/121) of patients were very satisfied with immediate postoperative results, while the rate decreased to 46.28%(56/121) during long-term follow-up. Family members of patients who were $\leq$ 18 years old had a higher proportion of dissatisfaction with the scars caused by surgery and a stronger willingness to undergo another surgery. The tumor recurrence was closely related to surgical benefits (OR=2.317, P<0.05). Further analysis found that the gender and age of patients were the main risk factors for the recurrence. The recurrence rate of patients $\leq$ 18 years old was 3.485 times higher than in that over 18 years old (P<0.01), and the highest in the 7–12 year-old group, reaching 68.42%(13/19). The recurrence in male patients was significantly lower than that in females [37.50%(24/64) vs. 54.00%(27/50), P<0.05].

Conclusion: Clinical diagnosis and treatment in PNF should focus on the applications in comprehensive methods such as full preoperative evaluation, active MDT cooperation and combined therapies in order to improve the safety and effectiveness of treatment and reduce tumor recurrence.

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Evaluation of Non-Monoexponential Diffusion Models for the Classification of Peripheral Nerve Sheath Tumors in Patients with Neurofibromatosis Type 1

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Background and Purpose: Diffusion-weighted imaging with monoexponential apparent diffusion coefficient (ADC) imaging is an established tool in tumor imaging and is able to distinguish benign (BPNST) from malignant peripheral nerve sheath tumors (MPNST) in patients with neurofibromatosis type 1 (NF1). Non-monoexponential models allow parameterization of additional tissue characteristics, with potentially lower numerical stability and longer computation times. The purpose of this study was to investigate the ability of the ADC model and six non-monoexponential diffusion models to differentiate BPNST and MPNST, to investigate their sensitivity to tumor perfusion, and their numerical properties.

Methods: In this retrospective study, 33 patients (mean age 31.0 years; 18 female) with 71 tumors (53 BPNST, 18 MPNST) were included. The investigated non-monoexponential diffusion models were intravoxel incoherent motion (IVIM), kurtosis, stretched-exponential, and the statistical models based on truncated Gaussian, gamma, and beta distributions. Fitted model parameters were compared by Mann-Whitney-U-tests. Numerical aspects were evaluated using the normalized analyzable pixels, the coefficient of determination, and the information criteria. Receiver operating characteristics (ROC) were analyzed.

Results: All modeled diffusion coefficients were significantly higher in BPNST than in MPNST (all $p < 0.05$) (Figure 1). The ADC model yielded a sensitivity of 94% and a specificity of 77%. The ROC analyses of all non-monoexponential models, except the IVIM model, equaled or exceeded the area under the curve of the ADC model of 93.8%, while their curves did not differ significantly from the ADC model (all $p > 0.05$) (Figure 2). The IVIM model showed the lowest number of analyzable pixels of around 75%. The stretched exponential model performed similarly to the ADC model and provided a heterogeneity index that appears to be sensitive to tissue perfusion.

Conclusion: The monoexponential ADC model and non-monoexponential diffusion models all sufficiently differentiate BPNST from MPNST. None of the investigated diffusion models performed best overall, therefore the choice of model depends on user requirements.
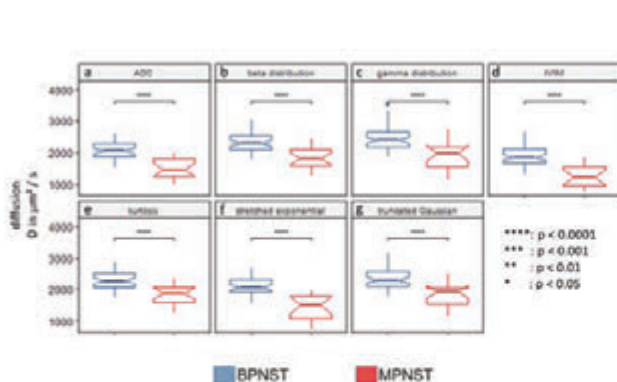
Figure 1: Comparison of benign (BPNST) and malignant peripheral nerve sheath tumors (MPNST) by fitting seven diffusion models to diffusion-weighted data. For all models applied, BPNST showed significantly higher diffusion values than MPNST.

[The "*" indicate statistical significance. BPNST: Benign peripheral nerve sheath tumor; MPNST: malignant peripheral nerve sheath tumor]
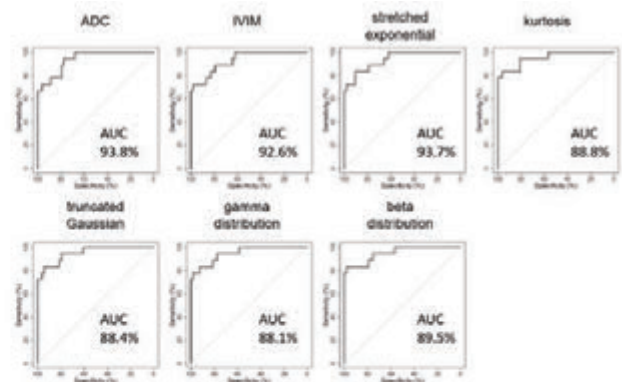
Figure 2: ROC analyses for differentiation of benign and malignant peripheral nerve sheath tumors in patients with NF1 by mono- and non-monoexponential diffusion models.

[ADC: Apparent diffusion coefficient; IVIM: intravoxel incoherent motion]

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Sphenoid Wing Dysplasia with Orbital Plexiform Neurofibroma: When to Consider MEK Inhibitor to Prevent Progression

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Introduction: Sphenoid wing dysplasia (SWD) occurs in ~3% of individuals with neurofibromatosis type 1 (NF1), typically characterized by pulsatile exophthalmos and diagnosed radiographically with bone defects of the orbital wall.[2,3] It has been proposed that SWD is a result of intrinsic bone tissue abnormalities associated with neurofibromin deficiency[4] and is often associated with orbital-periorbital plexiform neurofibromas (OPPN)[3-5] that can lead to cosmetic and visual impairment.[6-8] There is no standard treatment for SWD and surgical interventions are typically performed to improve cosmetic appearance and/or visual acuity.[9] However, patients often require multiple surgical revisions[5,9] and little advancements have been made on the role of medical management of OPPN to reduce recurrent deformities and progressive visual decline. We present a case of a patient with SWD and OPPN with persistent proptosis and orbital displacement despite initial surgical fixation.

Case Presentation: TB was clinically diagnosed with NF1 due to the presence of typical multiple cafe-au-lait macules and SWD. On imaging, she had a temporal encephalocele involving the orbit. At 23-months of age, she underwent left fronto-temporal-orbital craniotomy and repair of temporal encephalocele with autogenous bone graft and titanium plating. Operative reports indicate dysplastic bone in the temporal fossa consistent with 'fibrous dysplasia'. She continued to have significant exophthalmos of the affected eye and presented to our NF Clinic for initial evaluation at age 9 after referral to our surgical colleagues to evaluate for reconstruction of the orbit. Left anterior orbitotomy with tissue mass biopsy confirmed the presence of OPPN, consistent with MRI findings. Imaging showed marked left SWD with bony expansion of the left middle cranial fossa and mass effect and narrowing of the left orbit with proptosis and stretching of the optic nerve.

Conclusions: SWD represents a challenging manifestation of NF1 that can significantly impact both cosmetic appearance and visual function. Current management strategies focus on surgical interventions; however, these approaches frequently necessitate multiple revisions and may not address the underlying pathophysiology. The presented case represents the refractory nature of SWD with OPPN despite prior surgical intervention, highlighting the need to consider the potential role of MEK inhibitors or other medical therapy in preventing further tumor growth and resultant bone modulation. Exploration of medical management options alongside surgical interventions is warranted in future research efforts to elucidate the efficacy of targeted therapies in mitigating the clinical impact of SWD and OPPN in patients with NF1.

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Congenital Head and Neck Plexiform Neurofibromas in Neurofibromatosis Type 1: Complications and Response to Treatment with MEK Inhibitors

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Purpose: This is a retrospective review of the comorbidities, complications, and response to treatment of infants with neurofibromatosis type 1 (NF1) and congenital head and neck plexiform neurofibromas.

Methods: Data was extracted from the medical records of 3 consecutive patients born with large head and neck plexiform neurofibromas seen in the Comprehensive Neurofibromatosis Clinic at Children's Healthcare of Atlanta. Clinical and demographic data was abstracted.

Results: Patient 1 is a Black female born with corneal clouding and neck mas. Imaging showed large mass involving the mediastinum, neck, and orbits. She had biopsy confirmed plexiform neurofibroma. Complications from her plexiform neurofibroma included facial disfigurement, sphenoid wing dysplasia, bilateral corneal clouding, glaucoma and blindness, tracheal deviation requiring tracheostomy, dysphagia requiring feeding tube placement. She started on trametinib at 84 days of age. There was a period where she was sub-optimally dosed. One week after resuming full dose she developed infantile spasms and medication was discontinued. Patient died at 9.5 months of age due to tracheostomy plugging.

Patient 2 is a Black male with neck mass noted at 1 month of age. Imaging confirmed plexiform neurofibroma of the neck, face, skull base and right orbit. No biopsy has been obtained. Complications from his plexiform neurofibroma included facial disfigurement, sphenoid wing dysplasia, proptosis, and glaucoma. He had focal epilepsy and global development delays. He started on selumetinib at 5.5 years of age. No radiographic or clinical response has been noted after 7 months of treatment.

Patient 3 is a White male. He was noted to have proptosis at birth. Imaging reviewed a large plexiform neurofibroma involving the left orbit, skull base, face, and neck. Biopsy confirmed plexiform neurofibroma. Complications from his neurofibroma include proptosis and glaucoma of the right eye resulting in blindness, vascular dysplasia of left middle and anterior cerebral arteries, aneurysm of left middle cerebral artery requiring surgical resection, progressive atrophy of left cerebral hemisphere. He has focal epilepsy and global developmental delays. He started on selumetinib at 7 months of age, but this was stopped after 1 month due to concern for possible retinal detachment.

Conclusion: Patients with large congenital head and neck plexiform neurofibromas are at elevated risk for significant comorbidities, including glaucoma, blindness, developmental delays, epilepsy. Neither of the infants treated with MEK inhibitor remained on treatment long enough to determine efficacy.

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Tear Proteomics in Children and Adolescents with Neurofibromatosis Type 1

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Introduction: Optic pathway glioma (OPG) occurs in 15-20% of patients with Neurofibromatosis I (NF1). Although in NF1, OPG are generally considered low-grade, 20-30% of patients experience progressive vision loss. OPG in NF1 predominantly affect young children and require close monitoring during childhood. Currently, there is no reliable biomarker to assess the risk of NF1 patients developing OPGs.

Eyes are seen as an extension of the brain, suggesting that tear proteins could serve as valuable biological markers, reflecting central metabolism and offering potential insights into assessing neurological disorders. Moreover, tears are collected easily in a non-invasive fashion. Tears contain a complex mixture of proteins, lipids, electrolytes, and various organic molecules, produced by the lacrimal glands. Proteomic analyses have identified over 2500 proteins in tears, highlighting their potential richness for diagnostic insights.

Objective: This study aims to examine the tear proteomic profile of NF1 patients and its correlation with their clinical manifestations and to identify potential early detection biomarkers for OPG development in NF1.

Methods: Tears were collected using Schirmer strips. Proteomic analysis was conducted using state-of-the-art sample preparation and LC-MS/MS data-independent acquisition. We conducted a comparative analysis of tear samples from 35 NF1 patients, ranging in age from 6 months to 17 years old. Six of them were confirmed to have OPG on neuroimaging. We investigated variances in the tear proteomic profiles between NF1 patients with and without optic nerve glioma and we correlated the tear proteomic profile with the clinical and laboratory parameters of the patients. The quantitative proteomic results were processed within the Perseus software suite for statistical evaluation and data interpretation.

Results: Statistical analysis revealed overexpression of the **Serine/threonine-protein kinase A-Raf** (ARAF gene) protein in children with NF1 and optic glioma. This overexpression was statistically significant compared to NF1 children without optic glioma. The ARAF gene encodes a proto-oncogene protein belonging to the RAF family of serine/threonine-specific protein kinases. The ARAF protein plays essential roles in cell signaling pathways, particularly the RAS-RAF-MEK-ERK pathway, which regulates various cellular processes such as cell growth, proliferation, differentiation, and survival.

Conclusions: The observed overexpression of the **A-Raf protein** in the tears of NF1 patients with OPG suggests that the tear proteomic profile reflects the pathological cell growth, proliferation, and differentiation characteristic of OPGs. These preliminary findings suggest the potential utility of tear analysis in identifying biomarkers for OPGs in NF1 patients, marking a promising first step in this direction.

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The Dermatologist's Role for the Management of Plexiform Neurofibromas in Child Patients with Neurofibromatosis 1 in Japan

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Since the approval in Japan in 2022 of the MEK inhibitor selumetinib for treatment of child patients with inoperable plexiform neurofibromas (PNs), multidisciplinary team building and interprofessional work have been gradually established for the management of neurofibromatosis 1 (NF1) under the Japanese Society of Recklinghausen disease initiative. Our facility was certified as a member of the NF1 Japan Clinic Network in 2023. Courses of treatment for PNs, including application of selumetinib, have been determined by board members (dermatologist, pediatrician, plastic surgeon, orthopedic surgeon, head and neck surgeon, brain surgeon, neurologist, ophthalmologist, radiologist, clinical geneticist and pharmacist) at Tottori University Hospital. Here we report a case series of 3 pediatric patients with NF1. The details are as follows.

A 15-year-old girl was referred to our department for evaluation of PN on her left cheek. It was determined that there was no indication for treatment with selumetinib because resection of the tumor was technically possible. Finally, complete resection was performed by a plastic surgeon, one of the NF1 board members.

A 5-year-old girl presented with painful PNs on the right side of her neck. Administration of selumetinib was recommended by the NF1 board since it was judged that complete resection was impossible without functional disturbance. She continued to take oral medication for 6 months. She showed drug-related alopecia as an adverse event. The patient was followed annually by dermatologists with the help of a pediatrician and pharmacist.

A 15-year-old girl presented with large PN on the left side of her face. Although she underwent partial resection of the tumor 5 years ago, the tumor gradually re-enlarged. Oral medication of selumetinib was recommended by the board. Drug-induced acneiform eruption, paronychia and chalazion were treated by dermatologists with administration of selumetinib for 1 year.

In conclusion, we consider that dermatologists can play a key role in the management of NF1, especially in transitional care of PN that requires long-term selumetinib medication.

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Visual-Spatial/Motor Abilities of Individuals with Neurofibromatosis Type 1: A Systematic Review and Meta-Analysis

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Background: Previous studies have reported inconsistent findings regarding visual-spatial/motor abilities in children with Neurofibromatosis type 1 (NF1) compared with the general population. This study aims to provide a robust estimate of mean differences in visual-spatial/motor abilities between NF1 and general populations and explore how sample characteristics and study designs are associated with group differences, through meta-analyses.

Methods: Data for this study was derived from a broader systematic review project focusing on the neurobehavioral functions of individuals with NF1. Systematic literature searches were conducted in Scopus, PsycINFO, Web of Science, PubMed, and ProQuest, utilizing NF1-related terms and neurobehavioral functioning-related terms (e.g., visual-spatial). Papers measuring visual-spatial/motor abilities in individuals with NF1, and comparing them with a control group or using standardized scores, were further coded. Hedges' g values were computed to indicate differences between the NF1 and the control groups (healthy community control, unaffected siblings, and normative data) and were pooled using the robust standard error estimation technique and random model. Meta-regression and subgroup analyses were performed to examine potential moderators of group differences.

Results: The analysis included 71 unique samples, involving 3,084 participants with NF1 (Figure 1). The sample mean ages ranged from 0.53 to 41.40 years. Individuals with (vs. without) NF1 exhibited lower visual-spatial abilities ($k = 81$, $g = -0.90$; 95% CI [-1.02, -0.79], $p < .001$, $I^2 = 66\%$) and worse visual-motor abilities ($k = 108$, $g = -0.84$; 95% CI [-0.96, -0.73], $p < .001$, $I^2 = 70\%$, Table 1). Moderate to large group differences remained after adjusting for publication bias (Table 2). Larger group difference in visual-spatial abilities were significantly associated with a higher percentage of depression diagnosis ($\beta = -0.01$, $p = .020$) and anxiety diagnosis ($\beta = -0.01$, $p = .020$). A larger group difference in visual-motor abilities was related to a lower full scale IQ ($\beta = 0.04$, $p = .042$). Group differences in visual-spatial/motor abilities were also influenced by measurement types. However, mean age, gender composition, the percentage of the White, familial NF1 cases, ADHD diagnosis, and ASD diagnosis, presence of a brain tumor, and control types were not significant moderators ($p > .11$) (Table 3).

Conclusions: Individuals with NF1 exhibited lower visual-spatial/motor abilities, highlighting the necessity for increased support and interventions to enhance the visual-spatial/motor abilities of NF1 population. There is substantial between-study heterogeneity in group differences, underscoring the need to examine potential predictors of group differences between NF1 and general populations.

Table 1. Summary of Mean Effect Size across Studies

	Hedge g	LL	UL	SE	df	p-value	n	k	Tau²	I² (%)
Visual-Spatial Abilities	-0.90	-1.02	-0.79	0.06	48.23	< .001	51	81	0.12	66
Visual-Motor Functioning	-0.84	-0.96	-0.73	0.06	43.80	< .001	47	108	0.13	70

Notes: LL = lower limit of 95% confidence interval; UL = upper limit of 95% confidence interval; SE = standard error; df = degrees of freedom; n = number of studies; k = number of effect sizes; Tau² = Tau-square; I² = I-squared.

Table 2. Sensitivity Analysis of Effect Size

	Funnel Plot Symmetry Test (Egger's Test)		Trim and Fill Analysis						Robu Meta Regression				
	Z	p	N of trimmed studies	Filled N	Fill d ES	p	95% CI	t	B	SE	p	n	k
Visual-Spatial Abilities	0.17	.869	51	0	-0.91	< .001	[-1.02, -0.79]	-15.92	-0.01	0.70	.985	51	81
Visual-Motor Functioning	-1.94	.053	57	10	-0.71	< .001	[-0.85, -0.58]	-10.79	-1.51	0.63	.031	47	108

Note. Z = standard normal distribution score; p = probability value; filled N = number of filled studies; filled ES = effect size after filling the hypothetical unpublished studies; t = t-value; CI = confidence interval; B = estimated regression coefficient; SE = standard error; n = number of studies; k = number of effect sizes.

Table 3. Tests of All the Potential Moderators

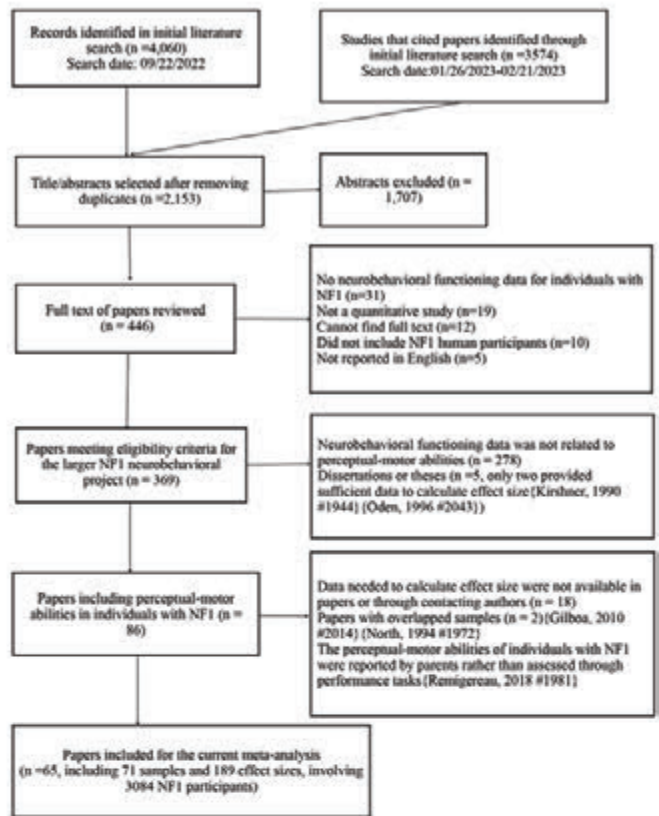| Moderator	n	k	β	SE	LL	UL	df	P value
Visual-Spatial Abilities								
Mean age	49	78	0.01	0.00	-0.01	0.02	2.97	.205
% Girl	45	73	-0.01	0.00	-0.02	0.00	7.28	.163
% White *	8	11	0.00	0.01	-0.04	0.04	2.58	.768
% familial NF1	16	28	0.00	0.01	-0.06	0.06	1.49	.707
% ADHD diagnosis	22	34	0.00	0.01	-0.02	0.02	4.13	.900
% learning disability diagnosis NF1	11	19	0.00	0.00	0.00	0.01	5.60	.381
% depression disorder diagnosis NF1	**6**	**11**	**-0.01**	**0.00**	**-0.02**	**0.00**	**3.95**	**.020**
% anxiety disorder diagnosis NF1	**6**	**11**	**-0.01**	**0.00**	**-0.03**	**0.00**	**3.95**	**.020**
% ASD diagnosis	11	22	0.00	0.01	-0.05	0.05	1.36	.796
Mean full scale IQ	45	73	0.02	0.01	-0.01	0.05	16.89	.212
Mean verbal IQ	35	59	0.02	0.02	-0.03	0.06	10.59	.384
Mean performance IQ	35	59	0.01	0.02	-0.03	0.04	11.69	.665
Excluded brain tumors (no vs. yes)	51	81	-0.10	0.11	-0.32	0.12	26.62	.371
Community (vs. siblings)	51	81	0.01	0.13	-0.28	0.30	11.98	.939
Community (vs. normative data)	51	81	0.07	0.12	-0.17	0.32	40.84	.544
Siblings (vs. normative data)	51	81	0.06	0.14	-0.24	0.36	12.00	.648
Children (vs. adolescents)	48	77	0.28	0.16	-0.08	0.63	8.21	.115
BBDT (vs. other)	**51**	**81**	**0.37**	**0.11**	**0.16**	**0.59**	**41.67**	**.001**
Visual-Motor Functioning								
Mean age	43	101	0.00	0.01	-0.03	0.03	3.50	.991
% Girl	41	98	0.00	0.01	-0.02	0.01	10.84	.354
% White *	10	16	-0.01	0.01	-0.02	0.01	3.59	.458
% familial NF1	18	45	-0.01	0.00	-0.02	0.00	2.46	.169
% ADHD diagnosis	14	41	0.00	0.01	-0.02	0.01	3.49	.534
% learning disability diagnosis NF1	7	19	0.00	0.00	-0.02	0.01	2.36	.458
% ASD diagnosis	8	20	0.01	0.01	-0.03	0.05	1.50	.296
Mean full scale IQ	**38**	**90**	**0.04**	**0.02**	**0.00**	**0.08**	**10.31**	**.042**
Mean verbal IQ	29	65	0.05	0.03	-0.02	0.12	4.52	.104
Mean performance IQ	29	68	0.03	0.02	-0.01	0.07	12.40	.122
Excluded brain tumors (no vs. yes)	47	108	-0.05	0.11	-0.28	0.17	42.84	.654
Community (vs. siblings)	47	108	0.23	0.16	-0.12	0.57	13.56	.184
Community (vs. normative data)	47	108	0.09	0.15	-0.21	0.39	30.34	.545
Siblings (vs. normative data)	47	108	-0.14	0.11	-0.38	0.11	11.61	.255
Childrens (vs. adolescents)	42	99	0.03	0.21	-0.50	0.56	5.28	.897
Childrens (vs. adults)	42	99	-0.01	0.24	-0.69	0.68	3.62	.979
Adolescents (vs. adults)	42	99	-0.03	0.30	-0.77	0.70	6.11	.911
BBDT (vs. GPT)	47	108	0.35	0.17	-0.12	0.82	4.15	.107
BBDT (vs. RCFT-CO)	47	108	0.25	0.17	-0.12	0.62	11.39	.172
BBDT (vs. WIS-BD)	**47**	**108**	**0.24**	**0.12**	**-0.02**	**0.49**	**19.81**	**.009**
BBDT (vs. WIS-OA)	47	108	0.12	0.20	-0.32	0.55	13.10	.569
BBDT (vs. other)	**47**	**108**	**0.39**	**0.11**	**0.17**	**0.61**	**20.60**	**.002**
GPT (vs. RCFT-CO)	47	108	-0.11	0.22	-0.64	0.42	5.95	.640
GPT (vs. WIS-BD)	47	108	-0.12	0.17	-0.59	0.35	4.28	.535
GPT (vs. WIS-OA)	47	108	-0.23	0.23	-0.81	0.34	5.47	.349
GPT (vs. other)	47	108	0.04	0.16	-0.46	0.53	3.36	.834
RCFT-CO (vs. WIS-BD)	47	108	-0.01	0.18	-0.40	0.38	11.99	.953
RCFT-CO (vs. WIS-OA)	47	108	-0.13	0.24	-0.65	0.39	12.30	.601
RCFT-CO (vs. other)	47	108	0.14	0.16	-0.22	0.50	9.94	.393
WIS-BD (vs. WIS-OA)	47	108	-0.12	0.15	-0.45	0.21	12.05	.452
WIS-BD (vs. other)	47	108	0.15	0.11	-0.08	0.39	22.13	.184
WIS-OA (vs. other)	47	108	0.27	0.18	-0.12	0.67	11.36	.157

Note: n = number of studies; k = number of effect size; β = estimated regression coefficient; SE = standard error; LL = Lower limit of 95% confidence interval; UL = Upper limit of 95% confidence interval; df = degrees of freedom; p = probability value. * The percentage of White individuals was only reported in American samples. Community = community healthy controls; siblings = unaffected siblings as controls; normative = normative data as controls.

BJOL = Benton's Judgment of Line Orientation; WIS-BD = Wechsler Intelligence Scale-Block Design; WIS-OA = Wechsler Intelligence Scale-Object Assembly; BBDT = Beery-Buktenica Developmental Test; GPT = Grooved Pegboard Test; RCFT-CO = Rey Complex Figure Test-Copy; CBTT = Corsi Blocks Tapping Test; CNTAB-SWM = Cambridge Neuropsychological Test Automated Battery-Spatial Working Memory; CNTAB-PAL = Cambridge Neuropsychological Test Automated Battery-Paired Associate Learning; RCFT-DR = Rey Complex Figure Test-Delay Recall.

We only reported results when there were at least six samples for continuous variables and at least four samples in each subgroup of a categorical variable.

Figure 1. Flow Diagram for Inclusion and Exclusion in Meta-analysis

Records identified in initial literature search (n =4,060) Search date: 09/22/2022

Studies that cited papers identified through initial literature search (n =3574) Search date:01/26/2023-02/21/2023

Title/abstracts selected after removing duplicates (n =2,153)

Abstracts excluded (n = 1,707)

Full text of papers reviewed (n = 446)

No neurobehavioral functioning data for individuals with NF1 (n=31)
Not a quantitative study (n=19)
Cannot find full text (n=12)
Did not include NF1 human participants (n=10)
Not reported in English (n=5)

Papers meeting eligibility criteria for the larger NF1 neurobehavioral project (n = 369)

Neurobehavioral functioning data was not related to perceptual-motor abilities (n = 278)
Dissertations or theses (n =5, only two provided sufficient data to calculate effect size (Kirshner, 1990 #1944) (Oden, 1996 #2043))

Papers including perceptual-motor abilities in individuals with NF1 (n = 86)

Data needed to calculate effect size were not available in papers or through contacting authors (n = 18)
Papers with overlapped samples (n = 2) (Gilboa, 2010 #2014) (North, 1994 #1972)
The perceptual-motor abilities of individuals with NF1 were reported by parents rather than assessed through performance tasks (Remigereau, 2018 #1981)

Papers included for the current meta-analysis (n =65, including 71 samples and 189 effect sizes, involving 3084 NF1 participants)

Full List of Authors: Liyan Yu[1], Dan Liu[1], Xian Wu[2], Jing Zhang[2], Julia Moreira[1], Benjamin Felipe Mujica[1], Elora Shelly Mukhopadhyay[1], Angelena Novotney[1], Mary-Mac Chown[1], Yang Hou[1]

[1]Florida State University, [2]University of Kentucky

The Application of Multi-Disciplinary Treatment (MDT) Program in Neurofibromatosis Type 1 (NF 1)

Zhen Zhang, *Shandong Provincial Hospital Affiliated to Shandong First Medical University*

Purpose: NF 1 pose a significant challenge globally. The unmet health care needs of patients and their families, including delayed diagnoses and difficulties in accessing specialized medical care, underscore the imperative for a coordinated and patient-centric approach to NF 1 management. In this program, the authors sought to enhance patient outcomes and reduce throughput time for individuals affected by NF 1 or undiagnosed conditions. Consequently, they implemented a patient-centric, coordinated care model at Shandong Provincial Hospital Affiliated to Shandong First Medical University, the MDT program, which streamlines the complex diagnostic and therapeutic strategies that are essential for these patients. Findings revealed that the MDT facilitated more interactions between different disciplines, promoting effective communication and discussion of diagnostic and therapeutic strategies for patients with NF 1.

Methods: Shandong Provincial Hospital Affiliated to Shandong First Medical University first determined the suppliers of MDT services for NF 1. We established a consultation expert database, which gathered more than 40 clinicians and experts in related fields from both internal and external institutions of the hospital, covering more than 10 professional areas, serving as an alternative expert database for NF 1 specialist consultation services. Then, a dedicated MDT module was established in the electronic medical record system, realizing the informatization of multidisciplinary consultation requests, execution, and records. The project also introduced multiple administrative assistants responsible for the coordination of multidisciplinary consultations, expert invitations, and MDT meeting organization, to maximize communication and communication between the treating team and specialty consultation doctors. In this project, the time limit from the treating doctor's consultation request to the MDT meeting was 2-3 days, in order to allow the treating team and multiple consultation specialties sufficient time to communicate and familiarize themselves with the patient's condition and appeals, truly implementing multidisciplinary collaboration.

Workflows of Conventional Specialist Consultation and the MDT Consultation for Patients With NF at Shandong Provincal Hospital
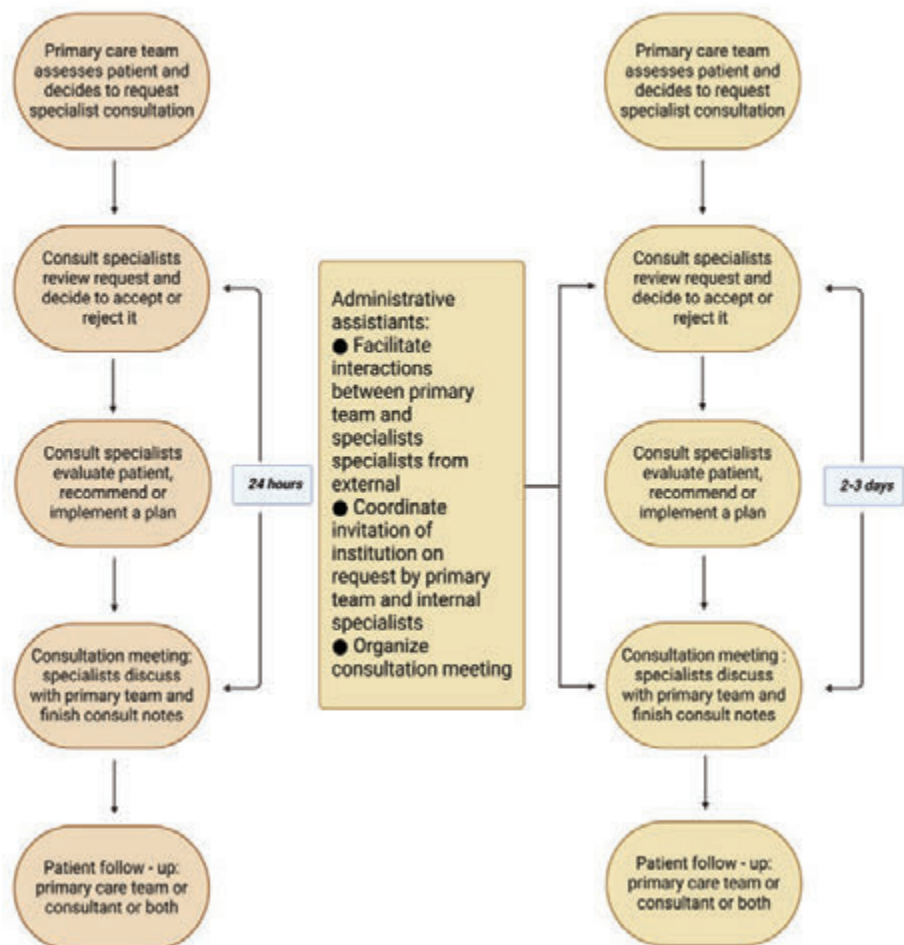
Results: The MDT program for NF 1 can shorten the diagnosis and treatment cycle for NF 1 patients. Through a single outpatient visit, all relevant departments can be consulted, enabling patients to receive homogeneous treatment.

Conclusions: Shandong Provincial Hospital Affiliated to Shandong First Medical University has demonstrated outstanding contributions in the diagnosis, treatment, and research of NF 1, especially in strengthening NF 1 diagnosis and treatment capabilities and hospital-wide collaboration. The hospital has successfully established and promoted the NF 1 MDT model, and promoted the MDT model throughout the province through the Rare Disease Collaboration Network. Additionally, the hospital has also conducted NF remote MDT and provided support to hospitals within the collaboration network in Shandong Province through consultation broadcasts.Supported by the Rare Disease Quality Control Center, the Chinese Rare Disease Alliance, and the Hospital's Eugenics and Genetics Department, this team helps economically disadvantaged families who need genetic testing for diagnostic assistance to apply for corresponding free genetic testing opportunities. It should be emphasized that this project is applicable to the consultation of rare and difficult cases with stable conditions. For critical and severe cases, the relevant emergency consultation or multi-disciplinary consultation system should still be followed. In addition, this project is currently exploring the use of artificial intelligence to assist primary hospitals in NF 1 diagnosis and treatment, so as to help primary hospitals better identify and handle rare diseases, and provide patients with more comprehensive medical services.

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Genotype-Phenotype Correlations of Neurofibromatosis Type 1: A Cross-Sectional Study From a Large Chinese Cohort

Beiyao Zhu, *Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*

Background: Neurofibromatosis type 1 (NF1) is a highly heterogeneous autosomal genetic disorder characterized by a broad spectrum of clinical and molecular manifestations. The correlations between genotype and phenotype in NF1 remain elusive. This study aimed to elucidate genotype–phenotype associations in a large Chinese cohort of NF1 patients.

Methods: We included NF1 patients from our center who underwent genetic testing for NF1 variants and systemic examination. Genotype–phenotype correlation analyses were performed, focusing on variation types and involved neurofibromin domains.

Results: A total of 195 patients were enrolled, comprising 105 males and 90 females, with a median age of 18 years. Truncating variants, single amino acid variations, and splicing variants accounted for 139/195 (71.3%), 23/195 (11.8%), and 33/195 (16.9%), respectively. Patients with splicing variants exhibited a significantly higher prevalence of spinal plexiform neurofibromas (spinal PNF) than those with truncating variants (76.4% vs. 51.8%; p = 0.022). Variations affecting the PKC domain were associated with higher rates of cutaneous neurofibromas (CNF) (100% vs. 64.9%, p < 0.001), Lisch nodules (100% vs. 61.2%, p < 0.001), plexiform neurofibromas (PNF) (100% vs. 95.7%, p = 0.009), and psychiatric disorders (11.8% vs. 1.6%, p = 0.042). Patients with mutations in the CSRD had an elevated risk of secondary primary malignancies (11.6% vs. 2.8%, p = 0.015). GRD involvement might enhance the risk of Lisch nodules (76.9% vs. 53.7%, p = 0.044). Variations in the Sec14-PH domain were correlated with a higher rate of CNF (76.8% vs. 58.6%, p = 0.014). Additionally, we found that the p.R1748* variants carry a high risk of malignancy.

Conclusion: Our study suggested some novel genotype–phenotype correlations within a Chinese cohort, providing innovative insights into this complex field that may contribute to genetic counseling, risk stratification, and clinical management for the NF1 population.

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Unveiling the Limitations of Hydrocephalus Imaging Biomarkers in Adults with Neurofibromatosis Type 1

Alicia M. Zukas, MD, *Medical University of South Carolina (MUSC); Charleston, South Carolina, USA*

Purpose: Patients with neurofibromatosis type 1 (NF1) may exhibit radiographic findings indicative of normal pressure hydrocephalus (NPH). NPH is more prevalent in the elderly population, affecting less than 1% of seniors over age 65. The symptoms of NPH can overlap with the complex symptoms seen in patients with NF1, including gait disturbance, urinary urge incontinence, and cognitive decline. The Evan's index radiographic measurement greater than 0.30 is the standard for hydrocephalus expansion, with multiple other radiographic measures also used to assess ventricular volume. After diagnosing radiographic NPH in two symptomatic seniors using these multiple assessments, negative confirmation through cerebrospinal fluid (CSF) testing followed. This prompted further investigation into the use of these radiographic biomarkers in the NF1 population.

Methods: Within our single-center population, brain MRIs from 33 individuals with NF1 were assessed for indices such as the Evan's index, frontooccipital-horn-ratio, frontal horn ratio, bicaudate index, and callosal angle. Patient ages ranged from 21 to 81 (mean 41), with no patients having shunts or known increased intracranial pressure from mass lesions. Relationships between these indices and age were further assessed through regression analysis.

Results: The Evan's index surpassed 0.30 in two-thirds of our patients, raising radiographic concerns for normal pressure hydrocephalus. A moderately positive correlation between age and Evan's index was observed, with values exceeding the 0.30 threshold in the fifth decade of life. As age increased, the bicaudate index substantially rose, peaking in the fifth decade of life. All patients exhibited a callosal angle suggestive of hydrocephalus ex vacuo.

Summary: These findings suggest that usual radiographic measures for assessing ventricular enlargement in NPH may be unreliable in NF1 patients, given the high proportion exceeding NPH thresholds. A significant number of NF1 patients exhibited values surpassing typical NPH thresholds, potentially leading to unnecessary invasive tests. Common radiographic indices for NPH appeared early in NF1 without clinical correlation, limiting their screening utility. More specific imaging guidelines may be necessary for both NPH and hydrocephalus ex vacuo assessments in NF1 patients.

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NF2-Related Schwannomatosis (*NF2*-SWN): Clinical Science

Clinical Presentations of *NF2*-Related Schwannomatosis in Children: Should Variant-Predicted Severity Influence Surveillance Initiation?

Alise Blake, MS, CGC, *St. Jude Children's Research Hospital*

Purpose: The purpose of this study is to investigate clinical presentations and mutation-predicted disease severity for patients less than 18 years of age with *NF2*-related Schwannomatosis (*NF2*-SWN), often prior to when surveillance is recommended.

Methods: Data were collected for patients with *NF2*-SWN followed at St. Jude Children's Research Hospital from 12/1/2013-2/1/2024. Clinical manifestations were compared to the expected phenotypes based on the genetic severity score, a tool used to predict *NF2*-SWN disease severity.[1]

Results: Eight patients were included (5 males, 3 females). The mean age at *NF2*-SWN diagnosis was seven years (range, 0.9-16 years). Six patients presented with tumors and two were diagnosed through cascade testing. Five patients (62.5%) were diagnosed with multiple, synchronous tumors at initial presentation. Hearing loss was noted in three (44.4%) patients (4 ears) at mean 8.7 years (range, 4-15 years). Four (50%) patients required surgery, three (37.5%) were started on Avastin, and one (12.5%) received proton radiation (Table 1).

Four patients (50%) had *NF2* variants predicted to incur a severe phenotype, two (25%) patients had moderate-predicting variants, and two (25%) patients were mild-predicting.[1] Three (75%) patients with severe-predicting variants meet severe clinical criteria and initially presented with a meningioma between two and seven years (Mean 4.33 years). The fourth patient with a severe-predicted genotype is 11 years with negative baseline surveillance imaging, affected family members have severe phenotypes, and as he is not yet 20 years old his phenotype remains unclear. No patients with variants predicted to confer mild or moderate severity have corresponding phenotypes. Notably Patient 5 who has an exon 14 truncation (predicted moderate) and Patient 7 who has a mosaic splicing variant (predicted mild) both have a severe phenotype (Table 1).

Conclusions: Consistent with the literature, this cohort demonstrates that variants predicting severe disease phenotypes are better indicators of clinical severity than variants predicting mild or moderate phenotypes.[2] Current *NF2*-SWN surveillance begins at 10 years of age. Nevertheless, half of the patients described here were diagnosed with symptomatic tumors before this age, many requiring interventions. Further, other studies report that ~18% of patients are diagnosed with *NF2*-SWN before 10 years.[3-7] Although this was a small sample, with bias due to a tertiary pediatric cancer hospital setting, earlier initiation of surveillance for *NF2*-SWN patients, especially those with variants predicting severe clinical phenotypes, may be warranted. Additional studies are needed to validate these findings and clarify tumor risks before the age of 10 years.

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Table 1: Patient Demographics and Medical History

Patient ID	Sex	Age at NF2 dx (current age)	NF2 Variant	Impact	Family History	Tumor History (Tumors at diagnosis in bold)	Medical History	Clinical severity (Variant-Predicted Severity if discordant)
1	M	7 (8)	c.900dup p.Ile301TyrfsTer5	Truncation of exon 10	No	**3 CNS Meningiomas** **8 Spinal Schwannomas** 3 Cranial Schwannomas – 2 at dx Cutaneous Schwannoma	R hearing loss R Vision loss Proton Radiation to CNS meningioma	Severe
2	F	4 (6)	c.1043delA p.Glu348GlyfsTer16	Truncation of exon 11	No	**CNS Meningioma** **CNS Oligodendroglioma** **Bilateral VS** **2 Spinal Schwannomas** 4 Cranial Schwannomas – 1 at dx	Headaches	Severe
3	F	2 (9)	c.1375C>T p.Gln459Ter	Truncation of exon 13	Yes	**CNS Meningioma** **Bilateral VS** 2 Cranial schwannomas	Cataracts and R vision loss Acid Reflux R hearing loss - Avastin 6-8yo Surgery at 2yo for meningioma	Severe
4	M	10 (12)	c.241dupG p.Val81GlyfsTer5	Truncation of exon 3	Yes	None- cascade testing	Craniosynostosis	TBD (Severe)
5	M	16 (19)	c.1471_1472delTT p.Leu491Ter	Truncation of exon 14	Yes	**Cervical Ependymoma** **Bilateral VS** **2 Spinal Ependymomas** 3 CNS Meningiomas – 1 at dx 5 Cranial Schwannomas – 2 at dx 4 Spinal Schwannomas – 3 at dx	Cataracts Exotropia Seizures Surgery for cervical ependymoma Avastin 17yo-19yo	Severe (Moderate)
6	M	1 (5)	c.447+1G>A	Splicing Variant in intron 4	Yes	None – cascade testing	Nystagmus	TBD (Moderate)
7	M	16 (22)	Mosaic c.363+2T>C	Mosaic splicing Variant in exon 3	No	**Spinal Ependymoma** **Bilateral VS** 6 Cranial Schwannomas – 3 at dx Innumerable Spinal Schwannomas Cutaneous Schwannoma Neurofibroma Plexiform Schwannoma	R Macular Pucker R Vision loss Deaf - Avastin 17yo-20yo Surgery for spinal ependymoma, R VS, cutaneous schwannoma, neurofibroma, and plexiform schwannoma	Severe (Mild)
8	F	7 (9)	c.-1787_114+659 9del	Deletion of Exon 1+ Promoter	No	**CNS Meningioma** **Bilateral VS** 4 Spinal Schwannomas	L Vision loss Surgery for CNS Meningioma	Moderate (Mild)

Note: Patients with TBD phenotype do not currently have any known tumors, but they are too young to characterize as a mild.
CNS=Central Nervous System, Dx = diagnosis, F=Female, L=Left, M=Male, R=Right, TBD=To Be Determined, VS=Vestibular Schwannomas, Yo = years old

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Designing a Novel Measure of Disease-Specific Quality of Life for *NF2*-SWN Clinical Trials: Analysis of Patient and Clinician Interviews

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Purpose: Prior research has shown that existing NF2-related quality of life (QOL) measures may not be sufficiently comprehensive or sensitive to change in clinical trials, highlighting a need for a novel measure. Regulatory guidance for developing new patient-reported outcome measures requires patient and clinician input to generate items.

Methods: Qualitative interviews were conducted with individuals with *NF2*-related schwannomatosis (*NF2*-SWN) enrolled in the INTUITT-NF2 clinical trial and *NF2*-SWN clinicians from the United States, United Kingdom, and Australia. Interviews included concept elicitation of NF2-related symptoms and impacts on QOL in the context of clinical trials. Interview transcripts were coded and analyzed using the Framework Method to document all concepts impacting QOL systematically. A modified scale-level content validity index (CVI-S) procedure was used to identify items with consensus among patients or clinicians. Questions from 6 existing NF2-specific and tumor-specific QOL measures were compared to concepts documented in interviews.

Results: We interviewed 16 individuals with *NF2*-SWN [69% female; age range: 15-54 years] and 10 *NF2*-SWN clinicians [3 neuro-oncologists, 2 neurologists, 2 surgeons, 2 advanced practice nurses, 1 psychologist]. Initial analysis of patient and clinician interviews yielded 83 unique NF2-related symptoms/impacts, with 48 items endorsed by both patients and clinicians (Figure 1). Statistical analysis indicated that 17 of the 83 unique items had sufficient consensus among patients, clinicians, or both (Figure 2). We grouped the 17 items into four domains: physical (e.g., mobility, hearing, balance), emotional (e.g., self-consciousness, uncertainty about the future, anxiety due to symptoms), social (e.g., ability to pursue leisure activities, interaction in social settings, achieving career goals), and medical care (e.g., frustration over care, quantity of surgeries). Of the 17 items, existing QOL measures include questions that assess between 6 (35.3%) and 15 (88.2%) of these items.

Conclusion: *NF2*-SWN has broad impacts across physical, emotional, and social well-being that should be measured through disease-specific QOL measures to evaluate treatment success. *NF2*-SWN symptoms/impacts identified in the study will be further evaluated for importance with a larger patient sample to prioritize items for inclusion in a new NF2-related QOL measure specifically designed for clinical trials. Future research will verify whether the novel measure is reliable, valid, and sensitive to change in clinical trials.

Figure 1. Overlap in Symptom/Impact Items Endorsed By Patients or Clinicians and Whether Assessed in Existing QOL Measures

Legend: Each vertical column indicates a single symptom/impact of *NF2*-SWN or NF2-related tumors, grouped into whether the symptom/impact items were endorsed by both patients and clinicians (48 items), only patients (20 items), or only clinicians (15 items). Shading within boxes indicates the presence (green) or absence (white) of the symptom/impact in patient interviews, clinician interviews, and existing QOL measures.

Figure 2. Most Commonly Endorsed *NF2*-SWN Symptoms/Impacts by Patients or Clinicians
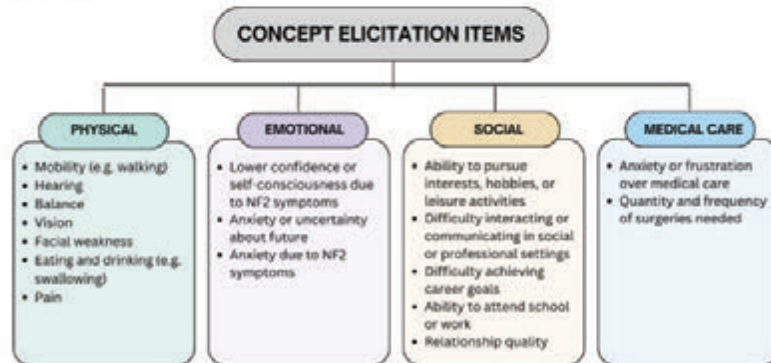
Legend: *NF2*-SWN symptoms/impacts most commonly endorsed by *NF2*-SWN patients or clinicians, as determined by the modified CVI-S procedure.

Complex Rearrangement of Chromosome 22 as Cause of Familiar *NF2*-Related Schwannomatosis

Maria Luisa Garau, *University of Padova, Department of Women's and Children's Health SDB, Padova, Italy*

NF2-related Schwannomatosis is an autosomal dominant condition predisposing to the development of schwannomas and multiple meningiomas. The detection of bilateral vestibular nerve schwannomas is considered enough to establish a clinical diagnosis of *NF2*-related Schwannomatosis. This case report highlights the relevance of considering classical cytogenetic techniques in the diagnostic algorithm of *NF2*-related Schwannomatosis.

Methods: We report a case of a 36 years old male who was referred to our center following diagnosis of recidivating schwannoma of the left VIII cranial nerve and RM anomaly in the right acoustic nerve zone, compatible with bilateral formation. The brother and the mother of our patient presented the same clinical and radiological picture. In addition, the patient spermiogram revealed azoospermia. No other clinical findings were reported, in particular no history of intellectual disability or neurodevelopmental delay.

Results: We proceeded with the sequencing of a panel of genes associated with schwannomatosis predisposing syndromes. This exam and the subsequent MLPA of *NF2* turned out negative. A routine cytogenetic analysis was then carried out from peripheral blood that uncovered the presence of a complex structural rearrangement involving chromosome 22, further characterized by Fluorescent In Situ Hybridisation (FISH) analysis. Molecular characterization of DNA from the left VIII cranial nerve schwannoma was also performed.

Conclusions: Azoospermia and peripheral nervous system tumors are described in patients with complex rearrangements involving the chromosome 22. The application of chromosomal analysis is rarely reported in cases of familial *NF2*-schwannomatosis mostly because complex rearrangements commonly cause impaired spermatogenesis or lead to meiotic arrest. Our case underlines the importance of considering cytogenetic analysis in case of vertical transmission mother-to-offspring.

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Perioperative Evaluation and Monitoring of Intracranial Pressure by Optic Nerve Sheath Diameter Measurements in *NF2*-Associated Intracranial Meningiomas of the Falx

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Objective: To evaluate long-term intracranial pressure in Neurofibromatosis Type 2 (NF2) patients who have been operated on intracranial meningiomas of the falx with/without infiltration of part of the cerebral venous sinus.

Methods: We retrospectively measured bilateral optic nerve sheath diameter (ONSD) with T2-weighted magnetic resonance imaging (MRI) scans of the orbit in 7 NF2 patients with 14 operated intracranial falx meningiomas during their pre- and postoperative follow-up. The mean ONSD of both eyes was calculated for further evaluation.

Results: All tumors were operated on due to critical growth progression. Headache was the most common symptom before surgery (5/14) in 36%, followed by new focal neurological deficits in 29% (4/14) and one patient with nausea and vomiting (7%). None of the patients were treated with a shunt system before surgery. 128 MRI-based ONSD measurements were performed in a mean follow-up of $68 \pm 67 (3\text{-}179)$ months before and 27 ± 35 (1-103) months after surgery. ~71% (10/14) of tumors were totally resected (Simpson grade 1) and the majority of tumors (10/14, ≙71%) infiltrated parts of the cerebral venous sinus. Symptoms postoperatively improved in 86% (12/14) and only 2 cases (2/14 ≙ 14%) exhibited new neurological deficits and postoperative seizures. Up to the last MRI directly before surgery, there was a non-significant ($p > 0.05$) and approximately 2% increase in mean preoperative ONSD values (6.32 ± 1.09 mm) compared to the mean baseline ONSD values (6.16 ± 1.16 mm). Nevertheless, a significant decrease ($p = 0.001$) in mean ONSD values after surgery could be observed (5.79 ± 1 mm). At the last follow-up MRI, values were still stably lower (5.69 ± 1.04 mm) with a minimal tendency to further decrease (approximately 2%).

Conclusion: Falx meningiomas with contact or infiltration to parts of the cerebral venous sinus seem to play an important role in increased intracranial pressure in patients with NF2. Timing of surgery is crucial to avoid the long-term effects of increased pressure. Regular ONSD measurements by ultrasound or MRI could help to find the ideal timing and assess postoperative outcomes. Other influencing and correlating factors such as volumetry, collateral formation, and intracranial tumor burden in general are under investigation.

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A Case of Spinal Ependymoma Associated with NF2

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Spinal ependymoma is a rare disease. Reports of this tumor in neurofibromatosis type 2 (NF2) are rare. A 31-year-old patients presented with gait disturbance, which had been present for 4 years. MRI revealed an intramedullary lesion within the upper cervical spinal cord, which was totally removed surgically. Pathological investigation revealed an ependymoma, WHO grade 2, whole exon sequencing for tumor and matched blood showed this patient harbored *NF2* germline mutation (c.1123-2A>G) and tumor has somatic *SMARCB1* mutation. However, this patient did not present symptoms and physical signs of NF2, brain and spine MRI both did not found neurofibroma.

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Prevalence of Mental Health and Developmental Disorders in Paediatric NF2

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Aim: Quantifying the presence of mental health and neurodevelopmental disorders in a cohort of children with NF2.

Methods: The NHS funded Highly Specialised NF2 lifespan service in Manchester (UK) was established in 2009. We have reviewed the records of all the children 0-18 years of age with a confirmed diagnosis of NF2 in 2024. Our aim is to quantify the presence of neurodevelopmental conditions including Autistic Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD), Learning Difficulties (LD) and mental health disorder including Anxiety, Depressive disorders and behavioural difficulties.

Results: 29 children were identified; 16 male, 13 female. Age range is 2 years to 18 years, median 13 years 7 month, mean 12 years 6 months. Approximately 45% (n=13, 9 males, 4 females) of the children seen by the service (with isolated NF2 or NF2 plus another diagnosis) received active (Child and Adolescent Mental Health Service) CAMHS input.

3 males, two of which are siblings have additional gene deletions (All of which have variable combinations of LD, ASD and ADHD.) These children have not been included.

Of the 26 children with genetically, clinically, and neuroradiologically isolated NF2, 10 children required CAMHS input.

Two children have a formal diagnosis of ADHD (one male and one female) and three a formal diagnosis of ASD (two females and one male). One female child with ASD also has severe behavioural difficulties and severe depression.

6 children have severe intrusive anxiety, three with behavioural difficulties particularly around hospital procedures needing venepuncture. One with school avoidance has been referred for assessment for ASD, which is still in progress. One has had a protracted functional gait disorder after successful surgery, which improved after extensive physiotherapy and support; he too has been referred for assessment of ASD.

38% of our Paediatric NF2 cohort required input from CAMHS. Intrusive anxiety is present in 23% of children and significantly affects their hospital journey and education. ASD in isolated NF2 is present in 11% of children and ADHD is present in 8% of children. Anxiety is a common association and can significantly impact the patient journey.

Conclusion: Mental health problems in paediatric NF2 is under recognised and can significantly impact the patient life journey. Children who have a combination of LD/ASD/ADHD should be investigated for additional genetic causes. NF2 services need to have a high level of paediatric CAMHS support to be able to provide holistic care for their patients.

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The Development of an Auto-Segmentation Tool for the 3D Volumetric Analysis of Vestibular Schwannomas

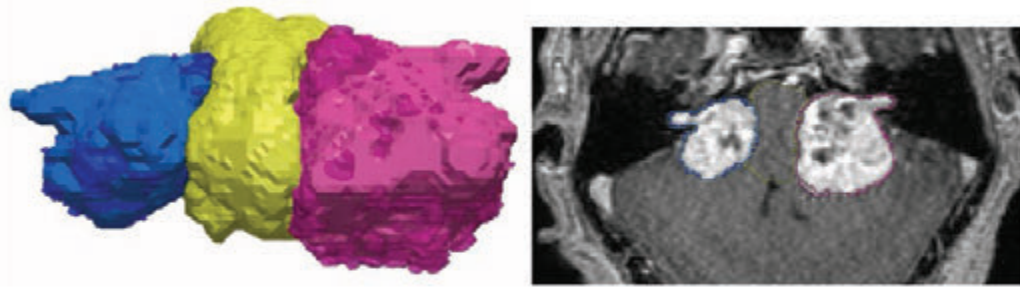**Noemi Jester**, *Yale University Department of Orthopedics and Rehabilitation*

Figure 1. Example of the AI modeler and the tumor models created from the MRI

Purpose and Aim: This project aimed to create an Auto-Segmentation Tool that enables the efficient and accurate three-dimensional (3D) volumetric analysis of Vestibular Schwannomas (VS).

Methods: 143 MRIs from 37 patients were used to create the ground truth data set. To create the tumor models an image processing software (Simpleware ScanIP, Synopsys, Mountain View, CA) was used. A final testing stage was completed using 30 new segmentations of MRI scans which had not previously been used in the creation of the ground truth data set to validate the segmentations produced by the tool to verify its ability to identify and segment tumors in previously unseen patient data. The accuracy of the auto-segmentation tool was measured using the DICE score.

Results: The DICE score calculation was calculated using the equation: Dice Coefficient $= 2 *$ the Area of Overlap / by the total number of pixels in both images.

A mean DICE score of 0.76 (standard deviation 0.21) was achieved in a proof-of-concept of the model. After the final testing stage, the final DICE score in the first version of the AI was 0.89 (standard deviation 0.04).

Conclusions: The results show that our AI prototype is an accurate tool for 3D volumetric analysis of VS tumors, providing a far more accurate visualization of tumor size and growth than other current methods. The usage of this tool and software would not only enable monitoring and surveillance of disease progression but also treatment planning using accurate volumetric measurements and tumor models.

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Project file	DICE	
	POC	Dpp V1.0
Image 1	0.89	0.9
Image 2	0.9	0.93
Image 3	0.91	0.92
Image 4	0.82	0.89
Image 5	0.85	0.93
Image 6	0.86	0.86
Image 7	0.14	0.84
Image 8	0.44	0.88
Image 9	0.47	0.92
Image 10	0.48	0.91
Image 11	0.75	0.87
Image 12	0.74	0.86
Image 13	0.78	0.88
Image 14	0.75	0.74
Image 15	0.92	0.88
Image 16	0.89	0.84
Image 17	0.88	0.83
Image 18	0.89	0.92
Image 19	0.91	0.88
Image 20	0.45	0.87
Image 21	0.91	0.93
Image 22	0.88	0.89
Image 23	0.9	0.92
Image 24	0.93	0.92
Mean	**0.76**	**0.88**
Standard Deviation	**0.21**	**0.04**

Figure 2. Table showing improvement in DICE scores between the initial tool and the current AI modeler.

Phase II Study of Axitinib in Patients with Neurofibromatosis Type 2-Related Schwannomatosis and Progressive Vestibular Schwannomas

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Purpose: Axitinib is an oral multi-receptor tyrosine kinase inhibitor targeting vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR) and c-KIT, which represent a clinically and/or preclinically validated molecular targets in vestibular schwannoma (VS).

Methods: Eligible participants were age >5 years with a clinical diagnosis of neurofibromatosis type 2-related schwannomatosis (*NF2*-SWN) and at least one volumetrically measurable, progressive VS. Axitinib was given continuously in 28-day cycles for up to of 12 cycles. Primary endpoint was objective volumetric response rate to axitinib, hearing response was a secondary endpoint, along with validated quality of life assessments (NFTI-QOL).

Results: Twelve participants were enrolled and eight completed 12 cycles, including two pediatric patients. Two patients were non-evaluable due to coming off study prior to the first scheduled response evaluation: one withdrew from the study for personal reasons and one for non-compliance. Due to slowing accrual, the study closed prior to meeting planned enrollment of 17 evaluable patients. Ten patients were evaluable for the primary endpoint, defined as ≥20% decrease in VS volume, with two volumetric responses observed; both were reached after three cycles and sustained during treatment. Best volumetric response was −53.9% after nine cycles. Maximum change in tumor volume during protocol therapy for all target VS compared to baseline (waterfall plot) is shown in Figure 1. Hearing response was evaluable in nine participants, with three hearing responses observed, one of which was sustained during treatment. All participants experienced drug-related toxicities, the most common were diarrhea, hematuria and skin toxicity, not exceeding grade 2, as well as hypertension, not exceeding grade 3. NFTI-QOL scores remained stable or improved during treatment in all participants.

Conclusions: Axitinib therapy targeting VEGFR, PDGFR and c-KIT is feasible in this population and associated with volumetric and hearing responses in a subset of patients. However, convenience of oral administration should be balanced with respect to efficacy and safety of axitinib in comparison with other molecular targeted therapies, such as intravenous bevacizumab.

Figure 1
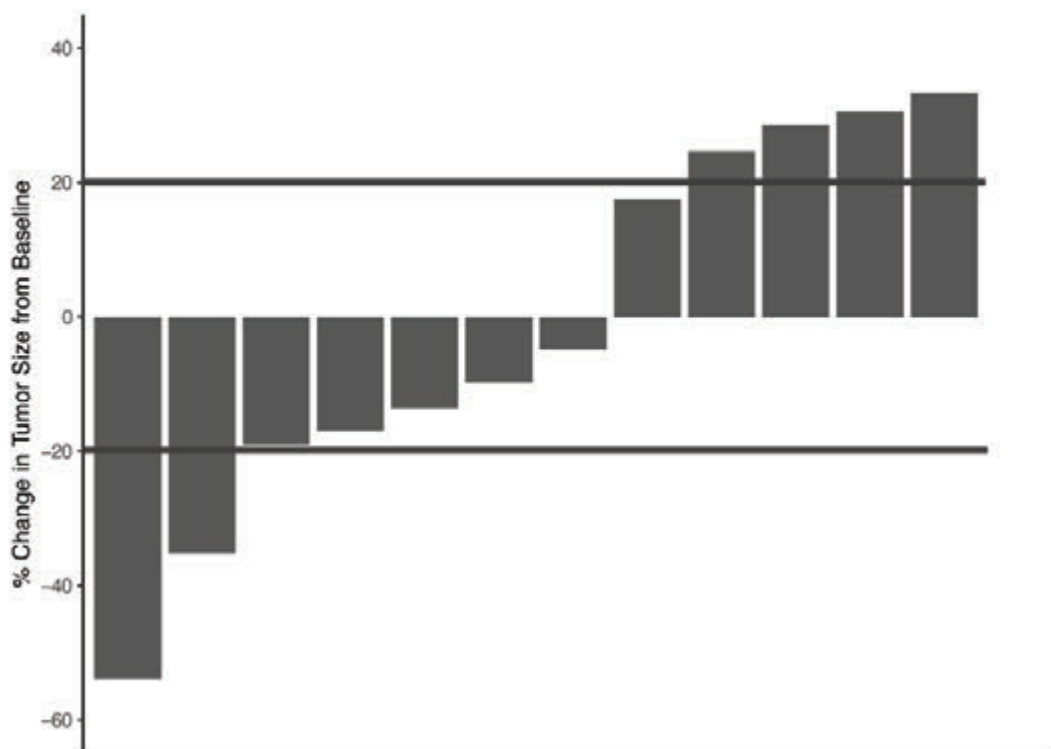
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Clinical Characteristics and Outcomes of *NF2*-Related Schwannomatosis

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Background: *NF2*-related Schwannomatosis (NF2) is a rare genetic disorder characterized by multiple tumors along the central and peripheral nervous system, particularly bilateral vestibular schwannomas. NF2 arises from heterozygous mutation in *NF2* gene located on chromosome 22, resulting in loss of merlin expression, with >50% occurring de novo. In the current study, the clinical and genetic features of 51 NF2 patients are described.

Methods: The medical records, laboratory findings, genetic evaluation and imaging studies of 51 NF2 patients (from 50 families) were reviewed. The following data were collected: onset of symptoms, age at diagnosis, sex, family history, accompanying symptoms, locations and types of tumors, frequency and methods of related management, genetic evaluation, detection rate of *NF2* mutation, and genotype.

Results: The average onset age of symptoms is 21.7 ± 13.4 years. Common symptoms at presentation were skin mass (n = 15, 29.4%), and hearing loss (n = 11, 21.6%). The patients had an average of 1.37 vestivular schwannomas, 2.28 non-vestibular schwannomas, 2.58 meningiomas, and 3.04 spinal tumors. Several managements were conducted, including surgery (n = 38), Gamma Knife surgery (GKRS, n = 24), and bevacizumab (n = 2), with the average age at the start of management being 23.57 ± 11.26 years. The pathogenic variant detection rate for the *NF2* gene was 64.5% (20/31) in blood samples and 81.8% (9/11) in tissue samples, suggesting that tissue-based genetic analysis may offer enhanced genetic diagnostic accuracy. Genotypically, nonsense and frameshift mutations were frequently observed.

Conclusions: Through relatively large-scale clinical data on NF2, it is possible to further expand our knowledge of the clinical characteristics of NF2. The onset of NF2 typically in early adulthood and the presence of multiple neoplasms underscore the aggressive nature. Despite a variety of management strategies, the challenge in achieving a cure highlights the complex pathology of NF2. Our findings underscore the need for ongoing research into targeted therapies for NF2.

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Duplex Sequencing Reveals Low-Level Mosaicism in Individuals with a Clinical Suspicion of *NF2*-Related Schwannomatosis and Negative Results from Standard Genetic Testing

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Background: Mosaicism is a common phenomenon in *NF2*-related schwannomatosis, impacting 25-30% of *de novo* cases where no pathogenic variant (PV) in the *NF2* gene in blood lymphocytes has been identified.[1] Schwannomatosis may also be associated with constitutional PVs in the *SMARCB1* or *LZTR1* genes. Approximately 50% of schwannomatosis cases without an identified constitutional *SMARCB1/LZTR1* PVs are mosaic for *NF2*.[2] The differential diagnostic strategy involves targeted resequencing of NF2 in blood DNA, based on genetic findings derived from the corresponding tumor analysis, contingent upon an identified *NF2* PV within the tumor.[3] However, the limited availability of tumor material for molecular testing poses challenges in providing patients with accurate diagnoses. Therefore, our objective was to optimize the ultra-sensitive duplex sequencing method to enhance the sensitivity of mosaic *NF2* variant detection in blood-derived DNA samples.

Methods: Thirty-nine reportedly sporadic probands with a clinical suspicion of mosaic *NF2*-related schwannomatosis and no constitutional PVs in the *NF2/SMARCB1/LZTR1* genes detected during routine diagnostic genetic testing performed in the reference laboratory at the Germans Trias i Pujol Research Institute in Barcelona were included in the study. This cohort comprised 19 individuals with known *NF2* PV(s) detected in tumor material but not in the corresponding blood and 20 cases with no identified *NF2/SMARCB1/LZTR1* PVs in blood, for whom tumor material was unavailable for testing. In addition, to determine the lower detection threshold for the duplex sequencing method, blood-derived DNA from mosaic *NF2*-related schwannomatosis with a known *NF2* PV of 7% was subjected to deep sequencing after serial dilution with commercially available human DNA. Blood-derived DNA from 20 healthy individuals served as a control for the study.

Results: Low-level (1-5%) and very-low-level (<1%) mosaic *NF2* PVs were detected in four and two unrelated individuals, respectively, with the lowest allele frequency (AF) of 0.06%. All these individuals met the Manchester diagnostic criteria,[2] except from a single case presenting solely with multiple intracranial and intraspinal schwannomas. Additionally, we validated the presence of *NF2* PVs in all prepared serial dilutions, with the lowest detected AF being 0.08%. Furthermore, none of the control samples had an *NF2* PV or variant of uncertain significance detected at the exact very low level.

Conclusion: Duplex sequencing emerges as a promising complementary method for enhanced detection of previously undiagnosed cases of low-level mosaic *NF2*-related schwannomatosis. This is particularly important for improving the diagnostic accuracy in cases with limited access to tissues other than blood samples for analysis.

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NF2-Related Schwannomatosis in Children: A Mono-Institutional Analysis of Clinical Presentation and Correlation with Pathogenic Variants

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Purpose: To increase awareness of pediatric *NF2*-related schwannomatosis (*NF2*-SWN) and accelerate the diagnosis, we highlighted the disease presentation in children; furthermore, since the *NF2*-SWN severity is genotype dependent, we stratified children by genotype, to better understand how pathogenic variants (PVs) affect age at onset and of diagnosis, and brain and spinal tumors burden.

Methods: We performed a retrospective phenotypic analysis of *NF2*-SWN patients, evaluated in our Department over the last 20 years, diagnosed <18 years using both the Manchester criteria[1] and the recently updated diagnostic criteria[2]. Patients were stratified using the validated UK NF2 Genetic Severity Score(GSS)[3].

Results: We collected data from 24 NF2 children(9 male).

The mean age of first clinical manifestation was 5.8 years and of diagnosis was 11.3 years with an early diagnosis in the 2 familial cases.

The presenting symptoms were ocular features(mainly epiretinal membrane and cataract) in 42%; dermatological features(>2 skin schwannomas) in 25%; neurological symptoms including gait disturbance in 17%, hearing impairment in 8%; seizures and intellectual disability in 2 cases.

The constitutional NF2 PVs were available for 66.7% patients.

Mean age at first manifestation was 4.3, 3.4, 7 years in groups 3(n=6), 2B(n=5), 2A(n=5), respectively, and at diagnosis was 11.5, 8, 15 years, in groups 3, 2B, 2A.

MRI was not available for 1 child of group 2B.

At the first brain-MRI, intracranial vestibular-schwannoma(VS), non-VS and meningioma occurred in 67%,33%,50% of group 3, and 75%,50%,25% of group 2B and 75%,60%,20% of group 2A.

At spinal-MRI, schwannoma occurred in 67%,75% and 80% of group 3, 2B, 2A; meningioma and ependymoma were present only in group 3(33%) and 2B(25%), while ependymoma was present only in group 3(33%).

Conclusions: Our data confirm that childhood-onset *NF2*-SWN is predominantly characterized by non-vestibular symptoms with ocular and dermatological features more frequent than neurological symptoms; identifying them could lead to an early diagnosis in the pediatric population in which the diagnosis is still late.

After applying the UK NF2 GSS, we confirm that the age of first manifestation and diagnosis is delayed in group 2A compared to groups 2B and 3. Intracranial and spinal schwannomas seem more frequent in group 2A, because in this group the *NF2*-SWN is diagnosed in adolescence, when the probability of developing schwannomas increases.

The group 3 phenotype manifests a greater burden of meningioma and ependymoma suggesting that these tumor types should be researched at an early age in the case of constitutional truncating PVs involving 2-13 exons.

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Efficiency of Clinical Criteria for Diagnosing Schwannomatosis

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Schwannomatosis (SHNT) is a rare phakomatosis characterized by the development of multiple tumors in the central and peripheral nervous system. Early diagnosis of the disease is challenging due to its slow progression and gradual manifestation of clinical signs. New diagnostic criteria proposed in 2022 show promise, but their sensitivity and specificity need further evaluation, particularly during the clinical debut. In this study, we assessed the effectiveness of various clinical criteria in patients with molecularly confirmed Schwannomatosis associated with a variant in the *NF2* gene, focusing on the stage of clinical manifestation. We retrospectively analyzed data from 54 patients (23 men and 31 women) with clinical manifestations of Schwannomatosis within a period of up to three months. Detailed descriptions and native data from contrast-enhanced magnetic resonance imaging of the brain were available for each patient. The clinical criteria used for phenotypic assessment included Baser 2011, Manchester 2016, and the latest 2022 criteria. The mutation spectrum was represented by changes in the copy number of DNA sections (n=9), as well as point genetic variants (n=45): nonsense mutations (n=18), frameshift indels (n=16), splicing mutations (n= 8), missense mutations (n=2) and indels without frameshift (n=1). Somatic mosaicism was detected in 24% of cases (13/54). Not all patients had access to all available criteria due to missing data at the time of clinical onset. The diagnosis did not meet clinical criteria in 10 (29.4%) of 34 patients for whom Baser criteria were available and 14 (29%) of 48 patients for Manchester 2016 and new criteria. Out of the 54 patients, the majority (47 or 87%) were considered de novo cases, indicating the absence of a family history. The analysis revealed the significance of the newly proposed 2022 criteria in aiding the diagnosis of Schwannomatosis. However, further research is needed to determine their sensitivity and specificity, particularly during the initial clinical presentation.

Conclusion: Schwannomatosis remains a challenging condition to diagnose, primarily due to its slow progression and non-specific symptoms during the clinical debut. The introduction of the 2022 diagnostic criteria shows promise in improving early detection and potentially reducing diagnostic delays.

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Natural History of Spinal Ependymomas in NF2 Patients

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Objective: Spinal ependymomas (SEN) are the most common primary spinal cord tumors. They can occur sporadically or associated to *NF2*-related Schwannomatosis (*NF2*-SWN). Their progression can be cystic or solid, with a significant morbidity and mortality associated with surgical management. In order to study the natural history of these tumors, we conduct a clinical and imaging study in patients with cystic and non-cystic SEN.

Methods: We included patients with *NF2*-SWN diagnosed with a SEN (minimal size: 12mm, tumor + cyst) in a retrospective study and added patients with sporadic SENs as a control cohort. We reviewed clinical, radiological and pathological data.

Results: Thirty-three patients had fifty-three SEN in the context of *NF2*-SWN and eleven control-cases harbored sporadic tumors. 25 (*NF2*-SWN) + 11 (sporadic) had relevant imaging data. Median age at surgery was 33 years in both groups. SEN in *NF2*-SWN were preferentially located in the bulbo-medullary (22.6%, n=12) and cervical locations (43.4%, n=23), while sporadic SEN were preferentially cervical (54.5%, n=6). The tumor portion of NF2 ependymomas is heterogeneous on imaging in about 2/3 of cases. Median ratio of cyst to tumor size was 49% in SEN *NF2*-SWN group and 32% in sporadic SEN. Median cyst number was one in both groups. SEN in *NF2*-SWN had cystic progression in 50% (n=12), mixed progression in 25% (n=6) and isolated tumor progression in 12.5% (n=3), surgery was performed upfront for 3 patients. We didn't have imaging follow-up to evaluate cystic growth in sporadic cases because they were discovered based on clinical symptoms that led to surgery. In *NF2*-SWN cystic cases, we observed that peripheral spinal cord edema was initially present at the periphery of the tumor nodules, before cystic formation (peritumoral edema was there before cyst in 90% of cases). Moreover, persistence of oedema at the cyst periphery was associated to the persistent growth of the cyst. From an anatomopathological point of view, within cystic SEN, there is a greater presence of CD-163 macrophages associated with edema (75% of cases presented macrophages infiltration). We performed FoxJ1 immunohistochemistry, which revealed strong positivity within *NF2*-SWN cases.

Conclusion: Descriptive analysis shows a predominant cystic nature in *NF2*-SWN SEN, associated with a continuous growth. Cystic growth is histologically characterized, by macrophagic infiltration with edema. This study raises questions about the right timing of a medical treatment, from the onset of peri-tumoral edema, which for the moment is based only on bevacizumab.

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Challenges in *NF2*-Related Schwannomatosis Treated with Stereotactic Radiosurgery

Katrina A Morris, MD PhD, *University of Sydney, NSW Health, Australia*

Introduction: The estimated risk of secondary malignancy of schwannomatosis-NF2 treated with radiotherapy is contentious in recent cohort reports[1,2]. Bin-Alamer et al (2023) in their stereotactic radiosurgery (SRS) cohort (median 59 month follow up) observed no malignant transformation. However, 27 of the 60 patients developed post-SRS progression requiring operative management (resection or shunting), an outcome which may be seen as malignant progression. Evans et al (2023) compare radiotherapy treatment of UK national schwannomatosis-NF2 specialist service, demonstrating a risk of malignant transformation/malignant progression in 5% of patients overall (vs <1% in non irradiated NF2 patients).

Case Presentation: We present two cases with deteriorating quality of life subsequent to SRS.

Case 1: presented with a growing cerebellopontine angle tumour complex despite previous surgery. Following SRS, tumour expansion was observed but reduced with glucocorticoids. Six months post SRS was noted to develop papilloedema with progressive loss of vision requiring acetazolamide, recurrent therapeutic drainage and optic nerve fenestration to prevent further deterioration. CSF protein was elevated at over 1.2g for several months.

Case 2: had fractionated radiotherapy for control of a large trigeminal schwannoma. At 12 months follow up the patient continues to have intractable trigeminal neuralgia and has developed intrusive trigeminal autonomic dysfunction with unremitting lacrimation, rhinorrhoea and shortness of breath.

Discussion: These cases demonstrate the challenges in managing patients with NF2 with radiotherapy. Tumour responses are less robust and risk of adverse events, including hearing deterioration following vestibular schwannoma SRS, appears higher in NF2. This, in addition to the potential longer term malignancy risks of radiotherapy, needs consideration when planning treatment in this cohort who may require more extensive follow up and symptom management. Further research is required to establish the reasons for malignant progression in schwannomatosis-NF2 post SRS.

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The Transorbital Neuroendoscopic (TONES) Approach for Access to *NF2*-Associated Pathology of the Anterior Cranial Fossa; Advancing a Minimally Invasive Surgical Approach with Maximal Access to Lesions of the Parasellar Region

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Purpose: Children with NF2 often require multiple revision transcranial surgeries for decompression of neurovascular structures which are associated with increased morbidity as compared to an index surgical approach. We aim to describe the use of the transorbital neuroendoscopic surgery (TONES) approach for access of parasellar pathology affecting children with NF2 having undergone prior anterior surgical approaches.

Methods: A retrospective single-institution analysis of patients with NF2 undergoing TONES for neoplastic lesions of the anterior cranial fossa were reviewed.

Results: A 15 year old boy with a history of NF2 and multiple prior anterior and posterior cranial fossa surgical approaches for resection of *NF2*-associated tumors presented with subacute decline of left-sided visual acuity. MRI revealed a growing planum and tuberculum sellae meningioma with compression of the optic nerve within the optic canal. Due to multiple prior transcranial approaches, additional transcranial approaches were felt to be high risk for neuro-vascular morbidity due to excessive scarring and adhesions. Preoperative ophthalmologic evaluation revealed compressive optic neuropathy with subsequent optic nerve atrophy. The patient underwent a multi-portal approach for endoscopic endonasal debulking of anterior cranial fossa meningioma and TONES approach for an "inside-out" bony optic nerve decompression. A 270-degree decompression of the optic nerve was accomplished with improvement in vision postoperatively. No manipulation of brain tissue was needed throughout the entirety of the operation and there was no surgical-associated morbidity. The patient was able to discharge 2 days following surgery.

Conclusion: The TONES approach affords a minimally invasive approach without the need for brain retraction or soft tissue dissection for access to parasellar structures without sacrificing visualization or working angles. This is especially important in patients having undergone prior anterior approaches to limit postoperative neurovascular morbidity associated with repeat surgery.

Figure 1: (A) Preoperative and (B) postoperative coronal CT showing the optic canal (A) prior to and (B) following multi-portal endoscopic endonasal and TONES approaches for optic nerve decompression. A 270-degree decompression of the left optic canal (black arrow) was achieved and the patients vision improved postoperatively.
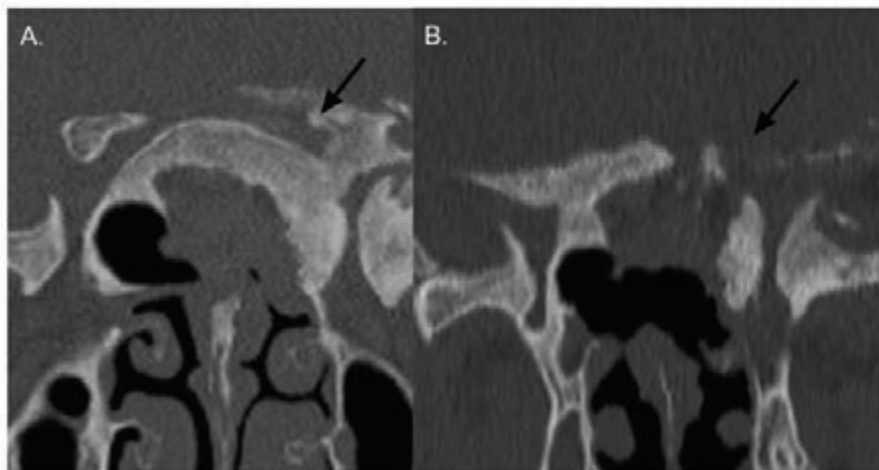
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Neurocognition in Children with *NF2*-Related Schwannomatosis: Parental Concerns of Academic, Social, Emotional, and Behavioral Functioning

Aimee Sato, MD, *Seattle Children's, UW Medicine*

Neurofibromatosis Type 2 related schwannomatosis (*NF2*-SWN) has been historically characterized as a disorder which does not impact cognitive function, though there are limited studies in children with *NF2*-SWN (Evans, 1998; Ruggieri et al, 2016). This study aims to better understand the neurocognitive profiles of children with *NF2*-SWN seen by Seattle Children's (SC) providers.

Demographics, developmental, and schooling information on patients with *NF2*-SWN seen at SC from 2018 to present were reviewed. In addition, starting from November 2023, parents of childrens with *NF2*-SWN (n=6) completed parental questionnaires prior to their appointment in *NF2*-SWN clinic. All parents were given the *Strengths and Difficulties Questionnaire (SDQ)* and 5 parents were given the *Colorado Learning Difficulties Questionnaire (CLDQ)*. One parent was excluded from completing the CLDQ due to the unavailability of a Spanish translation.

Preliminary results include review of 22 children with *NF2*-SWN, revealing that 14 (63%) were recommended to receive or were receiving developmental or academic assistance either through an Individualized Education Plan (IEP), 504 Plan, or equivalent. 5 children (23%) did not require school assistance and educational needs for 3 children (14%) were unknown.

The SDQ identifies concerns regarding social emotional difficulties including hyperactivity, prosocial behavior, conduct, emotional, and peer problems. Of the 6 completed parental questionnaires, there were concerns for hyperactivity (n=1, slightly raised), emotional problems (n=2, slightly raised), peer problems (n=3 slightly raised, n=1 very high), and low prosocial behavior (n=1).

The *CLDQ* contains questions regarding potential concerns in reading, math, and writing. An average score of 2 or greater is interpreted as requiring further attention. Of the 5 parent questionnaires reviewed in the present study, all (5) parents reported a score of 2 or higher in reading, 4 parents reported concerns in math, and 3 parents reported concerns in writing.

Preliminary results demonstrate that the majority of pediatric patients with *NF2*-SWN cared for at SC were recommended for or were receiving developmental or academic assistance. While these results cannot be generalized to all children with *NF2*-SWN, it does suggest that children with *NF2*-SWN who present to tertiary specialty centers would likely benefit from neuropsychological support. Also, the use of parent questionnaires have identified concerns regarding cognitive, academic, or social/emotional functioning in the *NF2*-SWN patient population seen at SC. We anticipate gathering additional data regarding the prevalence of these concerns in this patient population in future *NF2*-SWN clinics.
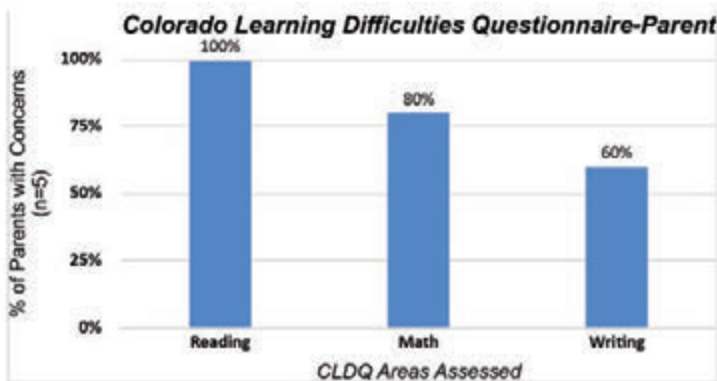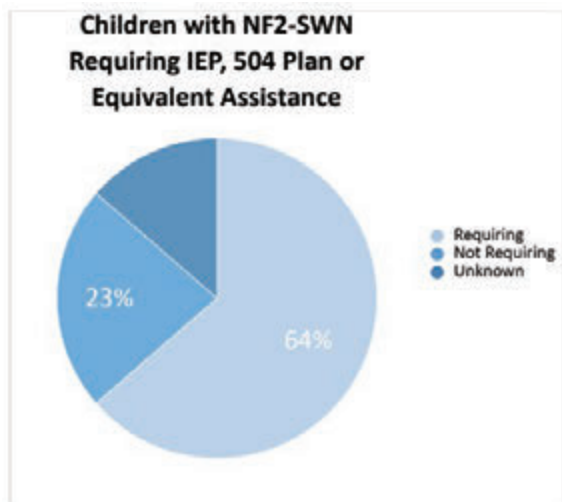
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Schwannomatosis: Clinical Science

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Targeted Sequencing of *LZTR1, SMARCB1* and *NF2* Genes in Multiples Tumors from Schwannomatosis Patients Harboring Spinal and Peripheral Nerve Schwannomas Reveals High Frequency of Mosaïc *NF2*-Related Schwannomatosis

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Purpose: Following the recent introduction of molecular diagnosis criteria for schwannomatosis, we decided to study the results of somatic molecular testing in patients with a tumor burden suggestive of *NF2*-independant schwannomatosis, with a particular focus on patients harboring multi-nodular schwannomas.

Methods: We selected 20 patients harboring multiple peripheral nerve schwannomas, with or without associated lumbar schwannomas after a brain and spine MRI workup ruling out cranial nerve schwannomas, meningiomas and ependymomas. For each patient, at least 2 separate anatomically unrelated tumor nodules were available for analysis. We classified tumors as mono-troncular when anatomically distinct nodules were located on the same nerve or nerve trunk or as pluri-troncular when located on different nerves. We performed targeted sequencing of the *NF2, SMARCB1* and *LZTR1* genes for all tumors and germline DNA when available (18 out of 20 patients).

Results: We analysed 47 tumors in 20 patients, 17 of whom had a non-familial disease. Twelve patients had a diffuse schwannomatosis and 8 presented with a segmental form of the disease. Constitutive analysis demonstrated a germline *LZTR1* mutation in 8 cases and no mutation in any of the known schwannomatosis driver genes in 10 cases. Following targeted sequencing of multiple tumors, 10 patients were diagnosed with *LZTR1*-related schwannomatosis (50%), 7 patients with mosaïc *NF2*-related schwannomatosis (35%) and 3 patients with 22q-related schwannomatosis (15%). Among patients with mono-troncular schwannomas (n=7), all with a non-familial disease, 5 harbored a mosaïc *NF2*-related schwannomatosis (71%).

Conclusion: This study illustrates the underestimated high frequency of mosaïc *NF2*-related schwannomatosis in patients harboring peripheral nerve schwannomas, especially when multi-nodular and mono-troncular. As multi-nodular schwannomas are associated with higher surgical morbidity, the pivotal role of the *NF2* gene in their tumorigenesis opens perspectives for Schwannomatosis research.

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Surgical Management of Peripheral Nerve Tumors in *NF2*-Independent Schwannomatosis

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Purpose: Pain is the cardinal symptom of *NF2*-indepedent schwannomatosis (non-*NF2*-SWN) and peripheral nerve schwannomas are the most frequent tumors encountered in this condition. The role of surgery in alleviating schwannoma-associated pain in schwannomatosis remains a matter of debate in the literature.

Methods: We conducted a retrospective chart review of all non-*NF2*-SWN patients followed up at our Schwannomatosis Reference Center and included all patients operated from a peripheral nerve tumor to report the results of surgery in this population. *NF2*-related Schwannomatosis was excluded on the basis of germline genetic study and/or absence of suggestive tumors (bilateral vestibular schwannomas, meningiomas, ependymomas) on brain and spine MRIs. All surgeries of major nerves were performed using intra-operative neuromonitoring.

Results: Fifty-nine patients were included, suffering mostly from a non-familial (49/59, 83%) and diffuse (40/59, 68%) non-*NF2*-SWN. A germline genetic study of the *NF2, LZTR1* and *SMARCB1* genes was performed in 34 patients (57%) and demonstrated a *LZTR1* gene mutation in 17 cases (50%), with no case of germline *NF2* or *SMARCB1* mutation. The patients were operated from 98 peripheral nerve tumors. The tumors were mainly located in major nerves (n=64, 65%) compared to subcutaneous (n=16, 16%) and intramuscular (n=16, 16%) cases. Most tumors were classical discrete tumors (n=84, 86%) while plexiform cases represented only 14% (n=14) of cases. Pathological analysis confirmed the diagnosis of schwannoma in all cases except 2 cases of hybrid neurofibroma / schwannoma tumors. A complete resection was performed in 89% of cases with a complete relief of pre-operative pain in 87% of cases. Post-operative motor and sensory deficits were encountered in 8 (8%) and 13 (13%) cases respectively. Plexiform schwannomas were characterized by a decreased rate of pain relief (64% vs. 90%, p=0.007) and an increased rate of post-operative motor deficit (3.5% vs. 36%, p < 0.001) compared to discrete tumors.

Conclusion: Nerve-sparing surgery using intra-operative neuro-monitoring remains effective in treating pain of non-*NF2*-SWN-associated peripheral nerve schwannomas, with the notable exception of plexiform tumors, also characterized by an increased rate of post-operative motor deficits.

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Real World Evidence for Quality of Schwannomatosis Care Within the United States

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Background: We previously published data about the variability in clinician awareness of, and agreement with, recommended care for patients with *NF2*-related schwannomatosis (*NF2*-SWN) and non-*NF2* related schwannomatosis (non-*NF2*-SWN)[1]. Here, we sought to assess whether patients with these disorders received care in line with published recommendations.

Methods: An electronic survey was sent in May 2021 and May 2022 to United States-based patients and caregivers affected by *NF2*-SWN (n=746) and non-*NF2*-SWN (n=204) who were enrolled in the Children's Tumor Foundation NF Registry. Survey topics included demographics, SWN care location, and self-reported clinical care in accordance with published recommendations. All data from survey responses were deduplicated to only include the most recent survey results and demographic data were taken from the associated registry entry. Data were examined in SPSS. IRB approval was obtained by Children's Tumor Foundation for this study.

Results: Survey responses were provided by 10% (76/746) of *NF2*-SWN registry participants and 15% (31/204) of non-*NF2*-SWN participants. Among *NF2*-SWN survey respondents, median age was 37 years, 37% were male, 84% were white, and 11% of cases were reported as inherited. Comparatively, for non-*NF2*-SWN respondents the median age was 51 years, 36% were male, 77% were white, and 7% were inherited. Among survey respondents with *NF2*-SWN, 47% reported undergoing genetic testing compared to 57% of non-*NF2*-SWN respondents. Forty percent of survey respondents with *NF2*-SWN reported being offered access to reproductive counseling services. Despite the complexity of these diseases, only 42% of survey respondents with *NF2*-SWN and 29% of survey respondents with non-*NF2*-SWN reported receiving care in a specialized NF clinic network site. Seventy-nine percent of non-deafened respondents with *NF2*-SWN reported receiving an audiogram in the last 12 months. Further, 85% of *NF2*-SWN respondents reported receiving a brain MRI in the last 12 months, 96% of respondents reported ever undergoing a spine MRI, and 85% of those with known spinal tumors reported receiving a spine MRI within the last 36 months. Of survey respondents with non-*NF2*-SWN, 80% reported ever having a brain MRI (63% within the last three years), and 83% reported receiving at least one spine MRI (71% within the three years).

Discussion: Our survey revealed that a large proportion of patients living with SWN in the United States do not attend a specialized SWN clinic and have not received recommended genetic testing and counseling. One-in-five non-deafened patients with *NF2*-SWN did not receive audiograms according to recommendations, and 15% did not receive neuraxial MRIs in alignment with published recommendations. More starkly, among patients with non-*NF2*-SWN, nearly 30% of patients did not receive spine MRIs according to recommendations and nearly 40% did not receive brain MRIs according to recommendations. These data serve as a benchmark for organizations focusing on expanding access to SWN care, and highlight the need for US-based SWN clinical care guidelines.

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Disclosures: Dr. Jordan serves as a paid consultant for Children's Tumor Foundation for work that does NOT pertain to the contents of this abstract. The other authors report no relevant disclosures.

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ABSTRACTS

Mobilizing Artificial Intelligence to Improve Understanding of Neurofibromatosis Research

Robert Allaway, PhD, *Sage Bionetworks*

Purpose: The technical language in scientific publications about neurofibromatosis and schwannomatosis (NF) often poses a barrier for non-scientists. Plain language summaries or lay abstracts are tools used to bridge this gap. Creating plain language summaries can be challenging due to the jargon-rich nature of scientific research, and the inherent challenge of communicating complex topics in plain language for different audiences. Large language models present a potential solution, as they can quickly synthesize plain language summaries of complicated text, making them more accessible to a broader audience.

Methods: We conducted a series of pilot experiments to assess the feasibility of using LLMs to simplify research text. We selected NF relevant research summaries from ten public grant abstracts on the NF Data Portal. We then generated plain language summaries for each scientific summary using GPT-4. We assessed readability using the Flesch-Kincaid Grade Level (FKGL) and CommonLevel methods, and quantified the semantic similarity between the original and simplified texts.

Results: GPT-4, when tasked with text simplification, significantly increased the readability of NF research summaries. The original abstracts, with a FKGL of 16.8 ± 3.3 (mean$\pm$standard deviation), were transformed into significantly more accessible versions averaging a FKGL of 9.6 ± 1.9 for the 8th-grade and 7.1 ± 1.4 for the 4th-grade reading levels. The CommonLevel metric also indicated a significant increase in readability, at -2.7 ± 0.29 for the original abstracts, -1.3 ± 0.57 for the 8th-grade, and -0.6 ± 0.47 for the 4th-grade. We also determined that the meaning of the original abstract is better preserved in the simplified abstracts than in a control dataset. The semantic similarity of the original abstracts and the 4th-grade abstracts averaged 0.629 ± 0.118 (mean$\pm$standard deviation, where 0 is completely dissimilar and 1 is completely similar).

Conclusions: We plan to determine whether our preliminary findings will replicate in larger or different types of NF research text, such as publications. Second, we need to determine if the content of the original text is sufficiently preserved in the LLM-generated plain language summaries. We intend to investigate both of these questions using a mixture of quantitative natural language processing and qualitative evaluation. Beyond this, it is not known how effective LLM-generated plain language summaries will be in making NF research outputs more accessible to both experts and non-experts. Until an independent benchmark is established, care must be taken when using LLMs to generate plain language summaries.

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Making AI/ML Ready Neurofibromatosis Genomic Datasets Available Through the NF Data Portal, cBioportal, and Other Computational Platforms

Jineta Banerjee, PhD, *Sage Bionetworks*

Purpose: Research in rare conditions like neurofibromatosis (NF) suffers from challenges related to inherent limitations in sample number. Recent funding initiatives have supported research projects generating large amounts of genomic data in NF1-related human tumors, animal models, and cell lines. However, individual high-dimensional genomic datasets with small sample sizes present considerable hurdles in analysis and interpretation. Coordinated efforts towards processing the data in a consistent way to ensure that it is comparable across different sources make small datasets more reusable. The NF Open Science Initiative (NF-OSI) has processed genomic and transcriptomic data from the multiple projects using standardized and publicly available workflows and released them on the NF Data Portal, cBioPortal, and Cavatica for maximizing use of these datasets.

Methods: Raw whole exome (WES), whole genome sequencing (WGS), and RNA sequencing (RNA-seq) data generated from human NF1 tumor samples and mouse models of the disease were shared on the NF Data Portal by principal investigators with appropriate regulatory approval. NF-OSI used Nextflow workflows from the nf-core community (nf-core/sarek v3.2 and nf-core/rnaseq v3.7) to align the data to the most recent human reference genome (GRCh38 or GRCm38). Somatic variant calls, copy number calls, and RNA-seq counts were generated from the raw data. After quality checks and annotation to meet FAIR standards, the processed files were made available on the NF Data Portal. The somatic variant calls from published samples were also formatted and submitted to cBioPortal. Furthermore, the processed data on NF Data Portal were linked to a cloud based computational platform called Cavatica making them ready for analysis.

Results: With this effort, NF-OSI released multiple datasets of consistently-processed genomics and transcriptomics data on the NF Data Portal, making it easily and rapidly reusable (https://tinyurl.com/nfprocessed). The formatted data on cBioPortal (tinyurl.com/NF1cBio) enables researchers and clinicians to explore NF1 specific datasets alongside other cancer datasets without extensive computational resources. The processed data linked to Cavatica are ready to be analyzed alongside other pediatric cancer datasets available on this platform.

Conclusion: This initiative is a significant step towards large-scale processing of NF data from diverse sources enabling researchers from various disciplines to use harmonized datasets for computational analysis. Datasets from multiple studies, including 2 WES, 3 WGS, and 6 RNA-seq datasets, have been processed and made available on the NF Data Portal. A subset of these datasets will be available on cBioPortal for researchers and clinicians to explore and compare with other cancer samples. All of these datasets are available to export to Cavatica for analysis alongside other pediatric cancer datasets on that platform. Overall, this effort empowers the NF research community to explore the genomic profiles of NF samples from multiple studies and enables people new to NF-OSI to use NF relevant data.

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Growth of Medical Consultations in the Last 13 Years at a Neurofibromatosis Interdisciplinary Center in Argentina

Patricia Ciavarelli, MD PhD, *Hospital de Clínicas, University of Buenos Aires School of Medicine, Buenos Aires, Argentina*

Introduction: After its foundation in 2010, the Center of Neurofibromatosis (NF) at the Hospital de Clínicas experienced a continuous growth in the number of patients treated therein. At the same place, the Argentine Association of Neurofibromatosis (AANF), a civil association of NF patients from the whole and neighboring countries, is also based. A multidisciplinary evaluation is carried out in a specially dedicated space so that all Neurofibromatosis patients can access a high number of specialists quickly.

Objectives:
1. To demonstrate the continuous growth of the NF Center following the creation of a space exclusively dedicated to the care of patients with this condition.
2. To inform the increasing number of conferences, webinars, and satellite centers organized under the sponsorship of the NF Center.
3. To show the care flowchart employed by the NF Center at present.

Materials and Methods: We reviewed the clinical histories of the patients having NF and being treated at our center in the studied interval of time. The NF center comprises a total of 20 medical specialties, with nutrition and psychology being the latest to join. Patients are initially seen by one of the directors of the center (PC), who is an experienced neurosurgeon. She triages and rules out potential surgical pathology, whether it's optic glioma, other brain gliomas, dystrophic scoliosis, or surgical neurofibromas. They are then selectively referred to the corresponding specialist. The average period in which patients are thoroughly evaluated by all the necessary specialists ranges from 2 days to 10 days in the more complex cases. To strengthen diagnostic and therapeutic criteria, monthly virtual case discussions are conducted, an activity that includes national and international physicians. According to the patients' unique medical needs, we formed a network of hospitals, including the Sarcoma Unit (Hospital Oncológico Roffo, Buenos Aires Hospital), and other high complexity and specialized monovalent centers.

Results: In total, our center recorded 6152 in-person consultations in the period of January 1st, 2010, to December 31st, 2023. In addition, 451 online interviews were performed since the start of the COVID-19 pandemic in 2020. Patients were adults and children meeting at least two clinical criteria for Neurofibromatosis. The origin of the patients was Argentina, Bolivia, Chile, Paraguay, Venezuela, Nicaragua, Costa Rica, Colombia, Perú, and Uruguay. The total number of NF patients treated by the NF center in the studied period was 1621, while 120 sustained SCH/NF2 and 49 schwannomatosis. Since 2021, the NF Center has refurbished and moved to a central part of the hospital (at the neurosurgical department floor) were all the medical and academic activities were more effectively and vastly organized. This change favorably impacted in the statistics of patient care: 28.9% of the total patients treated had their consultation in the last three years.

In parallel, nine workshops were organized in Buenos Aires and other Argentinian cities, involving a global total of: 90 conferences, three in-person congresses and one online meeting, where care of 1384 patients were given. The workshops lasted two days and had this organization: during the first day, patients were sequentially attended to by seven specialists (neurosurgeon, dermatologist, ophthalmologist, genetics, spine surgeon, cardiology, and peripheral nerve surgeon), while the second day was dedicated to patient and their families training through conferences given by the specialists. All the mentioned activities were completely free of charge.

Conclusions: The extensive growth in the number of NF patients treated at our NF Center occurred after opening a modern and multidisciplinary facility. The patients were initially evaluated by an experienced physician, a fact that was fundamental for the early detection of potentially surgical or malignant disease.

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Improving Curation and User Experience for NF Tools Central

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The NF Data Portal (nf.synapse.org) is a platform for sharing neurofibromatosis research data that features NF Research Tools Central, a comprehensive catalog of animal models, cell lines, genetic reagents, and antibodies. These tools are essential for studying the molecular and cellular mechanisms of neurofibromatosis, as well as developing potential therapies. However, finding and selecting the most suitable tools for a specific research question can be challenging, as it requires extensive knowledge of their characteristics, applications, and limitations. To address this challenge, we have cataloged 638 cell lines, 261 antibodies, 123 animal models, and 122 genetic reagents.

To overcome the challenges of displaying longitudinal data, we developed a visual timeline for natural history. This timeline lets users explore data like disease progression in animal models. Another significant challenge is the collection and curation of observational data. Historically, this process involved manual curation of information from scholarly articles, a method both time-intensive and laborious. To build upon the status quo, we are integrating advanced technologies, including large language models to rapidly enhance our database with a richer set of observational data.

A further complication in research is identifying the most appropriate tools for specific investigations, which necessitates detailed knowledge about these tools, such as their associated publications and details about genetic mutations. To mitigate this issue, we have facilitated easier access to critical information by creating direct links to essential research identifiers, including ClinVar, Research Resource Identifiers (RRID), and PubMed IDs. This enhancement ensures that researchers can swiftly locate external resources relevant to their work.

Looking ahead, we aim to improve the process for submitting new tools and to introduce a system for rating the tools most frequently used in the field. In summary, we believe our initiatives to document the application and findings associated with these tools are crucial steps towards supporting and propelling the field forward.

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Patient's Oriented, Coordinated and Comprehensive Multidisciplinary Care as a Result of Successful Perennial Cooperation Between Physicians and Parental Organization in the Field of Neurofibromatoses in Poland

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Almost 20 years ago the first two Medical University Centers of Excellence in Poland offered a multidisciplinary, patients'-oriented care for neurofibromatoses (NF) were settled in Bydgoszcz in 2004 and in Warsaw in 2005. The following year, in 2006 the Parental Association "Neurofibromatoses Poland – Alba Julia" was legally registered in the Bydgoszcz's District Court. Until now it is the only parental organization in Poland gathering patients affected by classical as well as mosaic and allelic forms of neurofibromatosis type 1, Legius syndrome and the whole range of the Schwannomatoses, and their families. From the beginning the close cooperation between NF-Centers and NF Poland build both medical community and general society awareness of the neurofibromatoses by themselves and the health, educational and social problems of the patients. The joint physician and parental actions resulted in significant improvement in disease awareness resulted in betted understanding of NF patients' health requirements, the significance of the NF patients' needs and finally, the better care and preventions of the disease complications. Thanks to effective lobbying treatments in Polish Parliament and both Ministry of Health (MOH) and The National Health Found, provided by the President of NF Poland and the management team of the Association, ultimately in 2020 the coordinated medical care program for NF patients hammered by the Nf specialist gathered in the NF Section of Polish Society of Pediatric Hematology and Oncology was introduced into the national health system in Poland by the MOH separate decree. Substantial work and close cooperation between NF specialist and parental organization resulted in many activities promoted patients' and their family's health and educational as well as social and psychological issues not only among the families and the specialist of the different medical specialties, but among the general society members too. It was reached by the countless media interviews, press conferences, society events, such as celebration of The NF Awareness Day or The Rare Diseases Day, as well as the country annually thematic symposia for physicians and Nf families, numerous lectures and presentations during the health professional symposia and congresses and introducing the NF issues into the pre and postgraduate medical education. Our joint action was rewarded by "The Angel of Medicine" Prize, "Health Visionary" Prize and others. At the poster we will present the way how to achieve the success assessed as a health gain for NF patients and building social awareness helping them to better survive in the society.

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Current Characteristics of the Children's Tumor Foundation NF Clinic Network in the US and Canada

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The Children's Tumor Foundation (CTF) established the NF Clinic Network (NFCN) in 2007 with the goal of standardizing and improving NF clinical care as well as integrating research into clinical care practice. Since it was established, the network has more than doubled in size, with 73 sites in the US and Canada. There are NFCN sites in 32 US states, the District of Columbia, and two Canadian provinces. Data submitted from 72 clinics is reported.

In the last year, 23,701 NF patients were evaluated in NFCN clinics (20,709 NF1, 2,149 *NF2*-SWN, and 843 Non-*NF2*-SWN). The number of unique confirmed and suspected clinic patients with these diagnoses was included.

Eighty-one percent of clinics see pediatric patients as a majority of their patient volume, with 28% of clinics indicating that there is a maximum age limit for evaluation of new patients (most frequently ages 18 or 21). Eight clinics provide almost exclusively adult care. The average wait time for an urgent visit at an NFCN site is 11 days. Telehealth visits for new patients are offered at 58% of centers.

The majority of clinic directors and co-directors are from specialty areas of medical genetics (26%), neuro-oncology (25%), and pediatric neurology (20%). Clinic directors from 72% percent of clinics attended the NF Conference in 2023 (in person or virtual.) Eighty-six advanced practice practitioners and 85 genetic counselors support patient care within the NFCN.

Sixty-one percent of clinics reported that the director and/or co-director were authors of an NF-related publication in the last two years. All clinics except one are involved with training and teaching activities. Eight in-person, three virtual, and seven hybrid educational NF symposia were held in 2023.

A vast majority (92%) of NFCN clinics inform patients of available research studies outside of their institution. On-site NF clinical trials or other NF-related research studies are available at three-quarters of the sites. Basic science research is done at 38 clinic sites. Ninety-four percent of sites promote the NF Registry.

In 2023, driven by the NF patient community, the Children's Tumor Foundation and members of its Clinical Care Advisory Board developed a Designation System to differentiate NF clinics and assist families in identifying clinics to meet specific patient needs. Relevant areas identified include Clinical Care, Research, Education and Mentorship, and Community Involvement. Each area is being assessed using an evaluation of clinic annual report data with required and/or recommended areas leading to two designation categories: *Comprehensive NF Center* and *NF Specialty Program.* NF1 and SWN will be considered separately for each category. Reviews and final determination of clinics using the Designation approach are in process.

Conclusions:
- The formation of an NFCN over 15 years ago has been highly beneficial in providing care to a large NF population with access to NF expertise, education, and research opportunities.
- There is still a need for additional NF centers in underserved areas of the US and Canada.
- There is a significant need to integrate more clinics providing care to adults with NF.
- The clinicians and researchers at NFCN sites are highly engaged with teaching, research activities, and clinical trials.
- The majority of NFCN clinics promote NF educational events for families and CTF initiatives such as the NF registry.
- Initiated by the NF patient community, care practices and activities provided at NFCN clinics will be differentiated to assist patients in meeting their specific needs.

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The Children's Tumor Foundation Experience with Post-Mortem Bio-Banking of Tissues from Individuals with Neurofibromatosis

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Purpose: The Children's Tumor Foundation (CTF) established a pilot program for planned recovery of tissues for research from recently-deceased individuals with neurofibromatosis or schwannomatosis (NF, collectively). This was in response to inquiries from terminally-ill individuals with NF who wanted to plan such donations.

Methods: After selecting groups to facilitate this work, a formal system was established in May 2014. This included regulatory paperwork, a notification system, and contracts with two academic labs (Wallace, Carroll), a commercial biobank, and tissue recovery service (NDRI) to follow our specifications. Tissues to be shipped to the Wallace lab were to include samples of MPNST, plexiform neurofibroma, normal nerve, cutaneous neurofibromas/schwannomas, and blood. Samples for the Carroll lab were to include brain, vertebral column (spinal cord nerve roots), some tumor specimens, blood, and skin with and without dermal neurofibromas. The Wallace lab's goal was to set up Schwann cell cultures, prepare FFPE blocks, and extract primary tissue DNA and RNA (blood too, plus save plasma). The Carroll lab's work included neuropathologic examination of tissues, and banking frozen neoplasms and non-tumor bearing tissue with Precision Bioservices. Those samples were transferred to Indiana University in 2019 for distribution for research by requesting investigators (nonprofit and for profit).

Outcomes: From Aug. 2015 – May 2017, of the six cases for which this system was utilized, one patient had schwannomatosis, one had *NF2*-related schwannomatosis (*NF2*-SWN) and the others had NF1. This procurement proved more difficult for NDRI subcontractors than anticipated, as tissues were mis-labelled and/or mis-directed in three cases. In one case, culture contamination resulted from a missed step in pre-shipment processing. After the first two cases yielded only a few live cells in culture, the post-mortem time-from-death-to-laboratory-receipt limit was reduced from 60 to 48 hours. From the subsequent cases, only one cell line was obtained, from an MPNST. The low and unpredictable resource yield, the difficulties in procurement, and the high costs led the CTF to formally end the program in 2020.

Conclusion: For NF, a national post-mortem tissue collection system is unlikely to work sufficiently well to merit the high costs.

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