ABSTRACT BOOK

2023 NF CONFERENCE

JUNE 24-27, 2023

FAIRMONT SCOTTSDALE PRINCESS
ARIZONA

CHILDREN'S TUMOR FOUNDATION
ENDING NF THROUGH RESEARCH

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CLINICAL CARE SYMPOSIUM – CME SESSION

Session Co-Chairs: Nicole Ullrich, MD, PhD, *Boston Children's Hospital;* Heather Radtke, MS, CGC, *Children's Tumor Foundation*

Mosaicism in Neurofibromatosis and Schwannomatosis – Clinical Manifestations and Implications for Testing

Saturday, June 24, 8:20am – 8:50am

Miriam Bornhorst, MD, *Children's National Hospital*

Mosaicism occurs when there are two different cell populations present in the body, some that carry a mutation and some that do not. Mosaicism is common in patients with Neurofibromatosis and Schwannomatosis, yet it can be difficult to both identify and diagnose, ultimately leading to gaps in management. In this presentation, the clinical manifestations of mosaicism in patients with NF1, NF2 and schwannomatosis will be reviewed, including implications for testing and subsequent management. Testing methodologies will also be discussed, including benefits and limitations of each method.

Diagnostic Odyssey in Schwannomatosis

Saturday, June 24, 8:50am – 9:20am

Vanessa L. Merker, PhD, *Massachusetts General Hospital*

People with non-*NF2*-related schwannomatosis may face a long and sometimes difficult journey to being diagnosed, called a diagnostic odyssey. Based on the review of medical records of 97 people with confirmed or probable non-*NF2*-related schwannomatosis seen in two U.S. tertiary care clinics, we found patients had a median time from first symptom to diagnosis of 16.7 years (95% CI, 7.5-26.0 years) and a median time from first medical consultation to diagnosis of 9.8 years (95% CI, 3.5-16.2 years). Thirty-six percent of patients were misdiagnosed at least once during this time; misdiagnoses were of underlying genetic condition (18.6%), pain etiology (16.5%), and nerve sheath tumor presence/pathology (11.3%) (non-mutually exclusive categories). Furthermore, 19.6% of patients had a clear missed opportunity for genetics workup that could have led to an earlier schwannomatosis diagnosis. Based on this data, we will discuss potential interventions for timelier and more accurate diagnoses of non-*NF2*-related schwannomatosis, as well as share qualitative findings from patients regarding how to effectively communicate a diagnosis of schwannomatosis once it is discovered. We will end by reflecting on how recent updates to the diagnostic criteria for schwannomatosis may affect the diagnostic process for patients moving forward.

OPG and MRI: To Screen or Not to Screen, That is the Question

Saturday, June 24, 9:40am – 10:10am

Cynthia Campen, MD, *Stanford University;* **Katie Metrock, MD**, *University of Alabama at Birmingham;* **Rosalie Ferner, MD, FRCP**, *Guy's and St. Thomas' NHS Foundation Trust London*

Objective: to discuss the controversies regarding screening for NF1-associated optic pathway glioma (OPG) in the context of novel therapies and neuroimaging advances.

Introduction: An overview of the current state of screening for NF1-associated OPG.

Discussion: With the aid of robust audience participation, we will discuss controversies and advances in the field that may have relevance when weighing decisions for screening and treatment of NF1-associated OPG.

1. Is waiting for vision loss too late?
2. Should advances in treatment options change our threshold for treatment?
3. Given the unpredictable nature of NF1-associated OPGs, could advances in neuroimaging techniques allow us to predict which patients will have aggressive OPGs that require treatment vs. those patients who have quiescent tumors?
4. Should we consider other screening techniques, such as optical coherence tomography (OCT) or clinical predictors of OPG behavior?
5. What evidence is there for the safety or toxicity of general anesthesia to the developing brain? How do these data inform our decision making?

Virtual Education Opportunities for NF Clinicians

Saturday, June 24, 10:10am – 10:30am

Amedeo Azizi, MD, *Medical University of Vienna;* **Pamela Trapane, MD**, *University of Florida at Jacksonville*

The CTF European and US Clinical Care Advisory Boards organize a series of online conferences for NF clinicians, including the INFER program and the Virtual Case Conference. These two programs offer healthcare providers the opportunity to expand NF knowledge and expertise and collaborate with other NF clinicians with the goal of improving the diagnosis, management, and treatment of neurofibromatosis and schwannomatosis.

KEYNOTE #1: DRUGS THAT TARGET RAS AND THEIR RELEVANCE TO NF1 – CME SESSION

Saturday, June 24, 12:30pm – 1:30pm

Frank McCormick, PhD, FRS, DSc, *University of California San Francisco and Frederick National Laboratory for Cancer Research*

Direct targeting of KRAS has emerged as a new paradigm in targeted oncology. Two drugs that target one specific mutant allele, KRAS G12C, have already been approved for treating non-small cell lung cancer, and many more are in clinical and preclinical development. These include drugs targeting the ON state of G12C covalently, non-covalent KRAS G12D inhibitors, pan-KRAS inhibitors, and others, some employing novel drug modalities. Drugs targeting other proteins in the RAS pathway are also under development, as single agents or in combinations. Targets include SOS1, SHP2, RAF and PIK3CA. I will discuss the relative merits of these drugs in the context of NF1 disease, and future prospects for treating NF1. I will also discuss the possibility that loss of neurofibromin provokes pathways unrelated to the classical RAS MAPK pathway and the roles of RAS superfamily members in the NF1 phenotype.

TIPS AND TRICKS FOR SUCCESSFUL PRECLINICAL DRUG ASSAYS – CME SESSION

Session Co-Chairs: Pau Castel, PhD, *NYU Langone School of Medicine;* Piotr Topilko, PhD, *INSERM*

Screening and *In Vivo* Validation of Drugs Targeting Cutaneous Neurofibromas

Sunday, June 25, 2:00pm – 2:25pm

Piotr Topilko, PhD, *Mondor Institute for Biomedical Research, Créteil, France*

Despite the prevalence and significant burden of cutaneous neurofibromas (cNFs), which develop often in large numbers in almost all patients, the initial focus of treatment with the MEK inhibitors was directed towards NF1 children's with non- operable plexiform tumors (pNFs). To determine the feasibility of such therapies in cNFs, it is crucial to conceive reliable animal models that faithfully recapitulate the characteristics and progression of cNFs in humans. Such models should accurately mimic the complex pathogenesis of cNFs including the role of tumor Schwann cells (SC) and their interactions with the microenvironment. Furthermore, deciphering the similarities and differences in the mechanisms driving the pathogenesis of cutaneous and plexiform tumors is essential. Finally, the generation of the large cohort of animals with numerous cNFs and the read outs and endpoint parameters for drug evaluation efficacy should be easy and precise. Thanks to the novel *Nf1-KO* mouse models of cNFs (expressing Tom+ in mutant SC), our and other studies has demonstrated that cutaneous and pNFs, despite originating from common SC lineage, are distinct entities. Unlike pNFs, which exhibit slow but permanent mutant SC proliferation, cNFs develop rapidly but transiently, with the majority of tumor SC quickly becoming quiescent due to low MAPK pathway activity. Such observations suggest that drugs targeting tumor SC proliferation may not be effective in treating cNFs. Additionally, we have observed significant differences in the architecture of both types of tumors, including distinct immune landscapes and abnormally dense innervation in cNFs. These observations point towards immune cells and sensory neurons as potential targets for their treatment. To validate candidate drugs for preventing or regressing the development of cNFs, we have developed a versatile in vivo platform. For each assay, *Nf1-KO* mice are generated and their back skin is scanned for Tom+ SC before and immediately after treatment to assess cNFs size, number, and location. The drug or drug combinations, delivered either systemically or topically, are administered to developing (preventive protocol) or established (curative protocol) cNFs, and the mice are closely surveyed throughout the treatment period for adverse effects. At the end of the treatment, the mice are sacrificed, and the tumors, along with the adjacent healthy-looking skin, are dissected and processed for quantitative and qualitative analysis of tumor cells and their complex microenvironment. Using this approach, we have tested the efficacy of variety of drugs targeting different checkpoints of the RAS signaling pathway. While MEK inhibitor binimetinib showed only a mild effect when topically delivered to mature cNFs, the same drug demonstrated high efficiency in preventing cNFs over long periods, providing strong evidence for the quiescent nature of tumor SC in mature tumors and the importance of designing drugs that target the crosstalk between tumor cells and their microenvironment, including the dense innervation.

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Tips and Tricks for Successful Drug Trials in Rare Disease

Sunday, June 25, 2:25pm – 2:50pm

Wade Clapp, MD, *Richard L. Schreiner Distinguished Professor and Chair, Indiana University School of Medicine and Riley Hospital for Children, Indianapolis, IN*

To date, over 7,000 rare diseases have been identified, affecting approximately 4% of the world's population or roughly 260–440 million people. Despite the significant morbidity and mortality associated with rare diseases and their collective prevalence worldwide, the development and translation of therapeutics for the treatment of patients suffering from rare disease has been hindered by several factors. Firstly, due to the lack of commercial return on investment, there is little to no economic incentive to develop novel agents for the treatment of orphan diseases. A potential solution to this issue is through the identification of established biochemical pathways known to be disrupted in other common malignancies as drivers of malignancies in rare cancer predisposition syndromes, thus making it possible to leverage the enormous investments in drug development through the repurposing of existing agents. A second major barrier is disease heterogeneity compounded by the low number of patients, resulting in a wide range of therapeutic response and a lack of statistical significance in clinical trials. Finally, the lack of a "critical mass" of committed investigators with complementary basic and clinical trial expertise at any single institution is a substantial impediment to conducting state-of-theart translational research in orphan diseases. With these barriers in mind, collaborative research groups that span the basic and translational spectrum of discovery are making progress by collectively developing accurate preclinical models, utilizing state-of-the-art biochemical tools, and designing preclinical trials that will inform novel phase 1-2 trials and, at the same time promote engagement with biotech and large pharmaceutical companies. Establishing a strategy linking preclinical and clinical studies is critical for translational innovation and efficiency. The utility of this 'linked' preclinical to clinical strategy is reflected in the identification of early and promising therapeutic strategies for the treatment of neurofibromas and JMML. Using the success of this preclinical to clinical strategy in the setting of NF1 as an example, this presentation will highlight some accomplished and developing strategies that could be applied to accelerate therapeutic discovery and development in neurofibromatosis and other orphan diseases.

PAIN & ITCH – CME SESSION

Session Co-Chairs: Jaishri Blakeley, MD, *Johns Hopkins University;* Scott Plotkin, MD, PhD, *Massachusetts General Hospital*

SMARCB1 in Schwann Cells Directly Represses the Transcription of Factors That Induce Pain Sensitivity in Sensory Neurons

Saturday, June 24, 3:30pm – 3:55pm

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

Schwannomatosis patients typically present with intractable pain. A significant proportion of patients with schwannomatosis have mutations in the *SMARCB1* gene (also called INI1, BAF47 and SNF5). We found that inducible conditional disruption of the *Smarcb1* gene in mouse Schwann cells causes increased sensitivity to capsaicin. Dorsal root ganglion (DRG) neurons and trigeminal ganglion neurons from mice with Schwann cell-targeted disruption of *Smarcb1* express elevated levels of TRPV1, a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin. We also find that TRPA1, an ion channel that acts as a sensor for environmental irritants, is elevated in the DRG and trigeminal neurons of these mice. Wild type DRG cells grown in *Smarcb1*-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients with *SMARCB1* mutations expressed elevated levels of TRPV1 and TRPA1 as indicated by immunocytochemistry. Proteomic analysis demonstrated that the secretome of *Smarcb1*-mutant Schwann cells is distinct from wild type Schwann cells and includes elevated levels of cytokines and chemokines that have been implicated in pain. *Smarcb1* interacts with the promoters of these genes and directly represses their transcription. Furthermore, agents that block at least some of these proteins can reverse the induction of TRPV1 in DRG cells treated with *SmarcB1*-mutant Schwann cell conditioned media and reduce pain responses to conditioned media in mice. Collectively, these data indicate that loss of *Smarcb1* 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 in patients with *SMARCB1* mutations.

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Nociceptors: Itch and Pain

Saturday, June 24, 3:55pm – 4:20pm

Matthias Ringkamp, MD, PhD, *Johns Hopkins University*

The sensations of itch and pain are closely related. In my presentation, I will give an overview of the types of nerve fibers that were found in primates to be involved in mediating these and describe some of their physiological properties. I will also summarize results from efforts to translate findings on the peripheral neuronal mechanisms of acute itch, previously reported in mice, to primates.

Imaging Pain: Identification of Painful Peripheral Nerve Sheath Tumors Using a Sigma-1 Receptor Radioligand and PET/MRI

Saturday, June 24, 4:20pm – 4:45pm

Thomas Wilson, MD, PhD, *Stanford University*

Background: Pain is often prominent in patients with neurofibromatosis and schwannomatosis. Pain control is best achieved with targeted treatment of the offending tumor(s), but the pain-generating tumor(s) can often be difficult to localize with currently available techniques. Thus, there is a need for improved diagnostics that allow for accurate identification of the pain-generating tumor(s). The sigma-1 receptor (S1R) is a molecular chaperone involved in the pain pathway and may be a candidate as a biomarker of pain in tumors and other conditions.

Methods: We performed immunohistochemistry on human benign peripheral nerve sheath tumor (BPNST) specimens, labeling S1R and compared S1R expression in painful versus non-painful tumors. We developed a novel radioligand, 18F-FTC-146, that is highly specific for S1R. Patients with a solitary painful (N=11) or non-painful (N=10) BPNST underwent 18F-FTC-146 PET/MRI. A painful tumor was defined as $\geq$3/10 on the numerical rating scale (NRS). PET and MRI statistics were compared against pain status.

Results: The mean SUVmax in the non-painful group was 1.39 versus 3.39 in the painful group ($p=0.004$). The mean SUVmean in the non-painful group was 0.62 versus 1.32 in the painful group ($p=0.007$). We generated Receiver Operator Chracteristic (ROC) curves for SUVmax and SUVmean on 18F-FTC-146 PET/MRI. The area under the curve (AUC) for SUVmax was 0.946 versus 0.846 for SUVmean. At a threshold level of SUVmax $\geq$2.13 for detecting a painful tumor, the sensitivity was 100%, specificity 90%, and accuracy 95%. Spearman's rho for the association of SUVmax and NRS pain score was 0.834 ($p<0.001$), indicating strong correlation.

Conclusions: S1R is differentially expressed in painful versus non-painful BPNSTs, and this differential expression can be detected in vivo using 18F-FTC-146 PET/MRI. This technique may prove useful in identifying sources of pain in patients with neurofibromatosis and schwannomatosis.

KEYNOTE #2: A FLY APPROACH TO NF – CME SESSION

Sunday, June 25, 8:30am – 9:30am

Ross Cagan, PhD, *University of Glasgow*

NF is a complex disease that involves complex whole body interactions. My laboratory has developed a suite of tools for exploring RAS-based diseases using Drosophila, chemistry, and organoid approaches. I will discuss how we are applying these approaches to take a whole animal approach to NF.

RASOPATHIES – CME SESSION

Session Co-Chairs: Marco Tartaglia, PhD, *Bambino Gesù Children's Hospital;* Hilde Brems, PhD, *KU Leuven*

The Selfish Origin of Germline Mutations in the RTK/RAS/MAPK Pathway

Sunday, June 25, 9:30am – 9:55am

Anne Goriely, PhD, *MRC-Weatherall Institute of Molecular Medicine; Nuffield Division of Clinical Laboratory Sciences, Radcliffe Department of Medicine, University of Oxford, Oxford, UK*

It is now well-established that *de novo* mutations (DNMs) are an important contributor to disease, causing a severe developmental disorder in ~1:300 births. Most DNMs (>80%) originate during spermatogenesis and their frequency increases with paternal age at the slow but constant rate of ~1-2 mutation/paternal year.

We have previously described a new disease-mechanism contributing to the paternal age-related increase in pathogenic DNMs called 'selfish selection', whereby specific DNMs arising in male germline stem cells (spermatogonia) lead to their clonal expansion, causing an apparent increase in mutation levels in human sperm over time. This mechanism relies on principles akin to oncogenesis and explains the paternal age-effect and high birth prevalence observed for several Mendelian disorders, including Noonan *(PTPN11),* Costello *(HRAS)*, Apert *(FGFR2)* syndromes and achondroplasia *(FGFR3),* that occur up to 1000-fold more frequently than background.

I will describe the strategies we have developed to show that the human testis is a repository of pathogenic *de novo* mutations and that selfish 'moles' are prevalent in older men's testes. Our data show that the male germline is particularly vulnerable to dysregulation of the RTK/RAS/MAPK pathway, which is a key regulator of stem cell homeostasis in the testis. Importantly, because the germline provides a source of heritable material (unlike somatic tissues), this phenomenon has implications that extend far beyond the individual in whom it takes place, and is predicted to affect disease prevalence and predisposition, genome heterogeneity and evolution of our species.

Molecular Genetics of RASopathies

Sunday, June 25, 9:55am – 10:20am

Marco Tartaglia, PhD, *Molecular Genetics and Functional Genomics, Ospedale Pediatrico Bambino Gesù, Rome, Italy*

Signaling through the RAS-MAPK signaling pathway is tightly controlled, and its enhanced activation has been known for decades to represent a major event in oncogenesis. Unexpectedly, discoveries derived from a massive disease gene hunting effort performed in the last 15 years have established a picture in which the upregulation of this signaling cascade underlies a group of clinically related developmental disorders collectively known as "RASopathies", which share cardiac defects, reduced postnatal growth, variable cognitive deficits, facial dysmorphism, ectodermal and musculoskeletal anomalies, and variably increased risk for certain malignancies as major features. Based on the relatively high prevalence of some of these disorders, the dysregulation of this signaling pathway represents one of the most common events affecting development.

RASopathies are caused by mutations in genes encoding RAS proteins and structurally/functionally related GTPases, regulators of RAS function, modulators of RAS interaction with effectors or downstream signal transducers of the MAPK backbone. A subset of these genes have been implicated in cancer. Many of these RASopathy-causing alleles encode proteins with upregulated functional behavior, but with less activating strength compared to those contributing to oncogenesis. In these genes, a largely non-overlapping spectrum of germline and somatic mutations is generally observed. On the other hand, a few disorders result from proteins with defective function. In these cases, the implicated proteins negatively control signal flow. Remarkably, RASopathy-causing mutations may affect protein function by multiple mechanisms and have different consequences on intracellular signaling.

Here is provided an overview on the genes implicated in this group of developmental disorders and the molecular basis underlying the differential impact of germline and somatic mutations in development and cancer. The circuits and players with previously unrecognized regulatory role on RAS-MAPK signaling are also discussed, together with the unanticipated molecular mechanisms converging toward the dysregulation of this signaling cascade, and major clinically relevant genotype- phenotype correlations.

Platform: Targeting KRAS to Treat Schwann Cell Tumors with *NF1* Loss

Sunday, June 25, 10:20am – 10:35am

Liang Hu, MD, PhD, *Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

The *NF1* gene mutation causes a genetic disorder called Neurofibromatosis Type I (**NF1**), characterized by tumor formation in the peripheral nervous system. These tumors include benign plexiform/cutatneous neurofibroma, and rarer malignant peripheral nerve sheath tumors (**MPNST**). Though the MEK inhibitor has been approved for plexiform neurofibroma treatment, a more effective and durable therapy for NF1-related tumors, MPNST in particular, is desperately needed. Though RAS activation has been implicated in NF1-related Schwann cell (**SC**) tumor formation and growth, the contributions of individual RAS proteins are poorly defined. We aimed to define the critical RAS protein(s) in *NF1* mutant SCs, and then target relevant RAS isoforms for treating NF1-related tumors. *NF1*-/- immortalized human SC lines and mouse *Nf1*-/- SCs showed increased GTP-H-RAS and GTP-K-RAS versus isogenic controls. Conditional suppression of individual RAS isoforms in SCs showed that reducing KRAS uniquely stopped the growth of and signicantly reduced the tumorigenesis of *NF1* mutated SCs. This suggested KRAS as a viable target for treating SC tumors with *NF1* mutations. Protein kinase C (**PKC**) agonism has been proposed as a tumor therapy in K-RAS mutant tumors. We find that PKC agonist also selectively impairs the viability of SCs with *NF1* mutations, and *NF1* mutant MPNST cell lines. Using knock-down approaches and pharmacology, we define novel PKC isoforms as the targets responsible for the effect of PKC agonist on *NF1* mutant SCs. In vivo, in a genetically engineered mouse model (**GEMM**) of plexiform neurofibroma short-term treatment with PKC agonist specifically induced cell death in mouse SCs; long-term treatment significantly reduced tumor number and tumor size. The PKC agonist also caused cell death/necrosis in xenografted MPNST cells and significantly, albeit transiently, slowed tumor growth in immunodeficient mice. Our results suggest that KRAS is a critical RAS isoform driving NF1 tumorigenesis, and that targeting KRAS activation by PKC agonists is a promising avenue for neurofibroma and MPNST therapy.

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Molecular Mechanisms of *LZTR1* in RASopathies and Schwannomatosis

Sunday, June 25, 11:00am – 11:25am

Pau Castel, PhD, *New York University School of Medicine, New York, NY*

RAS oncoproteins are drivers of tumor initiation and progression and therapies targeting these directly or their downstream pathways have resulted in unprecedented clinical benefit in cancer. However, recent advances in genomic medicine have revealed that many of the known RAS oncoproteins and their regulators are involved in the pathogenesis of a group of congenital disorders termed RASopathies. Our research is aimed at understanding the role of these oncoproteins at the biochemical and signaling level and we develop novel mouse models that allow us to study these in the context of cancer and genetic syndromes. Our previous work focused on the non-classical RAS protein RIT1, a poorly understood GTPase expressed in most tissues, that is frequently mutated in Noonan syndrome, a common RASopathy. While studying the signaling properties of this RAS GTPase, we identified LZTR1 as a novel interactor and regulator of RIT1. LZTR1 is a substrate adaptor protein that forms a complex with the RING E3 Ubiquitin Ligase Cullin3 and promotes the ubiquitination and degradation of RIT1. Therefore, in contrast to classical RAS proteins, RIT1 appears to be mostly regulated through proteasomal degradation by interacting with this novel complex. Interestingly, LZTR1 variants have been reported in both Noonan syndrome individuals and in some patients with schwannomatosis. We found that the LZTR1 complex is conserved in lower organisms and is necessary for the proper regulation of RIT1 protein levels in humans, mice, and fruit flies. We have developed mouse models to understand the role of LZTR1 at the organismal level. Heterozygous *LZTR1* knockout mice appear normal and do not exhibit any significant phenotypes; in contrast, homozygous mice are not viable due to cardiovascular and liver development defects, which can be rescued by crossing with *RIT1* knockout mice. We demonstrate that Noonan syndrome and schwannomatosis-associated *LZTR1* pathogenic variants are loss-of-function, as assessed by biochemical and functional assays. In addition, we show that LZTR1 loss in Schwann cells also results in dysregulation of RIT1-mediated hyperactivation of the Ras-MAPK pathway, which could have implications in the pathobiology of schwannomatosis. Overall, our work provides novel insights into the regulation, function, and pathogenesis of the LZTR1 adaptor protein and the RAS-like protein RIT1.

Platform: Interneurons that BiTE, a Cellular Therapy for NF1 High Grade Gliomas

Sunday, June 25, 11:25am – 11:40am

Thomas De Raedt, PhD, *Children's Hospital Philadelphia and the University of Pennsylvania*

NF1 associated High Grade Gliomas have a poor prognosis with limited therapeutic options. Cellular therapies, like CAR-T therapy, have unfortunately shown limited efficacy for brain tumors, due to the lack of unique tumor antigens, the downregulation of targeted antigens, and the immune suppressive tumor microenvironment (TME). To circumvent these brain tumor specific obstacles, we developed a cellular immunotherapy delivery system, derived from post-mitotic Migratory Cortical Inhibitory Interneuron Precursors (MCIPs), that induces a cytotoxic tumor response in high grade gliomas independent of unique tumor antigens. During fetal brain development, MCIPs are chemoattracted to migrate long distances from their subcortical origins into the cerebral cortex. The MCIP chemoattractant CXCL12 activates CXCR4 to induce this migration. Intriguingly, CXCL12 is secreted by High Grade Gliomas, suggesting they chemoattract MCIPs. Excitingly, our in vitro and in vivo data indeed show that MCIPs robustly migrate to the majority of glioblastoma cell lines evaluated. Crucially, this migration is independent of unique tumor antigens and the TME and provides us with the opportunity to use MCIPs as a delivery vector for cytotoxic agents. In one approach, we successfully eliminated EGFR expressing glioblastoma *in vivo* by modifying MCIPs to secrete EGFR Bispecific T-cell Engagers (BiTE). BiTE engagement with a tumor cell results in T-cell activation and elimination of the engaged tumor cell. Importantly, the T-cells have no innate recognition of the tumor cell, this is provided by the BiTE. Local EGFR BiTE secretion has limited systemic toxicity, thereby making EGFR a viable non-unique target. MCIPs can be equipped with a range of agents that eliminate High Grade Gliomas. As a therapy, we envisage injecting modified MCIPs, differentiated from patient or donor "off the shelf" induced pluripotent stem cells, into the margin of the surgical cavity to "clean-up" tumor cells remaining post-surgery. An interneuron clinical trial for epilepsy (NCT05135091) is ongoing, highlighting the translational potential of our MCIP cellular therapy.

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Funding: Department of Defense, Larson Foundation, Rally Foundation, NIH R25

Platform: Targeting the NF-kappaB Pathway to Treat *NF1*-Deficient Tumors

Sunday, June 25, 11:40am – 11:55am

Stephanie J. Bouley, PhD, *Massachusetts General Hospital*

Due to the limited number of therapeutic options available for treating patients with neurofibromatosis type 1 (NF1), there is a critical need for discovering novel targets for the rational design of strategies to treat *NF1*-deficient tumors.

To discover new therapeutic targets, we have taken a multipronged approach to identify molecular signatures associated with *NF1* loss in Schwann cells (SCs). Using a panel of isogenic pairs of *NF1*-deficient homozygous and heterozygous SCs derived either from plexiform neurofibromas or engineered using CRISPR gene-editing, we conducted profiling of the activated kinome, total and phospho-proteome, and transcriptome signatures. Numerous kinases with altered activity, proteins with altered phosphorylation patterns, and genes with altered transcription levels were identified. Comparative analyses between isogenic sets of SCs highlighted several signaling pathways with altered activity upon NF1 loss, including the NF-kappaB (NF-kB) pathway.

Results from our RNAseq data showing increased expression of NF-kB-associated receptors and downstream kinases were confirmed in a patient-derived isogenic pair of $NF1^{+/-}$ and $NF1^{-/-}$ SCs using RT-qPCR. Subsequent analyses are being used to explore whether components of the NF-kB pathway may represent potential biomarkers or therapeutic targets for NF1-associated tumors. Single agent therapeutics against these targets were tested and demonstrated preferential sensitivity to $NF1^{-/-}$ SCs compared to $NF1^{+/-}$ SCs. Additional isogenic pairs of $NF1^{+/-}$ and $NF1^{-/-}$ SCs, independent of those used in our initial analyses, are being used to confirm these results. We plan to analyze transcription of NF-kB target genes prior to and after treatment with our most promising inhibitors to identify potential biomarkers, as well perform combination treatments with the MEK inhibitor Selumetinib.

These data suggest that targeting the NF-kB pathway may be an effective strategy to treat NF1-deficient tumors such as plexiform neurofibromas and malignant peripheral nerve sheath tumors; further, this work may reveal novel biomarkers to measure successful treatment options in NF1 patients.

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KEYNOTE #3: TACKLING DISEASES DUE TO MUTATIONS IN LARGE GENES USING AAVs – CME SESSION

Sunday, June 25, 1:00pm – 2:00pm

Ivana Trapani, MD, PhD, *Telethon Institute of Genetics and Medicine (TIGEM); Dept. of Advanced Biomedical Sciences, University of Naples "Federico II", Naples, Italy*

Gene therapy with adeno-associated viral (AAV) vectors holds promises for treating inherited diseases. However, AAV packaging capacity limited to about 5 kb precludes use of these vectors for the treatment of those diseases which are caused by mutations in genes with a coding sequence exceeding 5 kb. We have recently developed strategies to expand AAV transfer capacity in the retina, which are based on the use of multiple AAV vectors and protein trans-splicing mediated by split inteins. Delivery of multiple AAV vectors, each encoding for one of the fragments of large therapeutic proteins flanked by short split-inteins, resulted in efficient protein trans-splicing and full-length protein reconstitution in the retina of small and large animal models and in human iPSC-derived retinal organoids. The levels of protein reconstitution achieved are therapeutically relevant in mouse models of inherited retinal diseases. This talk will present the development and our progress in using these systems, which support the capability to safely and effectively explore multiple AAV-based platforms to tackle common forms of inherited diseases due to mutations in large genes.

PRECLINICAL DEVELOPMENT: HOW TO CREATE A PATH FOR GENE THERAPY IN NF – CONCURRENT SESSION – CME SESSION

Session Co-Chairs: Renyuan Bai, MBBS, PhD, *Johns Hopkins University;* Deann Wallis, PhD, *University of Alabama Birmingham*

Developing an AAV Gene Replacement Therapy for NF1 Tumors

Sunday, June 25, 2:00pm – 2:25pm

Renyuan Bai, MBBS, PhD, *Johns Hopkins University*

Neurofibromatosis type I (NF1) is attributed to genetic alterations in the Nf1 gene, which subsequently lead to the formation of peripheral nervous system tumors following the loss of the second Nf1 allele. Plexiform neurofibromas (pNFs), a distinguishing feature of NF1, originate from Schwann cells (SCs), causing significant morbidity and mortality. They have the potential to transform into malignant peripheral nerve sheath tumors (MPNST). A subset of NF1 can result in countless cutaneous neurofibromas in or just beneath the skin, often leading to substantial cosmetic issues, pain, and other reductions in quality of life. No current therapies can prevent or cure this condition. However, restoring the function of the mutated Nf1 could ideally halt disease progression. Therefore, conceptually, NF1 gene replacement is a promising therapeutic strategy for this disease, despite two significant challenges. Firstly, no efficient vehicle exists to deliver genes to SCs, the tumor-initiating cells of NF1, and NF1 tumors. Secondly, the large 8.5 kb full-length Nf1 cDNA cannot be accommodated by viral vectors typically used for in vivo gene therapy, such as the adeno-associated virus (AAV).

In order to overcome these limitations, we created a membrane-targeting GAP-related domain (GRD- C24) that suppresses Ras activity potently in NF1 cells, serving as the payload for AAV vectors. Moreover, we utilized a combinatorial approach involving capsid DNA shuffling and biopanning in an orthotopic NF1 xenograft tumor model to develop a novel AAV vector that can strongly transduce NF1 tumors in vivo. Twelve naturally occurring AAV serotypes were used as templates for DNA shuffling and subsequent in vivo selection. One candidate, G557-2, exhibited improved biodistribution and transduction in NF1 xenograft models compared to the AAV9 vector. When packaged with GRD-C24, G557-2 significantly but transiently suppressed tumor growth. Further refinement of G557-2 was achieved through the screening of a 7mer peptide library inserted in the VR-VIII loop, leading to capsids with substantially improved biodistribution characteristics, including reduced liver retention and enhanced delivery in tumors.

Our results demonstrate AAV-mediated gene replacement as a promising systemic treatment for NF1 tumors, offering a viable path towards clinical translation.

Exploring Gene Replacement Therapy for NF2-Related Tumors

Sunday, June 25, 2:25pm – 2:50pm

Jeremie Vitte, PhD, *University of California, Los Angeles*

Homozygous loss of the tumor suppressor gene, *NF2* is associated with both hereditary and sporadic tumors of the nervous system. Germline mutations in *NF2* are responsible for *NF2*-schwannomatosis, a dominantly inherited disease characterized by the development of hallmark bilateral vestibular schwannomas, but also schwannomas on other cranial, spinal, and peripheral nerves, as well as meningiomas and ependymomas. Biallelic inactivation of the *NF2* gene is also almost always found in schwannomas from *SMARCB1*- and *LZTR1*-related schwannomatosis patients. Sporadic schwannomas are relatively common in adults and nearly all exhibit *NF2* inactivation. Current therapies involving surgery and radiosurgery are effective for individual tumors, but are not always a viable option for patients with multiple tumors and harbor significant risk of neurological deficits and morbidity. Gene therapy for hereditary diseases is becoming a promising new medical treatment, as in gene replacement therapy for vision loss, spinal muscular atrophy, and hemophilia as well as in clinical trials for a number of neurologic diseases. In this pilot study, we explored the potential of gene replacement therapy for *NF2*-related tumors using adeno-associated virus (AAV) vectors expressing merlin, the gene product of the *NF2* gene. We used the established schwannoma mouse model Postn-Cre;$Nf2^{flox/flox}$ to test the biodistribution and efficacy of AAV9-NF2. This proof-of-principle study demonstrated that reintroduction of merlin in deficient tumor cells can provide preclinical therapeutic efficacy.

Full List of Authors: Jeremie Vitte, Michael Wootton, Scott R. Plotkin, Marco Giovannini

Considerations for *In Vivo* NF Modeling for Gene Therapy

Sunday, June 25, 2:50pm – 3:15pm

Bob Kesterson, PhD, *Pennington Biomedical Research Center, Baton Rouge, LA*

There are numerous hurdles to overcome when developing a therapeutic to replace or correct a genetic mutation. To best match the type of mutation with the appropriate type of gene therapy as well as optimize the vector and route of delivery of the therapeutic, a robust animal model is needed that phenotypes the human condition. Approaches for NF1 *in vivo* modeling will be discussed including the development of numerous animal models (mice, rats, and zebrafish) that harbor NF1 patient-specific mutations representing the spectrum of mutation types. Genetic strategies for testing various phenotypes produced using NF1 conditional knockouts in mice will be reviewed and examples provided for different NF1 patient mutation models. Gene replacement using nanoparticles to package the large NF1 gene will be discussed and contrasted to the vectors to deliver the smaller NF2 gene. The need for NF2 avatar models will be presented as well as challenges for establishing therapeutic windows and limitations of CRISPR/Cas9 gene editing.

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

Sunday, June 25, 3:15pm – 3:30pm

Marc Moore, PhD, *National Horizons Centre, Teesside University*

Background: Neurofibromatosis type I arises from germline mutations across the *NF1* gene, which diminish expression of the tumour suppressor protein neurofibromin. 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.

Purpose: To provide pre-clinical validation of ASOs *in vivo* and facilitate translational development. The Kesterson lab created a mouse model (hG629R) with the insertion of a human exon 17 carrying the G629R pathogenic variant with partial flanking intronic sequences. Two routes of administration and three delivery platforms were explored to give proof-of-concept of *in vivo* exon skipping efficacy of our optimized ASOs.

Methods: The hG629R mouse model has been utilised to explore delivery platforms of antisense sequences, including i) Naked PMOs, ii) Adeno Associated viral (AAV) vectors with AAV9 serotype carrying U7-SnRNA expression cassettes and iii) conjugation of ASOs with morpholino chemistry to cell penetrating peptides (CPP-PMO). Two administration routes intravenous (IV) and Intracerebroventricular (ICV) injections were examined.

Summary of Results: Biodistribution studies: Viral delivery of AAV9-eGFP was administrated by both ICV and IV to adult and neonatal mice to evaluate biodistribution eGFP was detected in the liver, heart and brain, with ICV found to give greater expression in the brain relative to IV. Provisional evidence from an on-going sub-region analysis indicates eGFP detection in the cortex, cerebellum, and olfactory bulb in adult mice.

Exon Skipping efficacy: AAV9-U7-SnRNA encoding ASO sequences and CPP-PMOs were delivered to adult mice and harvested after one week. Viral delivery of U7-SnRNA constructs showed detectable exon skipping by RT-qPCR analysis and thus a reduction of the G629R variant at the transcript level in the optic nerve, liver and provisional evidence indicates the brain. Both routes of administration of the CPP-PMO produced exon skipping in brain, optic nerve, sciatic nerve and kidney, with exon skipping also evident in the liver following ICV delivery

Conclusions: We provide proof-of-concept evidence that efficient delivery of ASOs *in vivo* resulting in detectable targeted exon skipping is achievable across multiple tissues.

Future Plans: Optimization of the dosing regimen for both delivery strategies will be performed with examination of the efficacy and durability of the exon skipping response being used as readouts. Biodistribution studies of CPP-PMOs using fluorescent tagging
will also be investigated. Finally, efficacy in an acute or tumor model of NF1 loss would provide strong proof of concept for using exon skipping as a therapeutic.

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

Funding body: Gilbert Family Foundation

CLINICAL DEVELOPMENT: HOW TO CREATE A PATH FOR GENE THERAPY IN NF – CONCURRENT SESSION – CME SESSION

Session Co-Chairs: Renyuan Bai, MBBS, PhD, *Johns Hopkins University;* Deann Wallis, PhD, *University of Alabama Birmingham*

Gene Therapy for Epidermolysis Bullosa

Sunday, June 25, 3:45pm – 4:10pm

Peter Marinkovich, MD, *Stanford University*

This talk will describe the pathophysiology of dystrophic epidermolysis bullosa, the structure and function of the basement membrane and the current clinical features of dystrophic epidermolysis bullosa as well as the current state of therapy. Additionally the current state of the gene therapy field will be described as well as the concept of in vivo as well as ex vivo gene therapy approaches. Finally, this talk will also describe three programs in the development of gene therapy for epidermolysis bullosa, and will describe the4ri progress, from preclinical studies to phase 3 clinical trials and in the case of one therapy, FDA approval.

Development of Gene Therapies for Duchenne Muscular Dystrophy: Implications for Neurofibromatosis Type 1

Sunday, June 25, 4:10pm – 4:35pm

Linda Popplewell, PhD, *Teesside University*

The rare diseases Duchenne muscular dystrophy (DMD) and neurofibromatosis type 1 (NF1) share a number of similarities. They are both caused by a wide range of loss-of-function mutation types spread over the length of large genes. The disease phenotypes displayed are progressive in nature and severely impact patients' quality of life and reduce life expectancy.

DMD is caused by mutations in the X-linked *DMD* gene that normally encodes for dystrophin protein. Its loss from skeletal and cardiac muscle leads to sarcolemmal instability that, upon muscle contraction results in muscle fibre necrosis, inflammation and replacement with fatty and fibrotic tissue. The standard of care for DMD is treatment with corticosteroids which act to increase life expectancy but deleterious side effects with long-term use is reported. For NF1, a MEK inhibitor has been approved for use in children over 2 years of age who have symptomatic inoperable plexiform neurofibromas. Like corticosteroids for DMD, MEK inhibition acts on a downstream target rather than the mutated *NF1* gene itself.

Despite the affected gene being identified more than three decades ago, it is only in the last seven years that gene-targeting therapies have been approved for DMD. These approvals are for mutation-specific medicines based on antisense oligonucleotide-induced exon skipping and aminoglycoside-induced stop codon readthrough. Since these drugs target the RNA, repeat administration is required. Such treatments could hold applicability to certain mutations that cause NF1 and are being developed pre-clinically.

Understanding of the pathogenesis of a less severe allelic disease, where internally truncated but functional dystrophin is expressed, has enabled the design and optimisation of so called microdystrophin gene addition therapy for DMD. Delivery is mediated using adeno-associated viral (AAV) vectors with capsid serotypes that display muscle tropism. Clinical trials are producing encouraging results and it is expected that approvals will be imminent. Such a therapy is mutation-agnostic, but patients with pre-existing capsid antibodies are precluded from trial. Long-term episomal expression of microdystrophin results, but the therapy applicability is likely to be limited by the high costs of production. Since the dystrophin expressed is not full length, functionality is compromised. Other vector types are being explored pre- clinically for the delivery of the full length *DMD* cDNA. For NF1, the multiple functional motifs over the length of the NF1 gene and its instability, make gene addition therapy difficult to optimise but is the focus of much pre-clinical development.

Other strategies being explored for the treatment of DMD include genome editing, upregulation of expression of proteins that can compensate for the lack of dystrophin and targeting the muscular atrophy and fibrosis seen in the disease. It is likely that ultimately a combination of treatments may be required for fuller efficacy. Genome editing is also being explored for NF1, but it is as yet unknown whether another protein could compensate for the lack of neurofibromin. The diverse pathology displayed in NF1 is likely to require the optimisation of different treatments for different stages of the disease.

NF1 CLINICAL SCIENCE – CONCURRENT SESSION – CME SESSION

Session Co-Chairs: Carlos Romo, MD, *Johns Hopkins University;* Rianne Oostenbrink, MD, *Erasmus University Rotterdam*

Harnessing Innate Immune Responses in MPNST Therapeutics

Sunday, June 25, 2:02pm – 2:27pm

Ping Chi, MD, PhD, *Memorial Sloan Kettering*

Malignant peripheral nerve sheath tumors (MPNSTs), the most common and lethal malignant tumors in patients with Neurofibromatosis Type 1 (NF1), is characterized by recurrent biallelic inactivation of the *NF1, CDKN2A* and PRC2 components (*EED* or *SUZ12*). PRC2 loss in MPNST results in global loss of H3K27me2/3 and aberrant transcriptional activation of developmentally silenced master regulators, leading to enhanced cellular plasticity. PRC2 loss through epigenomic reprogramming also leads to aberrant activation of multiple signaling pathways (e.g., WNT signaling), an "immune desert" tumor microenvironment (TME), and primary resistance to immune checkpoint blockade (ICB). Using RNAi-based screen, we identified DNMT1 as a top synthetic lethal candidate with PRC2 loss in MPNST. We further demonstrated that DNA methyltransferase (DNMT) inhibitors led to significantly enhanced cytotoxicity and anti-tumor effects in PRC2 loss MPNST cells and tumors, at least in part through augmented activation of viral mimicry and innate immune responses. These observations have led to an investigator-initiated phase II trial of ASTX727 (an oral pan-DNMT inhibitor, recently FDA approved for treatment of myelodysplastic syndrome) in MPNST with PRC2 inactivation (Clinicaltrials.gov NCT04872543). We will further discuss novel recombinant engineered immunogenic viruses to activate innate immune response to capitalizing immunotherapy benefit and their clinical translation in MPNST.

Platform: Evidence for Reduced Incidence of Malignant Peripheral Nerve Sheath Tumors in NF1 Adults and Adolescents Under Close Surveillance

Sunday, June 25, 2:27pm – 2:42pm

Eric Legius, MD, PhD, *Center for Human Genetics, University Hospital Leuven and Department of Human Genetics, Catholic University of Leuven, Belgium*

Previous studies repeatedly showed severe increased risk for malignant peripheral nerve sheath tumors (MPNST) in individuals with neurofibromatosis type 1. A Finnish population study showed a cumulative incidence of 12.3% before the age of 50y and a life time risk of 15.8% (Uusitalo et al., 2016). It has been shown that many MPNSTs originate from pre-existing neurofibromas. Some neurofibromas go through the phase of atypical neurofibromatous neoplasia of unknown biological potential (ANNUBP). Some of these ANNUBPs further evolve into MPNST. ANNUBPs can be considered premalignant tumors and harbor a recurrent deletion of 9p including the *CDKN2A*/*CDKN2B* region (Beert et al., 2011; Higham et al., 2018). They frequently present as growing distinct nodular lesions (DNL) on whole body MRI (Fisher et al., 2022) and are FDG-PET avid (Miettinen et al. 2017; Higham et al., 2018).

We instituted a close surveillance of adolescents and adults using history, clinical examination and whole body MRI (at transition age or later if seen for the first time) to screen for potential hallmarks of ANNUBP or other relevant tumoral complications. In case ANNUBP was suspected the lesion was further screened by FDG-PET and if PET avid the lesion was removed if safely possible (Carton et al., 2023).

We studied a total of 276 individuals with NF1 who are under this type of surveillance in one center. The age range is 16-77y with mean age of 37y and mean follow-up of 4.8 years. The total follow-up period is 1328 person years. During this surveillance period 18 pathologically proven ANNUBPs were removed in 16 individuals (mean age 30.8y; range: 16.2-60.7), 11 secretory pheochromocytomas (mean age 39.1; range: 23-56.6) and 4 GISTs (mean age 53.5y; range:32.3-68.9). Two individuals are under close surveillance because their DNL showed FDG-PET avidity and could not be safely resected.

However we did not identify any MPNST in these 276 individuals during the surveillance period (1328 person years). The risk for MPNST is highest before the age of 50y and based on the population study of Uusitalo et al we would have expected 3.38 cases of MPNST during the 1073 person years (<51y) of follow-up taking into account the sex ratio of our cohort (p=.034; Poisson and Binomial distributions).

This is the first evidence of a reduced incidence of MPNST as a result of close surveillance in adolescents and adults with NF1 with a mean follow-up of only 4.8y. Further follow-up of this cohort is warranted to confirm that screening for ANNUBP followed by removal if safely possible decreases the incidence of MPNST in adolescents and adults with NF1. We hope that a longer follow-up will show an even higher benefit.

Full List of Authors: Pia Iasella, Daphne Hompes, Thomas Douchy, Frank Van Calenbergh, Thomas Decramer, Vincent Vandecaveye, Ellen Denayer, Hilde Brems, Eric Legius

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Platform: Early Detection of Malignant, Pre-Malignant and Benign Peripheral Nerve Sheath Tumors with Liquid Biopsy Cell-Free DNA Fragmentomics

Sunday, June 25, 2:42pm – 2:57pm

R. Taylor Sundby, MD, *National Cancer Institute*

Purpose: Early and accurate detection of neurofibromatosis type 1 (NF1) associated plexiform neurofibroma (PN), atypical neurofibroma (AN) and malignant peripheral nerve sheath tumors (MPNST) would improve prognosis, inform surveillance, and reduce treatment morbidity. Detection and precise classification, however, remain challenging by imaging and tissue biopsy due to tissue heterogeneity. We recently published that cell free DNA (cfDNA) copy number alterations (CNA) accurately distinguish MPNST from PN[1]. In this multi-institutional study, we hypothesize that the integration of cfDNA fragmentomics enhances early detection of MPNST and enables accurate identification of benign and pre-malignant disease states.

Methods: We performed whole genome sequencing (WGS) of plasma cfDNA from a novel cohort of healthy controls ($n = 21$), patients with PN ($n = 72$), AN ($n = 23$) and pre-treatment MPNST ($n = 30$). CNA analysis with *in silico* size selection was completed as we previously described[1]. We used DELFI[2] to integrate ratios of short (100-150bp) to long (151-220bp) cfDNA fragments in 5-megabase regions across the genome along with chromosome arm-level features. cfDNA fragment size profiles were also analyzed using unsupervised non-negative matrix factorization (NMF). One-versus-one (OVO) comparisons were made using logistic regression with 5-fold internal cross-validation followed by receiver operating characteristic area under the curve (AUC) analysis. RNA-seq differential expression analysis was performed with edgeR. 4-mer sequences were extracted from 5'-ends of each cfDNA read to determine end-motif frequencies.

Results: In this new cohort, CNA again accurately distinguished copy number aberrant MPNST from PN (AUC 0.78) but could not differentiate more copy-neutral AN from PN (AUC 0.5). Given our previous observation of differences in cfDNA fragment lengths[1], we analyzed fragmentomic profiles to better distinguish these copy-neutral states as well as to boost the sensitivity of detecting MPNST. Indeed, bin-wise fragmentomics with DELFI improved assay accuracy: MPNST vs. AN (AUC 0.86), MPNST vs. PN (AUC 0.83), MPNST vs. healthy (AUC 0.93), AN vs. PN (AUC 0.65), AN vs. healthy (AUC 0.57), and PN vs. healthy (AUC 0.80). NMF decomposition of global fragment length signatures further improved performance in OVO comparisons: MPNST vs. AN (AUC 0.91), MPNST vs. PN (AUC 0.89), MPNST vs. healthy (AUC 0.97), AN vs. PN (AUC 0.78), AN vs. healthy (AUC 0.70), and PN vs. healthy (AUC 0.78). Analysis of previously published RNA-seq of MPNST tissue[3] and normal tissue revealed that DNAses are differentially expressed in MPNST. Because DNases have specific preferences for nucleic acids motifs, cfDNA fragment end motifs were analyzed. The CTCA end motif was more commonly present in cell-free DNA from MPNST patients compared to healthy donors (Bonferroni-corrected p-value < 0.001). Strikingly, end motifs alone accurately distinguished MPNST from PN (lasso LOOCV accuracy 0.82).

Conclusions: This study demonstrates that the spectrum of benign, pre-malignant and malignant peripheral nerve sheath tumors have distinct, disease state-specific cfDNA fragmentomic signatures which facilitate accurate early detection. Ongoing analysis of end motifs and chromatin accessibility in cfDNA may provide further biological insights and novel therapeutic targets. Furthermore, this work validates our recently published CNA-based cfDNA assay[1] for detecting MPNST vs. PN in a novel cohort.

Full List of Authors: R. Taylor Sundby[1], Jeffrey J. Szymanski[2], Alex C. Pan[1], Paul A. Jones[2,3], Sana Z. Mahmood[1], Ling Liao[3], Peter K. Harris[2], Andrea M. Gross[1], Brigitte C. Widemann[1], Angela C. Hirbe[3,4,5,6], Aadel A. Chaudhuri[2,3,5,7,8], Jack F. Shern[1]

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Platform: SARC031: The Phase 2 Trial of Selumetinib and Sirolimus for Patients with Unresectable or Metastatic Malignant Peripheral Nerve Sheath Tumors (MPNST)

Sunday, June 25, 2:57pm – 3:12pm

AeRang Kim, MD, PhD, *Children's National Hospital*

Background: Novel therapies for malignant peripheral nerve sheath tumors (MPNST) are desperately needed. Combined mTOR and MEK inhibition, critical components of RAS effector pathway underlying the pathogenesis of NF1 mutant tumors, caused tumor regression in a transgenic MPNST mouse model. The glucose transporter GLUT1 was suppressed when both pathways were inhibited, which translated into early suppression of FDG-PET tumor uptake in a dose dependent manner that ultimately correlated with tumor shrinkage. Thus, we hypothesized that the combination of selumetinib with sirolimus, oral MEK and mTOR inhibitors respectively, would cause tumor regression in patients with MPNST. The primary objective aims to determine the clinical benefit rate (CBR) of this combination in patients with unresectable or metastatic NF1 associated or sporadic MPNST. Secondary objectives include defining the toxicities of the combination and assessing the impact on pain interference and pain intensity. Exploratory objectives include early FDG-PET response as a potential imaging biomarker, circulating tumor DNA (ctDNA) assessment, and pharmacodynamic and immune marker changes in blood and tumor tissue pre and on treatment.

Methods: This is a multi-institutional open label phase 2 study coordinated by Sarcoma Alliance for Research through Collaboration (SARC), funded by the Department of Defense, of selumetinib and sirolimus for patients ≥12 yrs with unresectable/metastatic MPNST. Selumetinib was given orally 50mg twice daily and sirolimus given orally 4mg once daily with a cycle 1 day 1 loading dose of 12mg in 28-day cycles. Tumor response was evaluated using RECISTv1.1. FDG-PET was obtained at baseline and cycle 1 day 11 to assess for early response via PERCIST1.0. Patient reported pain assessments (Numeric Rating Scale-11 and PROMIS Pain Interference short form), ctDNA, and blood and/or tumor tissue for pharmacodynamic (PD) and immune markers were collected at baseline and on treatment. A Simon's two stage design was used with a response defined as complete response (CR), partial response (PR), or stable disease (SD) ≥4 cycles. If ≥1/7 patient in stage 1 responded, enrollment would be expanded by 15 patients and if ≥ 3/21 responded, the combination would be considered of sufficient activity.

Results: 21 evaluable patients (7F; median age 41 y (range 16-72), 14 NF1 associated) enrolled on study at the 5 participating sites. Patients were heavily pretreated and majority had metastatic disease (n=17). Only 1/7 in stage 1 and 1/14 in stage 2 were considered responders (SD≥4cycles); median number of cycles was 2 (range 1-6). Most common Adverse Events (AE) related to investigational agents were grade ≤2 gastrointestinal (diarrhea, anorexia, nausea), acneiform rash, hypertriglyceridemia, mucositis, and transaminase elevation. Five patients had dose reductions due to toxicity. No patients came off study due to AE. Twenty of 21 evaluable patients had a cycle 1 early FDG-PET scan performed. Partial metabolic responses were seen in 8 patients (40%) but did not ultimately correlate with objective response. Majority of patients with MPNST had tumor pain (77%) but no meaningful differences in pain scores from baseline to pre-cycle 2 in the total cohort, although 5 of 15 patients had clinically meaningful (≥2 points) decreases in tumor pain intensity. PD-L1 expression increased in both CD3+ T and NK cells, while effector Treg and naïve Treg were decreased in pre and post treatment analysis.

Conclusions: The combination of selumetinib and sirolimus at the doses used appears to be tolerable with manageable and expected AEs. Promising early FDG-PET response did not correspond to RECIST imaging response. The combination did not meet study parameters for further evaluation in MPNST. ctDNA and tissue pharmacodynamic analyses are underway.

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Platform: Association of Serial Serum Cytokine and Chemokine Levels with Plexiform Neurofibroma Characteristics, Symptoms, and Response to Treatment with the MEK Inhibitor Selumetinib

Sunday, June 25, 3:12pm – 3:27pm

Steven D. Rhodes, MD, PhD, *Indiana University*

Background: Plexiform neurofibromas (PNFs) are complex tumors that exhibit variable clinical behavior and symptoms. Selumetinib, a MEK1/2 inhibitor (MEKi), is approved for treatment of symptomatic and inoperable PNF in children with neurofibromatosis type 1 (NF1). However, not all patients experience significant tumor regression or symptomatic improvement, such as pain relief, while on selumetinib, highlighting the need for biomarkers to identify patients most likely to derive clinical benefit. We evaluated associations between serum cytokines and chemokines with clinical phenotypes and PNF response in children with symptomatic PNF enrolled on a phase 2 trial of selumetinib (SPRINT, NCT01362803).

Methods: Serial serum cytokine samples were collected from all SPRINT participants at baseline and prior to cycles 3, 5, 9, and 12 of selumetinib treatment (25 mg/m^2/dose twice daily; 1 cycle=28 days). 65 cytokines/ chemokines were quantified using Luminex xMAP technology using pre-validated panels from Millipore and R&D. PNF response was evaluated by volumetric MRI analysis (partial response (PR) $\geq$20% decrease). Patient-reported outcome (PRO) measures were used to assess pain intensity (Numerical Rating Scale-11 [NRS-11]) and pain interference (Pain Interference Index [PII]). Target PNF growth prior to enrollment and response to selumetinib were analyzed using logistic regression models. Continuous outcomes including target PNF volume at baseline, percent shrinkage on treatment and PII scores were analyzed using generalized linear models for Gamma distribution. NRS-11 scores had excessive zero count, so a zero-inflated Poisson regression model was used to analyze the outcome. Potential confounders including age, gender, and weight were controlled across all models.

Results: Baseline serum cytokine/chemokine samples were collected on 49 of 50 participants, and 48 (96%) had at least one on-treatment sample. In this exploratory analysis, we identified cytokines including Leptin, VEGF, AXL, TGF-beta, and BMP9 that were significantly associated with multiple tumor characteristics and measures of pain, both at baseline and with changes over time. For example, baseline AXL levels were directly correlated with baseline target PNF volume ($p<0.0001$) and parent PII scores ($p<0.0001$). Leptin levels at baseline and change over time were associated with eight different clinical phenotypes and tumor outcomes, the most of any measured cytokine. These included direct correlations between the baseline leptin level and maximum response to treatment of the target PNF ($p=0.0233$) and baseline NRS-11 score ($p=0.0105$).

Conclusions: The decision of whether to initiate medical therapy for PNF can be complex, and variable treatment responses observed with MEKi and in other early-phase trials to date highlight the need for reliable biomarkers to forecast PNF behavior and treatment responses. In this exploratory analysis, several cytokines, including Leptin, VEGF, AXL, TGF-beta, and BMP9, were found to be associated with various clinical and PRO measures, including PNF growth status at baseline, PNF response to treatment, and PNF-related pain. Further studies are needed to validate these results and explore the underlying mechanisms by which these cytokines may contribute to PNF pathogenesis and treatment response. This study represents a critical first step towards the stratification of patients with PNF based on disease biology to ultimately inform risk-adapted care and improve treatment outcomes and quality of life for affected individuals.

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ERN GENTURIS Tumor Surveillance Guidelines for Individuals with Neurofibromatosis Type 1

Sunday, June 25, 3:45pm – 4:10pm

Rianne Oostenbrink, MD, PhD, *ErasmusMC-Sophia, Rotterdam, The Netherlands*

Background: Neurofibromatosis type 1 (NF1) is a multisystem genetic disorder, predisposing development of benign and malignant tumors. Given the oncogenic potential, long-term surveillance is important in patients with NF1. Proposals for NF1 care and its specific manifestations have been developed, but lack integration within routine care. This guideline aims to assimilate available information on NF1 associated tumors (based on evidence and/or expert opinion) to assist healthcare professionals in undertaking tumor surveillance of NF1 individuals.

Methods: By comprehensive literature review, performed March 18th 2020, guidelines were developed by the NF1 tumor management guideline group (NF1 experts n=32 and patient representatives (n=6) from Europe and 2 advisors from USA), conversant with clinical care of the wide NF1 disease spectrum. We used a modified Delphi procedure to overcome issues of variability in recommendations for specific (national) health care settings, and to deal with recommendations based on indirect (scarce) evidence. Experts in this exercise included the members of the NF1 Tumor Management Guideline Group, as well as an additional 60 external experts identified by the Guideline Group.

Findings: We defined 67 recommendations for personalized and targeted tumor management in NF1, ensuring appropriate care for those in need, whilst reducing unnecessary intervention. They achieved a consensus of 80% or higher and addressed general approach, psychosocial needs and the twelve most prevalent NF1 tumor manifestations. Most recommendations achieved the moderate evidence level; we could define strong levels for 5/11 recommendations for OPG, 5/8 for MPNST and 3/ 4 for periorbital PN. For all manifestations, we discussed i) appropriate clinical screening to detect tumors and its difference for NF1 patients, ii) usefulness of imaging screening, iii) the method and monitoring interval if a tumor is diagnosed; iv) the indication for treatment. In addition, we discussed and advised on the role of optical coherence tomography and whole body MRI in NF1 management. Finally, we addressed what type of psychosocial support is useful in people with NF1 and discussed the burden of living with the uncertainty considering possible tumor development or the monitoring and management of a tumor.

Interpretation: The guidelines reflect the current knowledge and standard of care for NF1 in Europe and are meant to be a tool to help to structure and justify the diagnostic and therapeutic path in NF1. They are not meant to be prescriptive and may be adjusted to local available resources at the treating center, both within and outside EU countries.

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Platform: Detection of Distinct Nodular Lesions on Whole-Body MRI in a High-Risk Population with Neurofibromatosis Type 1

Sunday, June 25, 4:10pm – 4:25pm

Carlos G. Romo, MD, *Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD*

Background and study purpose: Atypical neurofibromas (aNFs) and "atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP)" are pre-malignant tumors that may represent targets for early intervention to prevent MPNST in people with NF1.Prior MRI studies have identified "distinct nodular lesions" (DNLs) as potential imaging representations of aNF or ANNUBP. The purpose of this study is to prospectively evaluate the prevalence, natural history, and multi-parametric imaging features of DNLs on whole body magnetic resonance imaging (WB-MRI) in people with NF1.

Methods: Participants (n=80) are being enrolled at the Johns Hopkins Comprehensive Neurofibromatosis Center. Inclusion criteria include diagnosis of NF1 and high-risk for MPNST defined as: *NF1* microdeletion, personal or family history of ANNUBP, aNFs or MPNST, large pNF burden (≥3 cm on largest diameter), or prior radiation treatment. Participants undergo yearly WB-MRI using parallel imaging and total imaging matrix (comprised of isotropic volumetric T2 short tau inversion recovery (STIR) and diffusion weighted imaging (DWI) and apparent diffusion coefficient (ADC) mapping) at 3.0 Tesla. Participants are followed for 4 years and localized MRI and F18-FDG PET/CT are performed on suspicious DNLs for further characterization. Biopsy or resection is performed for DNLs with suspicious imaging features.

Results: Thus far, 54 participants (25 (44.6%) male and 31 (55.4%) female) have been recruited and 52 (96.4%) have completed baseline WB-MRI, with 2 (3.6%) unable to complete WB-MRI due to claustrophobia and body habitus. High-risk criteria included high internal pNF burden (47, 83.9%), microdeletion syndrome (4, 7.1%), personal history of ANNUBP/ANF or MPNST (17, 30.4%), family history of ANNUBP/ANF or MPNST (5, 8.9%) and prior radiation treatment (5, 8.9%). In this high-risk cohort, 82 DNLs were characterized on baseline WB-MRI in 30 patients (prevalence – 56% (30/54)). Among the 82 DNLs, 4 (5%) were biopsied or excised based on symptoms and suspicious WB-MRI features. Pathology was benign for 2 and malignant for 2. Seventeen participants had 1-year surveillance WB-MRI. One person had a new DNL (frequency of new DNL – 6%); pathology was consistent with low-grade MPNST.

Conclusions: Non-invasive biomarkers that identify tumors with high risk of malignant conversion are necessary for early intervention and improvement of clinical outcomes in people with NF1. WB-MRI can detect and characterize DNLs using DWI/ADC mapping. Screening WB-MRI in high-risk patients detects DNLs with high frequency (56%). Suspicious DWI/ADC features and the development of new DNL on surveillance WB-MRI can be useful in guiding clinical management.

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Platform: Social Determinants of Health and Neurocognitive and Psychological Outcomes in Youth with NF1

Sunday, June 25, 4:25pm – 4:40pm

Johanna Nielsen, PhD, *Children's National Hospital*

Objective: This study explored associations between social determinants of health (e.g., community resources and opportunities) and psychological and neurocognitive outcomes in youth with neurofibromatosis, type 1 (NF1).

Participants and Methods: The sample included 145 clinically-referred youth with NF1 living in the Washington, DC Metro Area who completed a neuropsychological evaluation (ages 2-23 years, M=10.5, 49.7% Male). Social determinants of health were indexed by the Childhood Opportunity Index (COI), a publicly available tool which measures neighborhood-level quality of environmental and social conditions that contribute to positive health. It includes an overall opportunity index and three component scores assessing distinct aspects of opportunity, which include educational opportunity (e.g., educational quality, resources, and outcomes), health/environmental opportunity (e.g., access to healthy food, healthcare, and greenspace) and social/economic opportunity (e.g., income, employment, poverty). COI values were extracted from electronic medical records based on home address using census tract geocoding. Using metro-based norms, children from all opportunity levels were represented (17.9% Very Low, 15.2% Low, 16.6% Moderate, 20.7% High, 29.7% Very High). Outcome data included an age-appropriate Wechsler Scale and parent-report questionnaires (Behavior Rating Inventory of Executive Function/BRIEF, Child Behavior Checklist/CBCL). Multiple regression analyses were conducted to examine main effect associations between COI and performance based cognitive outcomes (Wechsler Full Scale IQ [FSIQ], verbal reasoning [VIQ], nonverbal reasoning [NVIQ], working memory index [WMI], processing speed index [PSI]) and parent reported emotional and behavioral outcomes (BRIEF GEC, CBCL Internalizing Problems, Externalizing Problems, Attention Problems, Social Problems), controlling for age and gender. Additional regression analyses examined these relationships for the three COI subdomains, entered as simultaneous predictors.

Results: Overall COI was significantly positively associated with overall intellectual functioning (FSIQ: $t=3.279$, $=0.001$) as well as verbal and nonverbal reasoning (VIQ: $t=3.026$, $p=0.003$; NVIQ: $t=2.999$, $p=0.003$). Overall COI was also significantly associated with performance-based working memory ($t=2.038$, $p=0.044$), but was not associated with processing speed ($t=0.972$, $p=0.333$). Lower COI was also associated with greater parent reported externalizing problems ($t=-2.100$; $p=0.038$) but was not significantly associated with executive function problems ($t=-1.672$; $p=0.097$), internalizing problems ($t=-0.666$; $p=0.507$), attention problems ($t=-1.267$; $p=0.208$), or social problems ($t=-0.798$; $p=0.426$). Educational opportunity was significantly associated with overall intellectual functioning ($t=2.592$; $p=0.011$), verbal reasoning ($t=3.296$, $p=0.001$), nonverbal reasoning ($t=2.723$, $p=0.007$).

Conclusions: Consistent with a growing body of literature demonstrating the impact of social and environmental contexts to health outcomes, these results show inequities in adverse neurocognitive, emotional, and behavioral outcomes in youth with NF1 related to neighborhood-level social determinants of health. Living in communities with lower economic, environmental, and educational resources and opportunities is associated with lower performance on tests of intellectual functioning and working memory, as well as greater externalizing behavior problems. Examination of specific contributing factors highlight educational opportunity as a particularly important contributor to overall intellectual development. These preliminary findings highlight the need for continued efforts to understand community level risk and resilience factors specific to the NF1 population, as well as policy and advocacy efforts to address inequities and improve outcomes for youth in low resource communities.

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KEYNOTE #4: NEW MODELS FOR DRUG SCREENING AND NEW MODELS – CME SESSION

Monday, June 26, 8:30am – 9:30am

Min Jae (MJ) Song, PhD, *Senior Scientist, National Center for Advancing Translational Sciences*

Complex *in vitro* models (CIVMs), also called microphysiological systems (MPS) or 3D organotypic models, are emerging tools to study cell-cell and cell-microenvironment interactions within a three-dimensional context and to better translate research findings to clinical studies as they faithfully recapitulate human physiology and pathology. With the advent of human induced pluripotent stem cell (iPSC) and iPSC derived cells, CIVMs enable extensive access to engineered human organotypic tissues for basic and translational research. With iPSCs, it is now possible to engineer isogenic tissues to study complex mechanisms underlying diseases and understand the relationships between genetics and physiological/pathological phenotypes, for specific patient groups, including underserved communities and minorities. The 3D Tissue Bioprinting Laboratory (3D-TBL) at NCATS accordingly aims to establish 3D predictive human in vitro model for drug development and discovery. This presentation will cover a portfolio of 3D models at NCATS 3D-TBL, highlighting multiple levels of tissue complexity.

NOVEL MODELS / NOVEL TARGETS – CONCURRENT SESSION – CME SESSION

Session Co-Chairs: Alison Lloyd, *University College London;* Helen Morrison, PhD, *Leibniz Institute on Aging - Fritz Lipmann Institute*

The Development and Validation of a Patient-Derived 3D Meningioma Cell Culture Model

Monday, June 26, 9:55am – 10:15am

Laurien van de Weijer, MSc, *Faculty of Heath: Medicine, Dentistry and Human Sciences, Derriford Research Facility, University of Plymouth, Plymouth, Devon, UK*

Meningioma is the most common intracranial brain tumour. These tumours are very heterogeneous and encompass a wide spectrum of clinical aggressivity, ranging from benign WHO grade 1 tumours to malignant WHO grade 3 tumours, which typically cause severe morbidities and patient death. Treatment options are limited to surgery and radiotherapy, reflecting the need for development of new therapies.

Three-dimensional (3D) patient-derived cell culture models have been shown to closely recapitulate *in vivo* tumour biology and microenvironment interactions and can serve as a robust tool for the development of novel therapies. Therefore, we have developed a novel easy-to-use method to establish patient-derived meningioma spheroids that resemble essential features of parental tumours, including the tumour microenvironment, to better predict the toxicity and efficacy of novel therapies. Reproducible spheroids were generated using ultra-low adherence 96-well plates and low-serum media optimised for spheroid culture. Extensive characterisation using RNAseq, DNAseq, and IHC revealed spheroid cultures to closely recapitulate histological and molecular features and tumour-stromal composition profiles of their respective primary tumours. Finally, the application of this model was validated for use as *in vitro* tool for assessing drug effectiveness and studying important oncogenic cellular processes such as EMT. Patient-derived spheroids were treated with several experimental drug-based therapies, including the histone deacetylase inhibitor Trichostatin A (TSA) and the MerTK inhibitor UNC2025. Patient-derived spheroids displayed significantly different IC_{50} values following 72h TSA and UNC2025 treatment compared to matched monolayer cultures ($p<0.05$). Single-dose and combination treatment with MerTK and HDAC inhibitors synergistically reduced spheroid cell viability, proliferation, and invasion. Furthermore, combination therapy also showed the potential to revert the EMT phenotype, as evidenced by a significant increase in expression of the epithelial marker E-cadherin after treatment by western blot and qPCR experiments ($p<0.05$).

Spheroid culture remains an unestablished niche in NF2-null tumour biology research. Development of 3D cultures such as this one could ultimately result in bypassing the animal phase in drug development and serve to bridge the translational gap between *in vitro* and *in vivo* research.

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Platform: Merlin-Deficient iPSCs Show Altered Pluripotency and Constitute a Potential *In Vitro* Model for NF2-Related Schwannomas

Monday, June 26, 10:15am – 10:30am

Núria Catasús, PhD, *Clinical Genomics Research Group, Germans Trias i Pujol Research Institute*

The appearance of bilateral vestibular schwannomas (VS) is one of the most characteristic features of *NF2*-related schwannomatosis (*NF2*-related SWN), an autosomal dominant syndrome that predispose to the development of tumors of the nervous system. VS are caused by the bi-allelic inactivation of the *NF2* gene in a cell of the Schwann cell lineage. Our current understanding of VS initiation and progression as well as the development of new effective therapies is hampered by the absence of human non-perishable cell-based models. With this aim, we generated and characterized induced pluripotent stem cell (iPSC) lines with single or bi-allelic inactivation of *NF2* by combining the direct reprogramming of VS cells with the use of CRISPR/Cas9 editing. Our results show a critical function of *NF2* for both reprograming and maintaining a stable pluripotent state. Despite the difficulty of maintaining merlin-deficient iPSCs, we were able to differentiate them into neural crest (NC) cells. At this stage, the spontaneous expression of the SC marker S100B and the impossibility to generate Schwann cells in 2D cultures denoted also an altered differentiation capacity of merlin-deficient cells towards the NC-SC axis, in the used *in vitro* conditions. Nevertheless, by applying a 3D Schwann cell differentiation protocol, we successfully generated *NF2*(+/-) and *NF2*(-/-) spheroids homogeneously expressing classical markers of the NC-SC axis. Transcriptional analysis of these spheroids also showed differential expression of genes related to *NF2* pathways and cell adhesion. Overall, *NF2*(+/-) and *NF2*(-/-) spheroids represent a potential genuine *in vitro* model of NF2-related schwannomas.

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Protein Replacement Therapy for Schwannoma Treatment

Monday, June 26, 11:00am – 11:25am

Helen Morrison, PhD, *Leibniz Institute on Aging, Fritz Lipmann Institute, Jena, Germany*

We have shown previously a multifactorial concept for schwannoma formation emphasizing microenvironmental factors, incomplete nerve regeneration after injury. Specifically, we discovered a pro-tumourigenic axonal effects on Schwann cells in genetically engineered mice. We found patient- mimicking schwannomas could be induced by crush injury in animals with combined Nf2 deletion in Schwann cells and axons. Currently in our tumor model, we identified that the lack of axonal Neuregulin 1 beta (NRG1ß) constitutes a key contributor to schwannoma formation. Following injury, Schwann cells dedifferentiate into proliferative "repair" cells that support debris clearance and nerve regeneration. Once axonal regrowth is completed, "repair" cells normally re-differentiate into non-proliferative axon- associated Schwann cells, but we propose that they are unable to do so in the absence of sufficient Nrgß1, thus contributing to schwannoma development. We have devised a protein replacement therapy, (re-) supplying soluble recombinant human rhNRG1, that significantly inhibits schwannoma formation and improves nerve regeneration. We are currently validating our approach in a preclinical, multi-center, confirmatory trial following human clinical trial standards. In conclusion we demonstrate a non-cytotoxic reduction of schwannoma growth by administration of rhNRG1, which had been shown to be safe in human application in trials for chronic heart failure.

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Platform: Targeted STAT1 Therapy for the Treatment of *LZTR1*-Driven Peripheral Nerve Tumors

Monday, June 26, 11:45am – 12:00pm

Tonči Ivanišević, MBiochem, *VIB Center for Cancer Biology, Department of Oncology, KU Leuven, Herestraat 49, 3000 Leuven, Belgium*

Schwannomas are tumors of the Schwann cells that cause chronic pain, numbness, and potentially life-threatening impairment of vital organs. Germline mutations of Leucine Zipper like post Translational Regulator 1 (LZTR1), the 22q tumor suppressor gene, account for up to 40% of the cases of familial schwannomatosis. To assess the role of *Lztr1* in schwannomatosis, we generated a Schwann cell-specific *Lztr1* knockout model using a Cre recombinase driven by the P0/Myelin Basal protein promoter. However, no phenotypic changes were detected in *P0*-specific *LZTR1* knockout mice up to the age of 100 weeks. *LZTR1* mutations are frequently accompanied by a loss of the second 22q chromosomal region, containing *LZTR1* and the other 22q tumor suppressor genes, such as *NF2,* the key regulator of the Hippo pathway. To recapitulate the 3-step model of schwannoma development, we generated Schwann cell-specific triple *Lztr1, Large tumor suppressor kinase 1 (Lats1), and Lats2* knockout mice. We monitored schwannoma development non-invasively by magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET-CT). We found that the triple knockout causes the development of peripheral nerve tumors that partially recapitulate vestibular, sciatic, and seminal schwannomas. Immunohistochemistry analysis of the tumors revealed hyperactivation of the Mitogen-activated protein kinase (MAPK) cascade and macrophage infiltration. Consistent with the observed inflammatory phenotype, the proteomic analysis of LZTR1-depleted human Schwann cells showed a dramatic up-regulation of the Interferon/Signal transducer and activator of transcription (STAT1) signaling pathway. We found that *LZTR1*-mediated activation of the MAPK cascade leads to the phosphorylation of STAT1 at S727, whereas *LATS1/2* loss promotes the phosphorylation of STAT1 at Y701, leading to a full-blown STAT1 activation. Anti-STAT1 therapy decreased inflammation and tumor cell proliferation in seminal schwannomas. These results identified vulnerabilities of schwannoma with loss of LZTR1 function and propose anti-STAT1 therapy for schwannoma treatment.

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NF2-RELATED SCHWANNOMATOSIS (NF2) & SCHWANNOMATOSIS (SWN) CLINICAL SCIENCE – CONCURRENT SESSION

Session Co-Chairs: Jaishri Blakeley, MD, *Johns Hopkins University;* P. Leia Nghiemphu, MD, *University of California, Los Angeles*

Platform: Genetic Findings in People with At Least One Non-Vestibular Schwannoma and Not Meeting Clinical Criteria for *NF2*-Related Schwannomatosis

Monday, June 26, 9:55am – 10:15am

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

Approximately 1/500 people develop a schwannoma during their lifetime. Half of these arise from the vestibular nerve, often causing deafness and balance problems. The other half arise from non-vestibular cranial nerves, or spinal or peripheral nerves. Most schwannomas are isolated and occur in otherwise healthy people. However, bilateral vestibular schwannomas, or multiple non-vestibular schwannomas are often associated with a genetic predisposition. This is most commonly *NF2*-related schwannomatosis, caused by germline pathogenic *NF2* variants. Non-*NF2* related schwannomatosis is rarer and around half of these cases have been associated with a pathogenic germline variant in *SMARCB1* or *LZTR1.* Schwannomas occurring in non-*NF2* related schwannomatosis are much less likely to occur on the vestibular nerve.

We assessed the genetic findings in 133 people, from 129 families who did not meet clinical diagnostic criteria for *NF2*-related schwannomatosis, unless they were known to have an identified pathogenic germline *LZTR1* pathogenic or likely pathogenic (P/LP) variant, and who had at least one non-vestibular schwannoma that had undergone genetic testing for *NF2* variants.

We found that 2/133 (1.5%) people in this cohort had a non-mosaic *NF2* P/LP variant identified in blood and another 15 (11%) had a mosaic *NF2* variant. There were 18 (14%) people from 14 families who had a germline *SMARCB1* P/LP variant and 20 (15%) unrelated individuals who had a germline *LZTR1* P/LP variant. A further 10 people had a variant of uncertain significance (VUS) in *LZTR1* (mainly missense variants). The remaining 78 people had no germline P/LP variant detected in *NF2, SMARCB1* or *LZTR1.*

NF2 screening of schwannoma DNA showed that 98/133 had LOH involving chr 22. Of these, 84/98 (86%) also had an *NF2* variant detected on the trans allele. In schwannomas without identified LOH, only 5/35 (14%) had an identified *NF2* variant. In total 103/133 (77%) non-vestibular schwannomas had at least one *NF2* variant identified in tumour DNA.

Overall, similar proportions of people had an identified germline P/LP variant in *NF2* (mainly mosaic), *SMARCB1,* or *LZTR1.* However, when two tumours are available, half of schwannomatosis patients who have no mutation in blood are normally found to have a mosaic *NF2* variant. In addition, if further evidence shows that more of the *LZTR1* missense variants are pathogenic, then the proportion of people diagnosed with *LZTR1*-associated schwannomatosis would be significantly increased in this cohort, indicating the importance of comprehensive testing and improved *LZTR1* variant pathogenicity classification.

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Strategies for Clinical Trials for LZTR1, SMARCB1 Related and Other Schwannomatoses

Monday, June 26, 10:15am – 10:30am

Jaishri Blakeley, MD, *Johns Hopkins University*

There are multiple tumor conditions related to alterations on chromosome 22. These include NF2, LZTR1 and SMARCB1 associated schwannomatosis. All of these conditions are associated with schwannomas and pain. Pain is the dominant presentation in most people with LZTR1 and SMARB1 schwannomatosis seeking treatment. NF2 and LZTR1 associated schwannomatosis are also associated with meningiomas. Developing therapies for these conditions requires choosing a focus for therapeutic impact (symptom control, tumor control, genetic alteration). There are increasing opportunities for impactful clinical trials for the schwannomatose based on: (a) increased experience and success with NF2-SWN clinical trials, (b) development and incorporation of tools measuring the therapeutic impact on pain, (c) more therapies in development that are relevant to pain pathways or targets relevant to chromosome 22 alterations, and (d) more experience with novel clinical trial designs. Considerations for the challenges and opportunities inherent to clinical trials for the schwannomatoses are discussed.

Platform: Phase II Study of Axitinib in Patients with NF2 and Progressive Vestibular Schwannomas

Monday, June 26, 11:00am – 11:15am

Mekka R. Garcia, MD, *NYU Grossman School of Medicine*

Purpose: Vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR), and c-KIT represent clinically and/or preclinically validated molecular targets in vestibular schwannomas. We conducted a single institution, prospective, open-label, two-stage phase II study (ClinicalTrials.gov identifier NCT02129647) to estimate the response rate to axitinib, an oral multi-receptor tyrosine kinase inhibitor targeting VEGFR, PDGFR and c-KIT, in NF2 patients with progressive vestibular schwannomas (VS).

Methods: Adult and pediatric patients age >5 years with a clinical diagnosis of NF2 and at least one volumetrically measurable, progressive VS were eligible. The primary endpoint was to estimate the objective volumetric response rates to axitinib. Axitinib was given continuously in 28-day cycles for up to of 12 cycles. Response was assessed every 3 months with MRI using 3-D volumetric tumor analysis and audiograms. Volumetric response and progression were defined as ≥20% decrease or increase in VS volume, respectively. Serial audiological evaluations were used to assess hearing response, including determination of word recognition score (WRS). Hearing response or progression was defined as a clinically significant increase or decrease, respectively, in the WRS.

Results: Twelve eligible patients (ages: 14–56 years) were enrolled on this study. Eight patients completed 12 cycles. We observed two volumetric responses; both were reached after 3 cycles and sustained during treatment. Best volumetric response was −53.9% after nine cycles. We observed three hearing responses, one of which was sustained during treatment. All patients experienced drug-related toxicities, the most common adverse events were diarrhea, hematuria and skin toxicity, not exceeding grade 2 and hypertension, not exceeding grade 3.

Conclusion: Our study shows that while axitinib has modest anti-tumor activity in NF2 patients with progressive VS, it is more toxic and appears to be less effective compared to bevacizumab. Based on these findings, further clinical development of axitinib for this indication does not appear warranted.

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Noninvasive Brain Stimulation for the Treatment of Chronic Tinnitus

Monday, June 26, 11:15am – 11:35am

Amber Leaver, PhD, *Northwestern University*

Chronic tinnitus is a common phenomenon that can negatively impact quality of life, but few effective treatments are available. Neuroimaging research has reported differences in brain activity and structure in adults with chronic tinnitus in the auditory cortex and elsewhere in the brain. Researchers have also attempted to use noninvasive brain stimulation methods like transcranial magnetic stimulation (TMS) to "correct" these tinnitus-related differences to improve tinnitus symptoms, with some success. This talk will review this literature, and discuss ideas for using noninvasive brain stimulation to treat tinnitus as a chronic symptom vs. chronic condition.

Platform: INTUITT-NF2, an Adaptive Platform-Basket Trial for *NF2*-Related Schwannomatosis Patients with Progressive Tumors: Primary Outcome of the Brigatinib Treatment Arm

Monday, June 26, 11:35am – 11:55am

Introduction: *NF2*-related schwannomatosis (*NF2*-SWN, formerly known as neurofibromatosis 2) predisposes affected individuals to vestibular schwannomas (VS), non-vestibular schwannomas (NVS), meningiomas, and ependymomas. We conducted an adaptive platform-basket trial to screen multiple drugs against any type of progressive *NF2*-SWN tumor. We report the final analysis of the first treatment arm with brigatinib, an oral ALK inhibitor that inhibits multiple tyrosine kinases.

Methods: We conducted a multicenter, phase II, open-label study for participants ≥12 years old with *NF2*-SWN and progressive target tumors (baskets: VS, NVS, meningioma, or ependymoma). Up to 5 non-target tumors were followed in each participant. Radiographic response (RR) was defined as ≥20% decrease in target tumor volume from baseline. The primary outcome was RR rate. Secondary outcomes included safety and hearing response. Tumor response was evaluated by MRI every 3 months in year 1 and every 6 months thereafter. In stage 1, 20 participants (minimum of 2 participants per basket) were treated with brigatinib 180 mg daily. Given the observation of drug activity in stage 1, an additional 20 participants with progressive meningioma or NVS were enrolled in stage 2.

Results: Forty participants (median age = 26 years, 12 pediatric, 28 female) were treated. Prior treatment included surgery (90%), radiation therapy (20%) and chemotherapy (55%). Target tumors included 10 VS, 9 NVS, 19 meningiomas, and 2 ependymomas. Overall RR rate for target tumors was 10%. By tumor basket, target tumor RR was 11% for meningioma, 22% for non-VS, 0% for VS, and 0% for ependymomas. Annualized growth rates decreased for NVS and meningioma tumors during treatment. There were no grade 4 or 5 treatment-related adverse events (AEs). The most common AEs were diarrhea (63%), nausea (40%), muscle cramps (33%), increased LDH (35%), increased AST (33%), and increased ALT (23%).

Conclusion: Brigatinib treatment was associated with RR in meningiomas and NVS in a heavily pretreated cohort of participants with actively progressing *NF2*-SWN tumors. This novel design provides unique ability to assess treatments for hereditary syndromes with multiple primary tumors.

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Funding source: Takeda Pharmaceuticals and Children's Tumor Foundation

KEYNOTE #5: NEURON-GLIAL INTERACTIONS IN NF1: IMPLICATIONS FOR COGNITION AND TUMORIGENESIS – CME SESSION

Monday, June 26, 12:45pm – 1:45pm

Michelle Monje, MD, PhD, *Stanford University, Howard Hughes Medical Institute*

In the central nervous system, neuronal activity is a critical regulator of development and plasticity. Activity-dependent proliferation of healthy glial progenitors, oligodendrocyte precursor cells (OPCs), and the consequent generation of new oligodendrocytes contributes to adaptive myelination. This plasticity of myelin tunes neural circuit function and contributes to healthy cognition, while disruption of myelin plasticity contributes to cancer therapy-related cognitive impairment. In NF1 mouse models, we have found that NF1-mutant OPCs exhibit altered lineage dynamics and impaired activity-regulated oligodendrogenesis, which may contribute to learning differences in NF1.

The robust mitogenic effect of neuronal activity on normal oligodendroglial precursor cells, a putative cellular origin for many forms of glioma, suggests that dysregulated or "hijacked" mechanisms of myelin plasticity might similarly promote malignant cell proliferation in gliomas. Indeed, neuronal activity promotes progression of high-grade gliomas in preclinical models. To explore the role of optic nerve activity in the pathogenesis of *NF1*-associated optic nerve glioma, we employed an authenticated murine model of Nf1-assocaited optic pathway glioma (OPG) to demonstrate that stimulation of optic nerve activity increases optic glioma growth, while decreasing visual experience via light deprivation prevents tumor formation and maintenance. We found that *Nf1*-OPG initiation depends on visual experience during a developmental period susceptible to tumorigenesis. Germline *Nf1* mutation in retinal neurons results in aberrantly high optic nerve neuroligin-3 (Nlgn3) shedding in response to retinal neuronal activity. Moreover, genetic *Nlgn3* loss or pharmacological inhibition of Nlgn3 shedding blocks murine *Nf1* optic gliomagenesis and progression. Collectively, these studies establish an obligate role for neuronal activity in the development of NF1-associated optic pathway glioma, elucidate a potential therapeutic strategy to reduce OPG incidence or mitigate tumor progression, and underscore the role of *Nf1* mutation-mediated dysregulation of neuronal signaling pathways in the NF1 cancer predisposition syndrome.

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ALIGNING PRECLINICAL STUDIES WITH CLINICAL TRIAL OUTCOME MEASURES – CME SESSION

Session Co-Chairs: Cristina Fernandez-Valle, PhD, *University of Central Florida;* Matthias Karajannis, MD, MS, *Memorial Sloan Kettering*

NF2-Related Schwannomatosis Clinical Trials – Lessons Learned and Future Perspective

Monday, June 26, 1:45pm – 2:05pm

Matthias Karajannis, MD, MS, *Memorial Sloan Kettering*

The first successfully completed prospective clinical trial for people with *NF2*-related schwannomatosis, using the EGFR/ErbB2 inhibitor lapatinib, was published in 2012. Numerous additional clinical trials have been completed since or are currently in progress, the majority of which is based on preclinical data in NF2-deficient tumor models. However, it is notable that the most effective currently available medical therapy for treatment of NF2-related vestibular schwannoma, bevacizumab, was identified empirically and without any available preclinical data in schwannoma. Recent observations made in clinical studies with molecular targeted therapies, including "Phase 0" or pharmacokinetic/pharmacodynamic studies, have provided valuable insights into translational barriers including tumor heterogeneity, drug penetration, drug metabolism and off-target effects, which will inform the development of future trials.

Designing a Drug Screening Strategy For Schwannomas: Review of Synodos: Lessons Learned & Improvements – CUDC907/Fimepinostat

Monday, June 26, 2:05pm – 2:25pm

Cristina Fernandez-Valle, PhD, *University of Central Florida, Orlando, FL*

Pre-clinical identification and validation of small molecules that ultimately reduce the size of schwannomas in Neurofibromatosis type 2 (NF2)-related Schwannomatosis patients remains a challenge in the field. The screening strategy that identified CUDC907, a dual inhibitor of histone deacetylase and phosphoinositide 3 kinase (HDAC/PI3K), as a candidate for clinical trials will be described. The investigational drug, fimepinostat (Curis), induces apoptosis in three human schwannoma model cells and slows the *in vivo* growth of mouse model schwannoma cells in sciatic nerves by 40-60% of vehicle treated controls. Remarkably, CUDC907/fimepinostat induces caspase 3/7 activity in all 18 primary vestibular schwannoma (VS) cells studied to date. A comparative high-content screen of 15-20 NF2 target compounds using 2D and 3D spheroid apoptosis assays confirmed efficacy of HDAC inhibitors in human model schwannoma cells and primary VS and non-VS schwannoma cells.

Meningioma Cell, Organoid, and Animal Models for Preclinical Evaluation

Monday, June 26, 4:10pm – 4:20pm

Long-Sheng Chang, PhD, *Nationwide Children's Hospital & The Ohio State University*

Originating from the meningothelial cells of the arachnoid layer lining the brain, meningiomas are the most common brain tumors, constituting ~40% of primary intracranial tumors. The majority (~80%) of meningiomas are benign (WHO grade I), whereas the remaining are atypical (grade II) and anaplastic (grade III). Meningiomas can occur spontaneously or are frequently found in patients with neurofibromatosis type 2 (NF2), which is caused by mutations in the *NF2*/merlin tumor suppressor gene. Intriguingly, ~50-60% of sporadic meningiomas also have recurrent *NF2* mutations, suggesting an important role of *NF2*/merlin in meningioma genesis. Current treatment options for these tumors include surgery and radiation. However, patients with NF2 often have multiple tumors, and surgical excision may be difficult, especially for those located along the skull base. In addition, *NF2*-deficient meningiomas have an increased likelihood of malignant transformation. Therefore, the development of effective medical therapies to treat meningiomas is urgently needed and requires high-fidelity preclinical models that recapitulate genetic and histopathological features of these tumors and faithfully facilitate translation of preclinical drug evaluation into the clinic. Over the years, a few models, including cell culture, genetically engineered mouse (GEM), xenograft, and more recently, organoid models, have been developed. Each model has different strengths and weaknesses. For cell culture models, primary meningioma cells can be easily prepared from patient tumors, but these 2D cultures are not homogeneous, lack tumor microenvironment, and most seriously, have limited lifespan. Telomerase-immortalized grade-I meningioma cell lines have been generated from sporadic and NF2 patients' tumors and used to establish orthotopic xenograft models for drug evaluation. Several potential targeted therapies have been identified and advanced into clinical trials. Development of a panel of patient-derived meningioma cell lines would further facilitate identification of effective targeted therapies by accounting for variations in treatment response. Direct implantation of human meningioma tumor pieces into immunodeficient mice to generate patient-derived xenografts has been attempted; however, intracranial sites of inoculation may be challenging, and the growth potential of benign tumors is limited. GEM models could be very powerful as they develop genetically defined tumor formation in the presence of host immune environment. Interestingly, GEM models with conditional *Nf2* inactivation in leptomeningeal cells or in prostaglandin D2 synthase (PGDS)-expressing meningeal progenitor cells exhibit meningioma formation. However, meningiomas take several months to develop with variable growth and were detected only in a fraction of animals, limiting their use for therapeutic evaluation. Organoids are miniature versions of an organ grown in 3D culture. Recent advances in organoid technology suggest that it may serve as an alternative platform for meningioma research. Meningioma cells can be co-cultured with cerebral organoids or used to directly generate spheroids to study tumor cell behavior and therapeutic screening. Due to different strengths and weaknesses of various meningioma models, it is suggested that multiple models should be used for more reliable predicting of therapeutic outcomes.

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Funding: CancerFree KIDS, Children's Tumor Foundation, Department of Defense, NIH-NINDS, and Rally Foundation

3D Meningioma Spheroids to Bridge the Translational Gap Between *In Vitro* and *In Vivo* Studies

Monday, June 26, 4:20pm – 4:30pm

Laurien van de Weijer, MSc, *Faculty of Heath: Medicine, Dentistry and Human Sciences, Derriford Research Facility, University of Plymouth, Devon, UK*

Treatment options for meningiomas are limited to surgery and radiotherapy and the effectiveness of current available systemic therapies has not been confirmed. Hence, development of new drug-based treatment strategies for meningiomas is warranted. Despite the advances that have been made in the understanding of the genetic background of meningiomas, progress in the development of therapeutic approaches targeting genetically stratified tumours remains limited, demonstrating a translational gap between preclinical *in vitro* and clinical *in vivo* study results. Most drug studies have been performed using two-dimensional (2D) cell culture systems. However, these systems present substantial limitations for testing new drugs and do not represent the complex nature of tissues including the surrounding microenvironment or important cell–cell and cell-extracellular matrix (ECM) interactions. By contrast, three-dimensional (3D) models can overcome many of these limitations and bridge the gap between oversimplified *in vitro* 2D and clinical *in vivo* studies. Therefore, we have developed a novel easy-to-use method to establish 3D patient-derived meningioma spheroids that resemble essential features of parental tumours, including the tumour microenvironment, to better predict the toxicity and efficacy of novel therapies. Reproducible spheroids were generated using ultra-low adherence 96-well plates and low-serum media optimised for spheroid culture. By IHC and DNAseq approaches, we show that spheroids closely recapitulate histological and molecular features and tumour-stromal composition profiles of their respective primary tumours. Furthermore, we demonstrate the application of these patient-derived spheroids as tool for assessing drug response and serve to bridge the translational gap between in vitro and in vivo research.

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BASIC SCIENCE PLATFORM SESSION – CONCURRENT SESSION

Session Co-Chairs: Maria Franco, PhD, *Florida International University;* Cristina Fernandez-Valle, PhD, *University of Central Florida*

Platform: Contribution of Fibroblasts to Tumor Growth in 3D NF1 Plexiform Neurofibroma Cultures

Monday, June 26, 5:00pm – 5:15pm

Kyungmin Ji, PhD, *Department of Neurology, Henry Ford Health, Detroit, MI*

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 *($Nf1^{-/-}$)* grown with human PN-fibroblasts *($Nf1^{+/-}$)* in 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 long-term culture periods. We use live-cell assays and 3D quantitative analysis of temporal and dynamic changes in NF1 PN Schwann cell:PN-fibroblast interactions in correspondence with changes in their growth and invasion.

Results: We cultured NF1 PN Schwann 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 Schwann cells were significantly greater in NF1 PN Schwann cell: PN-fibroblast co-cultures than in NF1 PN Schwann cell mono-cultures. We detected a slight increase in growth of PN-fibroblasts in the co-cultures than in PN-fibroblast mono-cultures. In addition, NF1 PN Schwann cells grew faster in media conditioned by PN-fibroblasts, implicating that the secretome from PN-fibroblasts increases the growth of NF1 PN Schwann cells. The induction of NF1 PN tumor growth by PN-fibroblast conditioned media is reduced by exosome-depletion and treatment with chlorpromazine, clathrin-dependent endocytosis inhibitor, 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 preliminary results suggest that fibroblasts secrete exosomes that may be therapeutic targets for reducing NF1 PN growth.

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Platform: Aberrant Accumbal Neurocircuitry Underlies ADHD Phenotype in Translational Model of Neurofibromatosis Type 1

Monday, June 26, 5:15pm – 5:30pm

Jodi Lukkes, PhD, *Indiana University School of Medicine*

The purpose of this study is to investigate attention deficit hyperactivity disorder (ADHD) in Neurofibromatosis type 1 (NF1) using region-specific knockdown of NF1 in a preclinical model.

Introduction: NF1 is a common genetic disorder with varied symptoms including neurocutaneous lesions, tumors, and neurodevelopmental disorders. Learning differences are common, with ADHD being most prevalent, affecting around 60% of patients with NF1. Preclinical studies using murine experimental systems of ADHD have shown that lesions of the prefrontal cortex (PFC) or nucleus accumbens (NAc) increase impulsivity in a delay discounting task (DDT). However, there is limited preclinical data investigating ADHD in NF1, particularly in females.

Methods: The aim of the current study was to determine sufficiency of *Nf1* knock-down in cortical-striatal circuitry to cause executive dysfunction during clinically-relevant behavioral tasks through the use of $Nf1^{flox/flox}$ male and female mice. $Nf1^{flox/flox}$ male and female adult mice were injected bilaterally with either control virus (AAV5-CMV-GFP) or the Cre virus (AAV5-CMV-Cre-GFP) into the PFC, NAc, or ventral tegmental area (VTA).

Results: We found that selective deletion of the neurofibromin gene *(Nf1)* in the NAc increased hyperactivity to a novel open field and increased risky behavior in a cliff avoidance reaction test (CAR) in males but not females. However, both sexes of $Nf1^{flox/flox}$ mice injected with AAV5-CMV-Cre-GFP into the NAc exhibited deficits in behavioral inhibition measured by increased frequency of small reward choice in DDT. In contrast, selective deletion of *Nf1* in the PFC or VTA of males only increased impulsive behavior during the DDT in males but not in females. No effects of treatment nor sex were observed following selective deletion of Nf1 in the PFC or VTA on distance travelled in a novel open field. We also found that injection of Cre virus into the VTA of $Nf1^{flox/flox}$ female, but not male mice increased risk-taking and impulsive behavior in the CAR test.

Conclusions: These data suggest that selective deletion of *Nf1* has region- and sex-specific effects on hyperactivity and impulsivity. Furthermore, our data show that the NAc plays an integral role in modulating the observed deficits in behavioral inhibition of Nf1 animals. Overall, these studies will help elucidate underlying molecular and neural mechanisms driving impulsivity. By investigating impulsivity in preclinical NF1, we can better understand cognitive differences in clinical NF1, a significant concern for patients and families.

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Platform: Single-Cell Sequencing Reveals Transcriptomic Diversity That Facilitates the Malignant Transformation of NF1 Nerve Sheath Tumors

Monday, June 26, 5:30pm – 5:45pm

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

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 margins. The current genetic model for development of MPNST proposes: 1) About 50% of NF1 patients exhibit plexiform neurofibromas (PN, benign tumors), which are caused by the loss-of-function in *NF1* in the Schwann cells and the associated hyper-activated Ras and its downstream signaling pathway, 2) Atypical neurofibroma (ANF, premalignant tumors) arise from PNs and in addition to hyper-activation of Ras they frequently exhibit loss of *CDKN2A/B,* and 3) about 13% of NF1 patients develop MPNST during their lifetime, in which recurrent mutations in *SUZ12* and/or *EED,* two key components of the polycomb repressive complex 2, are often identified, leading to loss of tri-methylation of histone H3 lysine 27 in more than 80% of these tumors. Mechanistic understanding of how the malignant transformation occurs within the tumor microenvironment (TME) remains elusive.

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 all pathological stages. Integrative analysis was performed to correct for batch effects and to compare the transcriptomic profiles of 55 NF1 nerve tumors.

Results: After quality control and filtering, we analyzed 425,001 cells, including 244,611 cells from 32 PN, 105,921 cells from 18 ANF, and 74,469 cells from 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, ANF, 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 using the Harmony algorithm. These immune cells were further grouped 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 ANF. Notably, there was emergence of CTLA4+ Tregs and loss of activated macrophages in MPNST. Finally, we discovered a unique "transitioning" cell population in some ANF, marking them as potentially high risk of malignant transformation. Signatures of these "transitioning" cells are currently being implemented as biomarkers in a clinical tool for early identification of MPNST.

Conclusion: In summary, we describe the cellular intra-tumoral heterogeneity of NF1 nerve sheath tumors using data from scRNAseq of patient samples. 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 "transitioning" cell type in ANF presents a unique opportunity for early detection of MPNST.

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Platform: Combining Brigatinib with mTOR Inhibition to Effectively Treat *NF2*-Deficient Meningiomas and MPNST

Monday, June 26, 5:45pm – 6:00pm

Janet L Oblinger, PhD, *Nationwide Children's Hospital*

The Synodos for NF2 Consortium was established to discover potent drugs/drug combinations to treat NF2-related tumors. In collaboration with the NIH-NCATS, high-throughput screening of an oncology compound library identified the multi-kinase inhibitor brigatinib and its combination with the AKT inhibitor MK-2206 to potently suppress the growth of *NF2*-deficient meningioma and schwannoma. In addition, the dual mTORC1/2 inhibitor INK128 and its combination with the multi-kinase inhibitor dasatinib were found to durably impede meningioma growth. Since brigatinib and INK128 as monotherapies exhibited strong anti-tumor effects, we explored potential anti-tumor synergy of these drugs in combination using two *NF2*-deficient grade-I meningioma cell lines. Ben-Men-1 and AG-NF2-Men cell lines were telomerase-immortalized from a sporadic meningioma and an NF2 patient tumor, respectively. Both cell lines have no detectable *NF2*/merlin, and express several merlin-regulated RTKs, including EGFR, ErbB3, and IGF-1R. Brigatinib and INK128 alone inhibited AG-NF2-Men cell proliferation at IC_{50} values similar to those in Ben-Men-1 cells. Combining brigatinib with INK128 displayed growth inhibitory synergy. Mechanistically, brigatinib+INK128 decreased p-AKT(S473) more efficiently than either drug alone, prevented INK128-mediated p-AKT(T308), and suppressed downstream signaling from mTOR. Transcriptomic analysis on AG-NF2-Men cells treated with brigatinib, INK128, or brigatinib+INK128 showed that the combination treatment more efficiently suppressed several upstream regulators of the signaling networks important for meningioma growth. Similar to the Ben-Men-1-LucB meningioma model, we also generated an orthotopic, quantifiable, NF2-associated meningioma model using luciferase-expressing AG-NF2-Men-Luc2 cells. In both the AG-NF2-Men-Luc2 and Ben-Men-1-LucB models, single-agent brigatinib and INK128 effectively blocked tumor growth, and their combination enhanced tumor regression. Also, the brigatinib+INK128 combination can be repeatedly used to shrink meningiomas. Since brigatinib also has anti-tumor activity in schwannoma, we determined whether the brigatinib+INK128 combination was active against malignant schwannoma like MPNST. When compared to individual drugs, brigatinib+INK128 synergistically enhanced growth inhibition in sporadic and NF1-related MPNST cells. The combination-treated MPNST cells had superior suppression of p-IGF-1R, p-FAK, and the downstream targets AKT and ERK1/2. Using a patient-derived xenograft model of MPNST, we found that brigatinib alone suppressed tumor growth by ~82% compared with the vehicle-treated group over four weeks of treatment, while INK128 alone only modestly inhibited tumor growth by ~32%. Combining brigatinib with INK128 gave rise to a slightly stronger suppression of tumor growth (by ~86%) than brigatinib alone. Brigatinib is currently under clinical evaluation in the INTUITT trial for NF2 patients, and our results suggest that combining brigatinib with an mTOR inhibitor may provide further benefit to these patients.

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Platform: Conditioned Media from Painful Human Schwannomatosis Tumors Hypersensitize Sensory Neurons to Painful Stimuli; An *In Vitro* and *In Vivo* Study

Monday, June 26, 6:00pm – 6:15pm

Kimberly L Ostrow, *Department of Neurology, The Johns Hopkins University*

Purpose: The majority of schwannomatosis (SWN) patients experience debilitating and unremitting pain, though the sizes and locations of their tumors do not clearly correspond to the distribution and severity of pain. We do not presently understand why some schwannomas cause severe refractory pain and others are painless. Furthermore, while mutations in *SMARCB1* or *LZTR1* are detected in a significant proportion of familial and sporadic SWN patients, whether mutation status influences pain signaling pathways is unknown. We predict that painful but not non-painful schwannomas, secrete proteins that act to hypersensitize sensory neurons.

Methods and results: We established cell lines from SWN tumors resected from patients with varying degrees of pain and mutations in *SMARCB1* and *LZTR1.* Our *in vitro* studies showed convincing evidence that substances secreted by SWN tumors sensitize neurons and alter neuronal gene expression. Conditioned medium (CM) collected from "painful" – but not "non-painful" SWN tumors, (1) contained increased amounts of specific inflammatory cytokines (IL-6, IL-8, VEGF), (2) upregulated the expression of pain-associated genes in DRG cultures (PTGS2, BDKRB1, IL-1b), and (3) increased the neuronal response to noxious TRPV1 and TRPA1 agonists. To study this phenomenon *in vivo,* we used well-established methods to quantify pain levels in healthy mice exposed to schwannoma conditioned media. We examined the effects of 4 painful and 3 non-painful CMs on pain behaviors after CM injection. Mice injected with *Painful LZTR1 mutant* CM demonstrated an increase in acute pain behavior *($p=0.02$).* Hypersensitivity to **evoked** mechanical pain was examined in a separate cohort of 80 mice. Ten mice per group received hind paw injections of CM from a Painful *LZTR1* mutant tumor, a Painful *SMARCB1* mutant tumor, and a painful tumor with no detectable mutation in either gene. Three non-Painful tumor CMs, and non-conditioned medium (DMEM/ 10% FBS / 2uM forskolin) were also tested. One hour after injection, the mice were placed in a ventilated plexiglass box on an elevated metal mesh surface. von Frey monofilaments (0.02 to 2 g) were applied through the mesh to the glabrous skin of the hind paw for ~1 sec, with stimulation at each force repeated six times on each hind paw. The proportion of paw withdrawal responses evoked by each force was recorded to generate a dose response curve. The ***Painful*** *SMARCB1* mutant CM sensitized mice to mechanical stimulation compared to Non-Painful tumor CM and control media at low forces, 0.04g *($p<0.008$)* and 0.07g *($p=0.02$)*. ***Painful*** *LZTR1* mutant CM showed an increased response to mechanical stimulation than control media at 0.07g *($p=0.01$).* 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 (0.07g $p=0.0008$).

Conclusions: These experiments, provide the basis for expanding *in vivo* testing of additional painful and non-painful CMs with different mutation statuses. We aim to better understand of the nature of the type of pain (mechanical, thermal, or spontaneous pain) in mice treated with schwannoma-secreted substances and whether mutation status influences the painful phenotype.

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Funding: Pamela Mars Foundation, Blaustein Pain Foundation, Neurosurgical Pain Research Institute

Platform: WP1066 Induces Cell Death in a Schwannomatosis Patient-Derived Schwannoma Cell Line

Monday, June 26, 6:15pm – 6:30pm

Abdulrahman Allaf, BS, *University of Central Florida*

Purpose: The purpose of the study was to explore precision medicine approaches to develop personalized therapies for schwannomatosis patients by identifying targeted small molecules that could reduce cell viability and induce cell death in schwannoma cells derived from a patient with a germline point mutation in the *SMARCB1/INI* gene.

Methods: A surgically removed schwannoma sample was prepared to isolate and culture schwannoma cells to establish the immortalized cell line, STSW-01. An hTERT-luciferase expressing line (STSW-01-LUC) was also created for in vitro and in vivo studies and was studied in parallel with the hTERT line. The cell line was characterized via immunostaining and Western Blot. STSW-01 was then used to test the means of cell death using viability, necrosis, and caspase cleavage assays in response to several compounds. The selection of compounds was based on phosphokinase arrays of primary schwannoma cells and genomic analyses of a previously resected schwannoma. Western blot analysis was then used to confirm target inhibition and explore cell death pathways.

Results: The immortalized cell line (STSW-01) was characterized and found to be capable of substantial proliferation in the absence of certain growth factors such as forskolin and SMDF. Viability assays ran on the STSW-01 cells revealed ten out of the 14 compounds tested were considered active because they decreased cell viability in a dose-dependent manner by at least 60%. WP1066 was found to be the most effective compound at promoting membrane flipping, inducing time-dependent cell death, and modulating STAT3 activity in a dose dependent manner. WP1066 was also found to increase levels of phosphorylated mixed lineage kinase domain–like (MLKL), a key player in the necroptosis cell death pathway.

Conclusions: This study established the first hTERT immortalized schwannoma cell line from a schwannomatosis patient, which proves suitable for drug screening and led to the identification of a potentially effective cytotoxic compound for clinical trials. The cell line can be used to screen libraries of FDA-approved drugs for repurposing for off-label use. This study also provides insights into how WP1066 induces necroptosis and caspase-dependent apoptosis in this patient-derived cell line. These findings provide a foundation for a precision medicine approach to improve care for schwannomatosis patients.

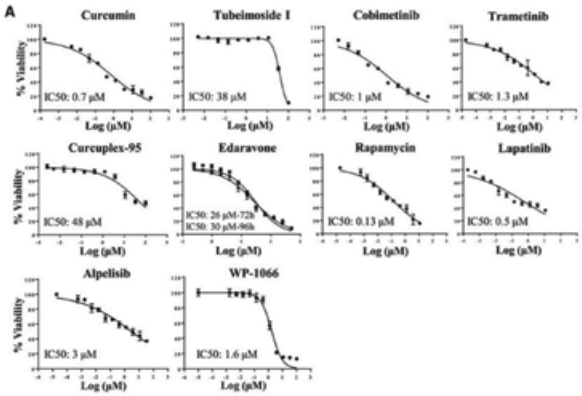

Figure 1: Viability curves for STSW-01-LUC cells treated with increasing doses of the active compounds.

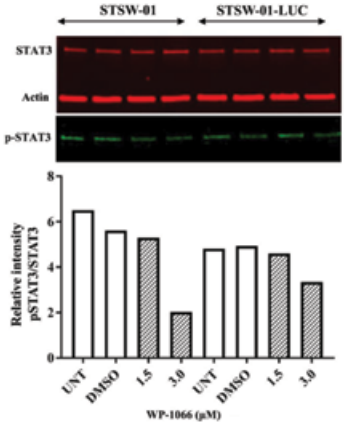

Figure 2: Western blots and quantitation for STAT3/p-STAT3 (Tyr 705) in both STSW-01 and STSW-01-LUC cells grown for 24 h in the presence of WP-1066.

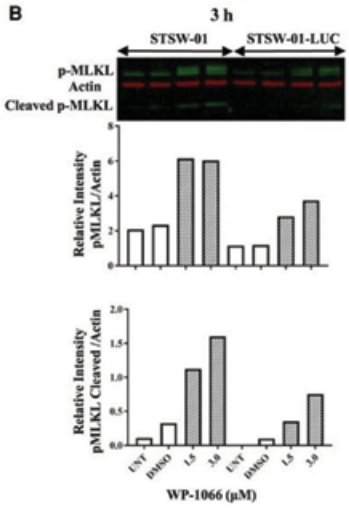

Figure 3: Western blots for full-length and cleaved pMLKL (Ser358) in STSW-01 and STSW-01-LUC treated with WP1066 for 3 hours.

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Platform: CDK4/6-MEK Inhibition in MPNSTs Causes Plasma Cell Infiltration, Sensitization to PD-L1 Blockade, and Tumor Regression

Monday, June 26, 6:30pm – 6:45pm

Joshua Lingo, BS, *University of Iowa*

Malignant peripheral nerve sheath tumors (MPNSTs) are lethal, Ras-driven sarcomas that lack effective therapies. Ras downstream effectors, CDK4/6 and MEK, were identified as actionable targets for combination therapy from patient MPNST analyses and preclinical drug studies. In MPNST cells, low-dose combinations of CDK4/6 and MEK inhibitors synergistically reactivated the retinoblastoma (RB1) tumor suppressor, induced cell death, and decreased clonogenic survival. In immune-deficient mice, dual CDK4/6-MEK inhibition slowed growth but did not shrink MPNSTs in 4 of 5 patient-derived xenografts (PDXs). By comparison, combination therapy of *de novo* MPNSTs in immunocompetent mice caused tumors to regress, delayed resistant tumor outgrowth, and improved survival relative to monotherapies. While drug-resistant tumors adopted an immunosuppressive microenvironment enriched with MHC II-low macrophages, drug-sensitive tumors that regressed contained plasma cells and increased T cell clustering. In other cancers, intratumoral plasma cells are associated with better response to immune checkpoint blockade (ICB). Impressively, CDK4/6-MEK inhibition sensitized MPNSTs to anti-PD-L1 ICB therapy with some mice displaying complete tumor regression. These results reveal a novel plasma cell-associated immune response and extended antitumor activity of combined CDK4/6-MEK inhibition in MPNSTs, which dramatically enhanced the efficacy of ICB therapy. This work highlights a promising, potentially curative treatment option for MPNSTs.

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Platform: Integrated Analysis of Cell-Free DNA for the Detection of Malignant Peripheral Nerve Sheath Tumors in Patients with Neurofibromatosis Type I

Monday, June 26, 6:45pm – 7:00pm

Derek Wong, PhD, *Princess Margaret Cancer Center*

Purpose: While all Neurofibromatosis Type 1 (NF1) patients exhibit a pathogenic germline variant in *NF1,* the clinical presentation is heterogeneous. Roughly 8-13% of NF1 patients will develop malignant peripheral nerve sheath tumor (MPNST). Despite advances in cancer therapies, early detection remains the best indicator of survival. Cell-free DNA (cfDNA), fragments of DNA released by cells into the bloodstream, are an emerging biomarker. One area of rapid development is fragmentomics, the analysis of fragment length and position to infer the cell-of-origin. Using a multi-assay approach, our goal is to develop a comprehensive and minimally invasive screening tool to both detect MPNST clinically stratify NF1 patients.

Methods: Through the CHARM Consortium (https://charmconsortium.ca), 45 plasma samples (pediatric = 15, adult patients = 30), and 30 healthy controls (HBC) underwent deep plasma whole genome sequencing (pWGS, 40x). A subset (n = 10) also underwent targeted panel sequencing (TS, 2,000x, 201 genes). We also secured an external dataset of 85 plasma samples from healthy controls and NF1 patients with plexiform neurofibromas (pNF) or MPNST *(1).*

Results: Using TS, a mean of 19 somatic alterations were detected per plasma sample which were validated using fragment size analysis to rule out germline, sequencing errors, and clonal hematopoiesis related variants. Somatic variants were not detected in MPNST-associated genes *(NF1, SUZ12, EED, TP53)* and could not be used to distinguish between pNF and MPNST.

As an alternative approach, we investigated a suite of fragmentomic features (fragment length, fragment ratio, fragment coverage) using pWGS. Across all fragmentomic features, pNF showed low levels and MPNST exhibited high levels of aberrant cfDNA fragmentation. Integrating these fragmentomic features using a machine learning classifier we observed robust distinction between 1) HBC and MPNST (AUC = 0.935) and 2) pNF and MPNST (AUC = 0.872). Additionally, patients with pNF exhibited MPNST scores across a spectrum of values.

Lastly, using a similar fragmentomic approach described above, we were also able to stratify patients classified as clinically mild and severe (AUC = 0.901) suggesting innate fragmentomic features indicative of clinical severity. Clinically moderate NF1 patients exhibited fragmentation scores intermediate to mild and severe patients.

Conclusions: Our approach demonstrates the clinical utility and potential of cfDNA fragmentomic analysis as a non-invasive method to monitor the malignant transformation of pNF to MPNST and assess clinical severity in NF1 patients.

Additional Authors: Ping Luo[1], Stephanie Pedersen[1], Clarissa Chan[2], Kirsten Farncombe[2], Maia Norman[2], Leslie Oldfield[1], María Carolina Sanabria-Salas[2], Julia Sobotka[1], Raymond H Kim[2], Trevor J Pugh[1]
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References:
1. J. J. Szymanski, R. T. Sundby, P. A. Jones, D. Srihari, N. Earland, P. K. Harris, W. Feng, F. Qaium, H. Lei, D. Roberts, M. Landeau, J. Bell, Y. Huang, L. Hoffman, M. Spencer, M. B. Spraker, L. Ding, B. C. Widemann, J. F. Shern, A. C. Hirbe, A. A. Chaudhuri, Cell-free DNA ultra-low-pass whole genome sequencing to distinguish malignant peripheral nerve sheath tumor (MPNST) from its benign precursor lesion: A cross-sectional study. *PLoS Med.* **18**, e1003734 (2021).

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CLINICAL SCIENCE PLATFORM SESSION – CONCURRENT SESSION – CME SESSION

Session Co-Chairs: Tamar Green, MD, *Stanford University;* Justin Jordan, MD, MPH, *Massachusetts General Hospital*

Platform: Improving Measurement of Quality of Life in NF2 Clinical Trials: Analysis of INTUITT-NF2 Participant Interviews and Patient-Reported Outcome Measures

Monday, June 26, 5:00pm – 5:15pm

Liesel Von Imhof, BA, *Massachusetts General Hospital, Boston, MA*

Purpose: The Response Evaluation in Neurofibromatosis and Schwannomatosis Collaboration recommended the NF2 Quality of Life scale (NFTI-QoL) to assess quality of life in NF2 clinical trials, but identified questions related to the scale's comprehensiveness and sensitivity to change. For this reason, we qualitatively assessed this scale in NF2 clinical trial participants.

Methods: We interviewed participants with NF2 enrolled in stage one of the brigatinib arm of INTUITT-NF2 (NCT04374305) — a multicenter, adaptive platform-basket trial targeting progressive NF2-related tumors — at cycle 7 and/or cycle 13 of treatment. Interviews included concept elicitation of NF2 symptoms/impacts and cognitive debriefing of the NFTI-QoL. Transcripts were coded by two analysts using a hybrid inductive/deductive framework. Data were analyzed qualitatively using the Framework Method to 1) identify themes related to the NFTI-QOL's content validity [i.e. relevance, comprehensiveness, and comprehensibility] and 2) to describe participants' perceptions of thresholds for meaningful change in NFTI-QoL symptom domains.

Results: We interviewed 16/20 stage one trial participants [69% female; mean age: 27 years (range, 15-54 years)]. More than half of participants endorsed the presence of each NFTI-QOL domain (**Figure 1**), confirming the relevance of these items. Three additional symptom domains were commonly identified during concept elicitation: voice/swallowing concerns, tinnitus, and muscle atrophy/weakness (**Table 1**). Common comprehension issues in NFTI-QOL items included how to interpret the phrase "usual activities" and whether outside assistance (e.g. glasses, mobility aids, non-study medications) should be considered when responding. Most participants acknowledged responding based on their experiences over the past week to 3 months instead of following instructions of "today." In an exploratory exercise, participants reported that small but meaningful improvements in their symptoms would not result in a change in their NFTI-QoL scores 9/31 (29%) times (**Figure 2**). Participants recommended rephrasing several NFTI-QoL response options (e.g. by changing "no problems" and "stop my usual activities" to describe less extreme states) and/or adding an additional, fifth response option to improve detection of symptom changes.

Conclusion: The NFTI-QoL is relevant to NF2 clinical trial participants but would likely benefit from revisions to improve its comprehensiveness, comprehensibility, and ability to detect within-person improvements in quality of life. Recommended revisions include: adding additional items and response options; rephrasing instructions as well as some item stems and response options and lengthening the recall period. Future research should verify whether proposed revisions to the NFTI-QOL retain adequate psychometric properties and increase sensitivity to within-person changes.

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Figure 1. Presence of NFTI-QOL Symptom Domains in a Sample of INTUITT-NF2 Patients

ID	Balance/ Dizziness	Hearing	Facial Weakness	Vision	Mobility /Walking	Outlook on Life	Pain	Depression /Anxiety	Score Max=8
20	■	■	■	■	■	■	■	■	8
11	■	■	■	■	■	■	■	■	8
14	■	■	■	■	■	■	■	■	8
02	■	■	■	■	□	■	■	■	7
05	■	■	□	■	■	■	■	■	7
10	■	■	■	■	□	■	■	■	7
09	■	■	□	■	■	■	■	■	7
12	■	■	■	■	■	■	□	■	7
01	■	■	■	□	■	■	□	■	6
06	■	■	□	■	■	■	■	■	6
07	■	■	□	■	■	■	■	□	6
17	■	■	□	□	□	■	■	■	6
03	■	■	■	□	□	■	□	■	5
13	□	■	■	□	□	■	■	■	5
15	■	□	□	■	□	□	□	□	4
08	□	□	□	□	□	□	□	□	0
Score Max=16	14	15	9	11	9	15	11	13	

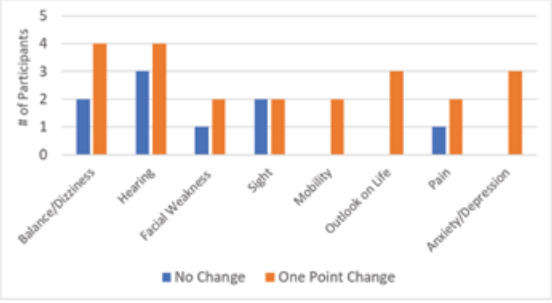
Legend: Boxes indicate whether a participant had (green) or had not (white) experienced NF2 symptoms within each NFTI-QOL domain. Sums in the bottom row indicate the total number of interviewees who endorsed each symptom. Sums in the rightmost column indicate the number of NFTI-QOL domains endorsed by each interviewee, ranked from highest to lowest.

Table 1. Common NF2-related Quality of Life Concerns Not Included in the NFTI-QOL

Symptom Domain	Quality of Life Impact	N	Exemplar Quote
Lower cranial nerve dysfunction (e.g., vocal fold weakness/ paralysis)	Altered voice quality, difficulty swallowing	5	MGH02: "In my neck, I've had vocal cord paralysis . . . Once I had the vocal cord implant, I had to be a little bit careful swallowing—you know, I've probably paid more attention to my swallowing than the average person, because I had choking incidents . . . [Also] my tongue's kind of paralyzed on one side."
Tinnitus	Difficulty hearing, psychological distress	4	MGH11: "The tinnitus is probably one of the largest, inescapable daily symptoms that I can't work around, I can't solve…Things that are really, really quiet are completely masked by the tinnitus. Things that are of moderate volume, things that I can physiologically hear, I can't understand, because of that interference and that garbled nature of the tinnitus."
Muscle Atrophy	Weakness, decreased endurance, fatigue	4	MGH09: "I have an atrophied quad so that is because of my NF, so stuff related to that - weakness, I guess, fatigue easier and more easily tired kind of stuff…"

Legend: N refers to the number of participants endorsing each concern (out of 16).

Figure 2. Change in NFTI-QOL Response Resulting from Smallest Meaningful Improvement in Corresponding Symptom Domain



Legend: Number of participants who reported that a meaningful improvement in their symptoms would result in no change in their NFTI-QOL response (blue), or a 1-point improvement in their NFTI-QOL response (orange). Differing Ns per item reflect that not all participants had time to complete each exercise, and participants who were already at the ceiling for an item were not asked to complete this exercise.

Disclosures/Funding: SP is a co-founder of NF2 Therapeutics; the remaining authors report no relevant financial disclosures. This qualitative sub-study was funded by Children's Tumor Foundation (CTF) Clinical Research Award 2020-10-001 to VM; Takeda Pharmaceuticals and CTF provided funding for the INTUITT-NF2 trial.

Platform: Single-Cell Transcriptomic Analysis of NF2-Asscoiated Schwannomas Reveals Novel Changes in Tumor and Immune Cell Subpopulations After Bevacizumab Treatment

Monday, June 26, 5:15pm – 5:30pm

Long-Sheng Chang, *Nationwide Children's Hospital & The Ohio State University*

Vestibular schwannomas (VS) are Schwann cell (SC) tumors originating from the 8th cranial nerve and cause significant morbidities, including hearing loss, facial paralysis, and brainstem compression. VS can occur sporadically or are commonly seen in patients with NF2, a debilitating tumor predisposition syndrome in which afflicted patients can also develop multiple meningiomas and other nervous system tumors. Invasive surgery and radiation have been the treatment options; however, due to multiple tumors in NF2 patients and complications from these treatments, drug therapy may be more desirable for some patients. Recent studies show that the anti-VEGF monoclonal antibody bevacizumab improves hearing and slows tumor growth in ~30-40% of patients with NF2 and progressive VS. To better understand the effects of bevacizumab treatment, we analyzed a right VS surgically excised from an NF2 patient with bevacizumab treatment for five years and a left VS from the same patient after 12 years of treatment due to tumor growth. Next-generation sequencing shows that these NF2-related VS maintain low mutational burdens and stable microsatellite status with no new gene mutations. Comparing with the right VS from this patient prior to bevacizumab treatment, the bevacizumab-treated right VS and left VS have a greatly increased number of blood vessels. In addition, the 12-year bevacizumab-treated left VS shows extensive extravasation and hemosiderin-laden macrophages, as well as vessels with obstruction and recanalization. Consistently, the patient experienced significant blood loss during recent surgical removal of this left VS. Single-cell RNA-sequencing was performed to comprehensively analyze the transcriptional landscape of the bevacizumab-treated left VS and three other naïve NF2-associated schwannomas from patients that have not received any drug therapies. Analysis of cellular diversity via unsupervised clustering in Seurat reveals that these VS harbor several tumor SC subpopulations with gene signatures resembling repair SCs in peripheral nerve injury, along with various immune subsets and stromal cells, including fibroblasts, within their tumor microenvironment (TME). Macrophages were highly represented along with a small population of T cells and NK cells. Macrophages can be further divided into subpopulations, including those expressing different immune signatures. Both M2 and M1 macrophages were present as corroborated by immunostaining. Importantly, we identified three new or highly enriched tumor SC subpopulations with high VEGF expression, mesenchymal, or immune phenotype, respectively, in the bevacizumab-treated VS. In addition, NF2-related VS appear to contain an exhausted immune ME as they express little immunostimulatory IL-2 but high levels of TGFβ, TNFα, and interferon-γ in the immune cell subsets. Also, we detected robust expression of several immune checkpoint receptors, including TIM3 in macrophage and T cell subpopulations and VISTA and LAG3 in T cells within the TME of both naïve and bevacizumab treated VS. Together, our results suggest that targeting these negative immune checkpoints may be a viable treatment strategy for VS. The emergence of a high VEGF-expressing tumor SC subpopulation in bevacizumab-treated VS supports the idea of a novel treatment-related resistance mechanism.

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Funding: CancerFree KIDS, Department of Defense, and Rally Foundation

Platform: Identification of Immune-Related Candidate Biomarkers in Plasma of Patients with Sporadic Vestibular Schwannoma

Monday, June 26, 5:30pm – 5:45pm

Konstantina M. Stankovic, MD, PhD, Bertarelli Foundation Professor and Chair, *Department of Otolaryngology–Head & Neck Surgery, Stanford University School of Medicine*

Background: This study compared the plasma concentration levels of 67 immune-related factors between patients with sporadic vestibular schwannoma (VS) and controls to identify potential biomarkers. The associations between biomarker levels and pre-operative hearing and tumor volume were assessed along with their diagnostic utility.

Patients and Methods: Blood from controls and from patients with unilateral, sporadic VS was analyzed via Luminex, electrochemiluminescence, and ELISA assays to quantify factor concentrations. Patient characteristics were obtained from medical charts and pre-surgical imaging, audiometric thresholds, and word recognition (WR) scores closest to resection. The VS cohort was classified into good hearing (GH) and poor hearing (PH) subgroups. Statistical comparisons were conducted between VS groups (overall, VS-GH, VS-PH) and vs. controls, and the relationships between biomarker levels, tumor volume, and hearing were assessed with regression models.

Results: A total of 163 VS patients (n=34 VS-GH, n=124 VS-PH) were included in the study. Twenty-two factors were considered candidate biomarkers based on their detectability in the plasma of >75% of VS patients; of these, MMP-14 and FGF-2 were the most elevated among VS patients vs. controls. IL-16 was significantly higher in VS-GH vs. VS-PH. MDC levels were correlated with WR score, while IL-16 and S100B levels were correlated with tumor volume. The 7-biomarker panel composed of MCP-3, BLC, S100B, FGF-2, MMP-14, eotaxin, and TWEAK showed the outstanding discriminatory ability for VS, reaching an AUC of 0.934 with 87.5% sensitivity and 95.8% specificity.

Conclusion: This is the first study conducting robust immune profiling of blood plasma from a large cohort of patients with sporadic VS for comparison with controls, between VS-GH and VS-PH patients, and between sexes. The findings of this study revealed possible therapeutic targets for VS-induced hearing loss and provided a unique diagnostic tool that may predict hearing change and tumor growth in VS patients and may help inform the ideal timing of tumor resection to preserve hearing.

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Disclosure: This work was supported by National Institute on Deafness and Other Communication Disorders grant R01 DC015824; Bertarelli Foundation Endowed Professorship at Stanford University; Remondi Foundation, and Larry Bowman (KMS).

Platform: Evaluation of cNF Burden and its Impact on Quality of Life in People with NF1 Using 3D Whole-Body Photographs and Modified-Skindex

Monday, June 26, 5:45pm – 6:00pm

Mandi Johnson, MBA, *Departments of Dermatology and Neurology, Johns Hopkins University School of Medicine, Baltimore, MD*

Purpose: The purpose of this study was to evaluate quality of life (QoL) as assessed by the modified-Skindex tool in relation to cutaneous neurofibroma (cNF) burden allocation using digital 3D whole-body (WB) photography in people with neurofibromatosis type 1 (NF1).

Background: cNFs are the most common tumor in people with NF1 and can significantly impact QoL. Despite their benign histology, cNFs are often reported as the most bothersome manifestation of NF1. 3D WB imaging allows for the evaluation of the entire skin surface and has been applied in research for other dermatologic conditions.

Methods: People with NF1 are being recruited at the Johns Hopkins Comprehensive Neurofibromatosis Center in a natural history study of cNF (NCT05581511). Participants completed the 19-item modified-Skindex with questions in 3 domains (emotions, functioning, symptoms) and 3D WB photographs were obtained. cNF burden was classified based on evaluation of photographs as: low ($\leq$10 cNF measuring $\geq$4mm); moderate (11-50); and high (>50). To explore possible association between cNF burden and QoL, chi-square analysis was used and participants were grouped as having low or moderate-severe burden.

Results: As of March 2023, 172 participants were enrolled, 95 (55%) female and 77 (45%) male, with a median age of 30 years [1-72]. cNF burden was classified as moderate-high in 55 (32%), and low in 117 (68%). cNF burden was directly related to age ($p=<0.005$) with only participants older than 10 years in the moderate-high cNF burden category. In the low cNF burden group, 50 (43%) reported no effects in QoL and 67 (57%) some impairment. In the moderate-high cNF burden group, 9 (16%) reported no QoL concerns vs 46 (84%) with impact in their QoL. The scores in the three modified-Skindex domains (emotions, symptoms, functioning) were consistently higher (more affected QoL) in the participants with a moderate-high cNF burden ($p=<0.005$) when compared to those with a low burden of cNF. In the low cNF burden group, 67 (57%) reported having an impact in their emotions, 44 (38%) in functioning, and 52 (44%) in symptomatology.

Conclusions: In a diverse population of people with NF1, 3D WB photography is a reliable method for assessing cNF burden. A moderate-high cNF burden was associated with impact in all QoL domains as assessed by the modified-Skindex tool. However, a significant number of participants reported some impact in QoL despite having low cNF burden.

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Granting agency: The Neurofibromatosis Therapeutics Acceleration Program at Johns Hopkins

Platform: High Intensity Focused Ultrasound (HIFU) Treatment of Cutaneous Neurofibromas (cNF): Preliminary Results from a Prospective Dual-Center Clinical Investigation

Monday, June 26, 6:00pm – 6:15pm

Katrine Elisabeth Karmisholt, MD, PhD, *Department of Dermatology, Bispebjerg University Hospital, Copenhagen, Denmark*

Purpose: Investigation of the safety, local tolerability, and efficacy of high intensity focused ultrasound (HIFU) for treatment of NF1 associated cutaneous neurofibromas (cNFs).

Methods: Adult patients having at least 8 treatable cNFs were recruited in two centers. Focused ultrasound treatment utilizing a 20 MHz HIFU-device with integrated dermoscopic guidance was performed using a handpiece with a focus depth of 2.3 mm below the skin surface. Single dose acoustic energy of 0.7 J/dose of pulse duration 250 ms/dose was manually positioned with distance of 1-2 mm between each applied dose, at repetition frequency of 1-2 seconds until the full cNF including a 1 mm perilesional margin was covered. No anesthetic was applied. Primary endpoint was evaluation of safety and tolerance of the HIFU-treatment. Post-treatment effects were assessed immediately after treatment and at follow-up visits including on-site clinical evaluation, patients' evaluation and clinical photography for 6 months. Further evaluation of the included cNFs was performed by ultrasound scanning (US) in one center and histopathology in the other center.

Results: 20 patients with a total of 147 cNFs (mean 7.35/patient; diameter 2-9 mm) received one treatment of each selected lesion. Mild wheal-and-flare reaction was observed immediately after treatment. Occasionally, erosions/crusts were observed and rarely dyspigmentation after 1 week and 3-6 months post-treatment respectively. No serious adverse events occurred, and no significant scarring was observed. The median reduction in cNF thickness measured by US was 0.62 mm (range -100% to +14%). Visual rating of treated cNFs by the clinical investigator at 6 months showed that 30 out of 92 cNFs (32.6%) had full or substantial reduction; biopsied lesions excluded. Patient reported pain-score during treatment was median 3.5 (range 1-7) on a 0-10 point scale. No pain was reported post-treatment.

Conclusions: HIFU treatment is a new non-invasive treatment modality that with high precision targets intradermal lesions; it is a rapid and tolerable treatment. This study demonstrates the safety, local tolerability, efficacy, and feasibility of HIFU for the treatment of cNFs in adults with NF1. The variation in cNF reduction after HIFU-treatment and the occasional erosions and crusts in the treatment area indicate that dosing needs to be further adjusted. Follow-up pre-clinical and clinical studies to optimize the dose response in adults with NF1 are underway with a goal of applying this therapy to both established and developing cNFs in the future.

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Funding: This study was funded by The Neurofibromatosis Therapeutic Acceleration Program, Baltimore, MD. Equipment for treatment has been provided by TOOsonix A/S, Hoersholm, Denmark.

Platform: TRAM-01: A Phase 2 Study of Trametinib for Pediatric Patients with Neurofibromatosis Type 1 and Plexiform Neurofibromas

Monday, June 26, 6:15pm – 6:30pm

Sébastien Perreault, MD, MSc, FRCPC, *Departement of Neurosciences, Université de Montréal, CHU Sainte-Justine, Montréal, Québec, Canada*

Background: Plexiform neurofibromas (PN) are observed in up to 50% of patients with neurofibromatosis type 1 (NF1). Trametinib has been used widely to treat PN but limited data has been reported on its efficacy within a clinical trial.

Methods: A part of this multicenter phase II trial includes pediatric patients with NF1 related PN. Patients received daily oral trametinib for eighteen 28-day cycles. The PN volumes were centrally quantified using a new semi-automatic 3D segmentation method.

Results: In total, 46 patients were recruited. The median age was 10.1 years (range 0.7-19.8). Median duration of treatment was 16.6 months (range 1.8 to 16.9). Median duration of follow-up was 32.3 months (range 7.8 to 48.9). Thirty-nine (86.7%) completed the planned treatment, two patients (4.4%) discontinued due to adverse reactions, three patients (6.7%) refused to continue treatment and one (2.2%) discontinued treatment based on physician decision. When based on modified RECIST criteria 32.6% (15/46 patients) had minor response ($\geq$ 10-$<$30% reduction in the sum of the greatest tumors diameters) and the rest had stable disease 67.4% (31/46 patients). Forty-five patients were available for volume analysis. The median PN volume at baseline was 89.2cm^3 (range 15.4 to 487.6). Volumetric assessment demonstrated an overall response rate (volume decrease of $>$20%) of 53.3% (24/45patients), and 59.2% for PN (29/49 PN). Median volume change was -24.2% (range -93.5 to 14.3). After discontinuation of treatment, 13 (28.9%) patients required intervention including three patients that underwent surgery and nine patients restarted MEK inhibitor. The median time of reintervention after discontinuation was 13 months (range 1.1-31.2 months).

Conclusion: We report outcomes and volumetric response of PN treated with trametinib within a phase II trial. Based on these results, trametinib is effective and offers durable response during treatment.

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Platform: Auditory Dysfunction Among Individuals with Neurofibromatosis Type 1 and Their Treatment in Children

Monday, June 26, 6:30pm – 6:45pm

Alice Maier, MPsych, *The Murdoch Children's Research Institute*

Purpose: To characterise the auditory neural phenotype and functional hearing abilities of individuals with neurofibromatosis type 1 (NF1), and to investigate possible treatment options in children with NF1.

Methods: This study comprised a case-controlled study (N=88) investigating (i) auditory neural function; (ii) monaural/binaural processing; and (iii) functional hearing in children and adults with NF1 by measuring auditory brainstem responses and performances on the Listening in Spatialized Noise tasks. We also conducted a pilot randomized, blinded, two-period crossover study of a remote-microphone listening device to determine whether speech perception deficits in children with NF1 are amenable to treatment (n=10; aged 7-17 years). Speech perception was assessed with and without the hearing device at a baseline using the CNC-Word speech perception task. Children were then randomized to one of two treatment sequences: active device use for two weeks at school followed by inactive device use (i.e., control condition) for two weeks; or vice versa. The Listening Inventory for Education – Revised (LIFE-R), questionnaire was completed at baseline and after each condition to assess functional hearing benefits.

Results: 25% of NF1 participants demonstrated auditory neural dysfunction, including absent, delayed, or low amplitude electrophysiological responses from the auditory nerve and/or brainstem compared with 2% in the control group (odds ratio [OR], 13.03; 95% CI, 1.59-106.95). 32% of NF1 participants showed clinically abnormal speech perception in background noise compared with 2% in the control group (OR, 20.07; 95% CI, 2.50-160.89). In the trial, remote microphone listening devices provided significant perceptual benefits in the active versus inactive conditions during in-clinic audiology testing ($p<0.001$). 60% of the cohort improved such that their perceptual skills normalized when wearing the device. Compared to the control condition, participants self-reported significantly better classroom communication skills in the active versus inactive condition ($p=0.017$). All parents indicated they would recommend the hearing device to families of children with similar hearing difficulties.

Conclusions: Individuals with NF1 demonstrate auditory neural abnormality and perceptual dysfunction severe enough to impede developmental progress in children and restrict communication in older participants. Remote microphone listening systems are a tolerable, feasible intervention that may provide significant speech/communication benefits to children with NF1.

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Funding: Children's Tumour Foundation (Barney Fellowship); HEARing CRC (2014-19) project support (XR1.2.2)

Platform: MRI Shape and Intensity Features are Associated with Vision Loss in Children with NF1-OPG

Monday, June 26, 6:45pm – 7:00pm

Purpose: Children with optic pathway gliomas associated with neurofibromatosis type 1 (NF1-OPG) are at risk for permanent vision loss. While OPG volume has been recently associated with vision loss, it is unclear if changes in shape and other OPG imaging features are associated with the likelihood of vision loss. We propose a fully automatic framework using multi-sequence magnetic resonance images (MRIs) to determine their association with VA loss secondary to NF1-OPGs.

Methods: This study includes brain MRI of 75 children with NF1-OPG from Children's Hospital of Philadelphia, acquired using Siemens platform. Each subject in the dataset contains multi-sequence MRI: T1-weighted isotropic volumetric sequence, low-resolution anisotropic T2-weighted and T2-FLAIR sequences. Manual segmentation of the anterior visual pathway (AVP) was performed to measure the surrogate ground truth of the NF1-OPG. The average resolutions of the three sequences were $0.91\times0.83\times0.83$ mm3, $0.54\times0.69\times1.93$ mm^3, and $0.6\times0.61\times3.69$ mm^3, respectively. A subset of 25 children underwent neuro-ophthalmic evaluation to determine the presence or absence of VA loss as ground truth. OCT images were acquired using a spectral-domain OCT device (Spectralis; Heidelberg Engineering, Heidelberg, Germany) in addition to standard VA testing. Automatic AVP segmentation was performed using a knowledge-transferred transformer-based segmentation network (Swin transformer). Evaluation of the results was performed using the Dice volumetric overlap (DVO), normal surface distance (NSD), and relative volume error (RVE). In total, 14 shape-based and 279 intensity-based radiomic features were extracted from the segmented AVP, and VA risk factors were identified using the three sequences and univariate statistical tests (ANOVA).

Results: The average DVO, NSD (mm) and RVE for automatic AVP segmentation were 0.791 ± 0.075, 0.335 ± 0.179 and 0.176 ± 0.183, respectively. Multiple radiomic features were inter- correlated and eliminated from analysis. Two final features were included in the classification, namely the AVP with NF1-OPG sphericity (shape) and local homogeneity in T2 measured by normalized inverse difference moment (intensity). VA loss association by cross-validation resulted in an accuracy of 0.8 with 0.69 sensitivity, 0.92 specificity and AUROC of 0.77. To put these results in perspective, in a previous independent study, the reported inter-observer variability for AVP manual segmentation was $DVO=0.75\pm0.06$.

Conclusion: Our deep learning-based automatic framework using multi-sequence MRI demonstrates new shape- and intensity-based features associated with VA loss in NF1-OPGs. If validated through longitudinal studies, this framework has the potential to accelerate and guide the treatment decisions of children with NF1-OPGs.

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LATE BREAKING ABSTRACTS – CONCURRENT SESSION – CME SESSION

Platform: Can MEK Inhibitors Help to Avoid the Need for Surgery in Neurofibromatosis Type 1 (NF1) with a Spinal Phenotype?

Tuesday, June 27, 9:30am – 9:50am

Alexander Lee, MD PhD, *Highly Specialised Service for Complex NF1: Manchester, UK*

Pathognomonic germline mutations of *NF1* form the basis for pathological constitutive activation of the MAPK signalling pathway, playing a central role in the growth of plexiform neurofibromas (PN) in neurofibromatosis type 1. Small molecule inhibitors of MEK (MEKi), a signalling intermediary of the MAPK pathway, have been successfully developed for the treatment of inoperable complex PN in children, while prospective randomised trials for the treatment of adult patients are underway. The optimal timing and sequencing of MEKi with other non-pharmaceutical interventions remains to be established. This is particularly the case in spinal phenotype, where the growth of multi-level neurofibromas at the origin of nerve roots can lead to compressive myelopathies and the requirement for morbid surgical intervention.

We report two cases of young patients with spinal phenotype NF1 who commenced treatment with selumetinib (provided through managed access program) with the aim of preventing the otherwise pressing need for further surgical spinal decompression: One, a woman aged 26 (de-novo variant *NF1* c.273A>C) who, over the preceding 5 years, since the age 21, had undergone 3 major neurosurgical procedures to relieve cord and nerve root compression related to growing PNs; the other, a 20 year old man (*NF1* c.7909C>T) who, at age 18, had required posterior cervical laminoplasty for severe cord compression and who had developed further progressive clinical and radiological evidence of multilevel myelopathy.

In both cases, selumetinib therapy has been well tolerated and associated with clinical and radiological evidence of reduced burden of symptomatic PN. Exhibited benefits included reduced PN-related pain and reduction in size of superficial neurofibromas. In one case, marked improvement of neurological debilitation has been seen; in the other, an absence of significant neurological dysfunction and attendant avoidance of further neurosurgical intervention has been seen. NF1-related quality of life has been shown to improve per serial completion of INF1-QoL questionnaire.

MEKi appear to have significant clinical activity in spinal NF1. Further prospective investigation is required to establish the optimal approach to the use of MEKi in preventing spinal morbidity in such patients.

Additional Authors: Judith Eelloo, Dr John Ealing, Dr Calvin Soh, Dr Grace Vassallo – affiliations as per lead author

Disclosure: AL has received consultancy fees and support with conference attendance from Astra Zeneca and Alexion

Platform: Magnetic Resonance Elastography Predicts Tumor Composition, Behavior, and Patient Outcomes in Vestibular Schwannomas

Tuesday, June 27, 9:50am – 10:10am

Bailey H. Duhon, MS, *Division of Otology, Neurotology, and Cranial Base Surgery, Department of Otolaryngology – Head and Neck Surgery, The Ohio State University Wexner Medical Center, Columbus, OH*

Introduction: Variations in biophysical characteristics of vestibular schwannomas (VS), such as tumor stiffness, can influence the difficulty of surgical resection, which remains the mainstay treatment of large, symptomatic, or growing NF2-related tumors. Here, we utilize magnetic resonance elastography (MRE) preoperatively to non-invasively predict the biomechanical properties of VS and correlate with intraoperative findings and postoperative outcomes.

Methods: In a prospective study of adult VS patients, we analyzed the association between MRE stiffness obtained within 48 hours prior to tumor surgery with: a) intraoperative assessment of tumor consistency and stiffness, b) postoperative outcomes including extent of tumor resection and facial nerve function and c) histological markers of tumor fibrosis. MRE scans (3.0mm isotropic resolution, 16 slices) were taken using a 3T MRI and pneumatic pillow-like driver (**Fig. 1**). A blinded reviewer obtained local-frequency estimation (LFE) inversion to estimate tumor stiffness (**Fig. 2**). During microsurgical resection, two surgeons blinded to MRE results recorded intraoperative characteristics using a quantitative survey. Tumors were stained for fibroblasts (alpha-SMA), hyaluronan (HABP), and Masson's Trichrome using immunohistochemistry.

Results: MRE stiffness measurements strongly correlated with intraoperative tumor stiffness assessments (r^2=0.66, p=0.05). Tumors were classified into "Stiff" and "Non-stiff" (Above or below the whole tumor mean stiffness value of 4.02 kPa). Stiff VS were significantly larger (70 cm^3 vs. 17 cm^3, p=0.048) and had worse FN function postoperatively (%HB Grade III or higher, 75% vs. 0%, p=0.035). Additionally, stiffness was elevated in aggressive VS that were adherent to the brainstem compared to non-adherent VS (5.18 kPa vs. 3.57 kPa, p=0.13) and in VS that required subtotal resections rather than gross total resections (5.15 kPa vs. 3.35 kPa, p=0.055). Histopathological analysis revealed that activated fibroblast (alpha-SMA) (r^2 =0.63, p=0.029) (**Fig. 3**) and Masson's trichrome stain (r^2 =0.63, p=0.029) were significantly correlated with stiffness, while hyaluronan (HABP) trended towards significance (r^2 =0.56, p=0.051).

Conclusion: In a feasibility study of patients undergoing microsurgical resection of VS, the hallmark tumor of NF2, pre-operative MRE accurately measured the biophysical properties of intracranial extra-axial tumors which correlated with intraoperative assessments. Tumors with higher stiffness exhibited more adhesions to the brainstem, which may result in higher incidence of subtotal resections and poorer clinical outcomes. On a molecular level, stiffer VS showed higher fibroblast activation and increased collagen and hyaluronan deposition. These findings motivate future studies to investigate how MRE can be utilized to assist in surgical planning and ultimately improve surgical outcomes.

Fig. 1

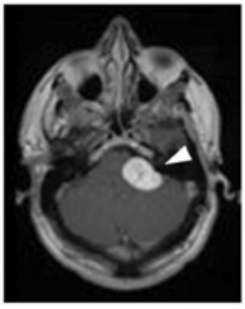

Figure 1: Contrast-enhanced T1 MRI showing a large vestibular schwannoma (white arrow) (VS#7).

Fig. 2

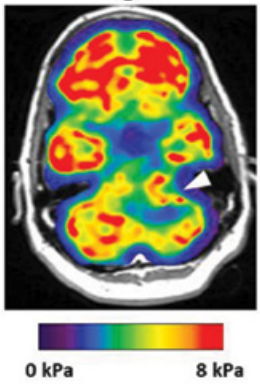

Figure 2: T1 MRI overlaid with local-frequency estimation (LFE) map showing stiffness of the vestibular schwannoma (white arrow) (VS#7).

Fig. 3

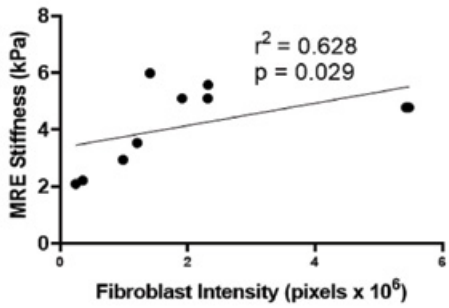

Figure 3: Correlation of MRE stiffness with whole tumor alpha-SMA (activated fibroblast) intensity.

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Platform: Impact of Fibroblasts on Cell Proliferation of *NF2*-Mutant Schwann Cells and Tumor Progression in Vestibular Schwannoma

Tuesday, June 27, 10:10am – 10:30am

Olena Bracho, BS, *University of Miami Department of Otolaryngology*

Objective: Because the role of tumor-associated fibroblasts (TAF) on tumor progression in vestibular schwannoma (VS) is unknown, the purposes of this study are to: (1) determine the effect of cultured fibroblasts on cell proliferation of *NF2*-mutant Schwann cells *in vitro,* and (2) examine the association of TAFs on tumor size and progression in VS.

Methods: For in vitro studies, tumor spheroids were cultivated on 3-dimensional culture plates using *NF2*-mutant human Schwann cells, normal human fibroblasts, and their co-cultures. Time-lapsed brightfield imaging was performed over 14 days. Tumor spheroid volumes were calculated from cross-sectional areas. Viability assays and immunocytochemistry for S100A4 (fibroblast marker) were performed on 2-dimensional cultures. Statistical analysis was performed using 95% confidence intervals.

For clinical studies, fresh tumors from 76 VS patients were harvested during surgery, fixed, and embedded in paraffin. Immunohistochemistry was performed for Ki67 (cell proliferation marker) and S100A4 (fibroblast marker). Age, gender, and NF2 status were obtained from the medical record. Tumors were categorized as small-to-medium and large-to-giant tumors by maximum linear tumor dimension on pre-operative contrast-enhanced MRI. Statistical analysis was performed using t-tests and one- and two-way analysis of variance with Bonferroni correction.

Results: Our *in vitro* studies showed tumor spheroids consisting of *NF2*-mutant Schwann cells demonstrated significant growth over time. Addition of normal fibroblasts to *NF2*-mutant Schwann cell cultures suppressed growth of tumor spheroids. Findings were further supported by viability assays and immunocytochemistry for S100A4 on 2-dimensional cultures.

Our clinical study showed the mean age at surgery was 51 years, 55% were female, 11% had NF2, and 47% had small-to-medium tumors. Of 76 tumors, 21 demonstrated tumor growth, while 3 had no radiographic evidence of progression. Ki67 index $\geq$3% was associated with larger tumors ($p=0.0370$) and higher expression levels of S100A4 ($p=0.0289$). Although no association between tumor size and S100A4 expression was found, in small-to-medium tumors, we found non-growing, sporadic VS expressed higher levels of S100A4 than growing tumors ($p=0.0054$).

Conclusions: This is the first study to demonstrate that the presence of normal fibroblasts can suppress tumor growth of *NF2*-mutant Schwann cells. We also showed that in small-to-medium tumors, non-growing sporadic VS expressed higher levels of tumor-associated fibroblasts, when compared to growing tumors. Together, these findings suggest that fibroblasts may be tumor suppressive in NF2-associated schwannomas and early VS development. Further investigations focused on elucidating the role of TAFs in NF2-associated schwannomas may reveal novel targets for therapeutic intervention to improve tumor control in NF2 patients.

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Funding: Sylvester K-Supplement Grant

Platform: Developmental Trajectories in Infants and Pre-School Children with Neurofibromatosis 1

Tuesday, June 27, 10:30am – 10:50am

Shruti Garg, MD, PhD, *Division of Neuroscience and Experimental Psychology, Manchester Academic Health Science Centre, University of Manchester, Manchester, United Kingdom; Royal Manchester Children's Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom*

Objective: Little is understood about the early developmental emergence of cognitive, social and behavioural impairments in children with NF1. This study aims to examine the social, cognitive, behavioural and attentional development in infant and pre-school children with Neurofibromatosis 1 (NF1) compared with typically developing children without a family history of neurodevelopmental difficulties.

Methods: This prospective cohort study enrolled children with NF1 and low-risk controls from 5 months of age. Data from standardised tests was gathered at 5, 10, 14, 24 and 36 months. Tests included the Mullen Scale of Early Learning, Vineland Adaptive Behaviour Scale, Autism Diagnostic Interview-Revised (ADI-R), Autism Diagnostic Observation Schedule-2 (ADOS-2), Brief Observation of Symptoms of Autism (BOSA) and the Child Behaviour Checklist (CBCL). Developmental trajectories of cognitive and adaptive behavioural development from 5 to 36 months were analysed using linear mixed modelling to estimate group differences over time. Descriptive analyses of social communication and attentional development at 24 and 36 months were explored.

Results: Cognitive skills (Mullen) were significantly lower in children with NF1 at each time point when compared with typically developing controls. The developmental trajectory over time of cognitive skills also differed significantly between children with NF1 and controls at 24 and 36 months. Adaptive behavioural skills (Vineland) were not significantly different in children with NF1 when compared with typically developing controls, however infants with NF1 did follow significantly different developmental trajectories of these skills within the model. The developmental trajectory of behavioural skills differed significantly between children with NF1 and typically developing controls. Maternal education was significantly associated with cognitive and behavioural skill development in both groups.

On analysis of social development aged 24 months, the NF1 cohort was significantly more likely to demonstrate social communication concerns on the ADOS or BOSA compared to controls. However, this difference did not reach significance at 36 months. The NF1 group gained higher mean scores on individual ADI-R subscales at 36 months, however the overall level of concern between the groups did not reach significance. The NF1 cohort demonstrated significantly higher levels of attentional impairment at 36 months (CBCL).

Conclusions: This study is the first to investigate trajectories of cognitive and behavioural development in children with NF1 from infancy up to pre-school age (5 to 36 months). Children with NF1 followed significantly different developmental trajectories of cognitive and behavioural skills. Our results demonstrate that overall cognitive trajectories are significantly different in the NF1 group at 24 and 36 months compared with controls. Attentional development is impaired at 36 months in the NF1 group, however while social differences are more pronounced at 24 months, these differences became non-significant at 36 months.

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On behalf of the EDEN-STAARS team

INDUSTRY PLATFORMS – CONCURRENT SESSION – NON-CME SESSION

Platform: Treatment Patterns and Healthcare Resource Utilization of Pediatric Patients with Neurofibromatosis Type 1-Associated Symptomatic Inoperable Plexiform Neurofibroma in the United States

Tuesday, June 27, 9:00am – 9:15am

Theresa Dettling, *Alexion, AstraZeneca Rare Disease, Boston, MA*

Introduction: Neurofibroma type 1 (NF1) is a rare genetic disease with onset in early childhood. Plexiform neurofibromas (PN), benign tumors along the nerves, are a common and chronic complication of NF1 occurring in 20% to 50% of NF1 patients (Nguyen et al., 2011; Tchernev et al., 2016; Miller et al., 2019). PN can be painful, potentially life-threatening, and often cannot be surgically removed, depending on the size and proximity to vital tissues. The aim of this study was to describe treatment patterns and healthcare resource utilization in pediatric NF1 patients with symptomatic inoperable PN in the US prior to US FDA approval of selumetinib.

Methods: This was a retrospective chart review of pediatric NF1 patients with symptomatic inoperable PN from 3 NF1 treatment centers in the US. Patient records were abstracted if the patient (1) received NF1 diagnosis from 1.Jan.2000 to 31.Dec.2018, (2) was 2 to 18 years old at the time of diagnosis, (3) was diagnosed with ≥1 symptomatic inoperable PN, (4) received care on-site for ≥12 months, and (5) had ≥3 site visits prior to the end of the study period on 31 Dec 2019. Records were abstracted at the patient's first, last, and "midpoint" visits (the visit nearest the midpoint between the first and the last).

Results: There were 102 pediatric patients with at least one symptomatic inoperable PN, of which 84 (82.4%) received treatment for their PN. Most patients were male (n=66, 64.7%) and the median age at diagnosis of first symptomatic inoperable PN was 6 years. On average, patients received 3.4 monitoring/imaging scans per year with MRI (2.0 per patient per year [PPPY])]) and x-rays (1.3 PPPY) being the most common. Patients attended 6.1 outpatient visits per year, on average, with 0.3 emergency room visits PPPY. Nearly half (n=50, 49.0%) were hospitalized at least once for an average rate of 0.2 hospitalizations PPPY. Among those who received treatment, 53 (63.1%) received pharmacotherapy. The most common pharmacotherapies were gabapentin (n=16, 30.2%) and NSAIDs (n=7, 13.2%) for pain, and selumetinib (n=13, 24.5%) and trametinib (n=9, 17.0%) for PN shrinkage. Chemotherapy was used to treat PN in 6 (5.9%) patients, half of which had PN progression despite treatment.

Conclusions: There is a substantial burden of monitoring and treatment of NF1 PN on patients and the healthcare system. Access to effective long-term pharmacological treatment to control PN progression may reduce the healthcare burden on patients and the healthcare system.

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References:
1. Miller DT, Freedenberg D, Schorry E, Ullrich NJ, Viskochil D, Korf BR; Council on Genetics and American College of Medical Genetics and Genomics. Health supervision for children with neurofibromatosis type 1. Pediatrics. 2019;143(5).
2. Nguyen R, Kluwe L, Fuensterer C, Kentsch M, Friedrich RE, Mautner VF. Plexiform neurofibromas in children with neurofibromatosis type 1: frequency and associated clinical deficits. J Pediatr. 2011; 159(4):652-5.e2.
3. Tchernev G, Chokoeva AA, Patterson JW, Bakardzhiev I, Wollina U, Tana C. Plexiform neurofibroma: a case report. Medicine (Baltimore). 2016 Feb;95(6):e2663.

This study was funded in full by AstraZeneca. TD, RR, MB are salaried employees of Alexion, AstraZeneca Rare Disease. XY is a salaried employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc. SDC and MIJ are employees of RTI Health Solutions.

Platform: HLX-1502: A Novel Potential Treatment for Neurofibromatosis Type 1 Plexiform Neurofibromas

Tuesday, June 27, 9:15am – 9:30am

Emma Davies, *Healx Ltd*

Healx has identified a novel small molecule that we plan to investigate for the treatment of neurofibromatosis type 1 (NF1): HLX-1502, also known as nitroxoline. It has a significant amount of human use data, a known safety profile and reported anti-cancer properties; it is not FDA approved.

HLX-1502 has anti-proliferative activity in human Schwann cells derived from a plexiform neurofibroma (ipNF95.6) and it significantly reduces nerve volume and tumor number in the *Postn-Cre*$^{+}$ *Nf1*$^{fl/fl}$ mouse model of plexiform neurofibroma (**Figure 1 & 2**). The degree 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 MPNST. The mechanism of action of HLX-1502 in NF1 is currently being investigated, with initial multi-omic data suggesting HLX-1502 modulates a range of pathways and processes in NF1 null Schwann cells. Notably, HLX-1502 does not inhibit the RAS/MAPK pathway (**Figure 3**). We have identified a number of target engagement biomarkers of HLX-1502 that we are currently using to build the PK/PD/efficacy relationship of HLX-1502 in NF1. Clinical development planning is in progress, as well as further preclinical *in vivo* combination studies with MEK inhibitors.

About us: Healx is a biotech company that has redesigned drug discovery to develop treatments for rare diseases. Healx's approach, made possible through the combination of artificial intelligence and digital sciences, builds a deep biological understanding of drug-disease relationships to uncover more effective treatments (**Figure 4**).

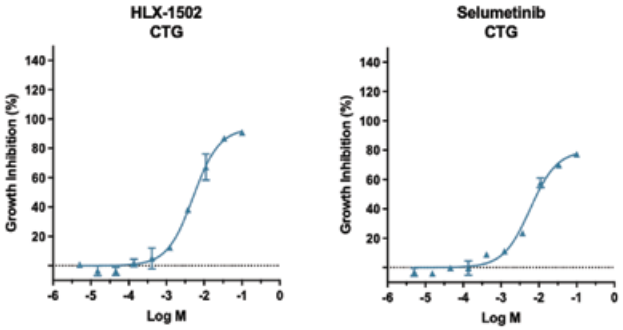

Figure 1. The growth inhibitory effects of HLX-1502 and selumetinib on *NF1*$^{-/-}$ plexiform neurofibroma Schwann cells

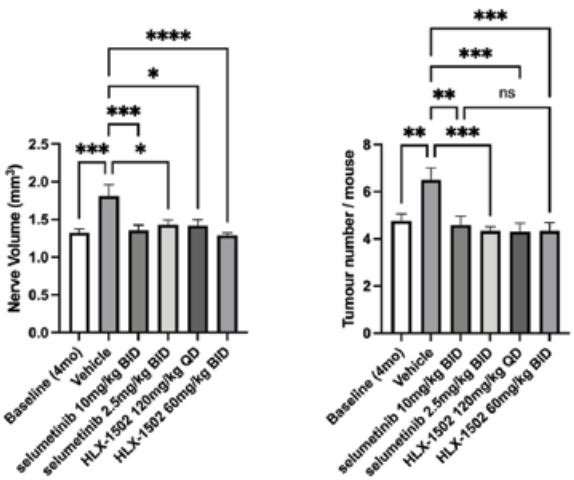

Figure 2. The effects of HLX-1502 treatment on proximal nerve volume and tumour number in the *Postn-Cre*$^{+}$ *Nf1*$^{fl/fl}$ mouse model of plexiform neurofibroma (n=12, * = p value <0.05, one-way ANOVA)

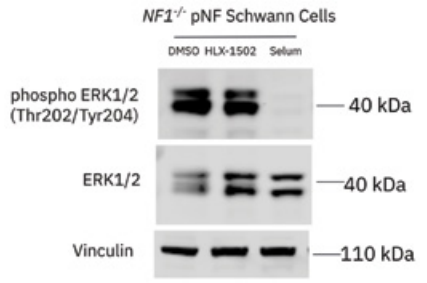

Figure 3: The effect of HLX-1502 and selumetinib on phospho-ERK1/2 levels in *NF1*$^{-/-}$ plexiform neurofibroma Schwann cells

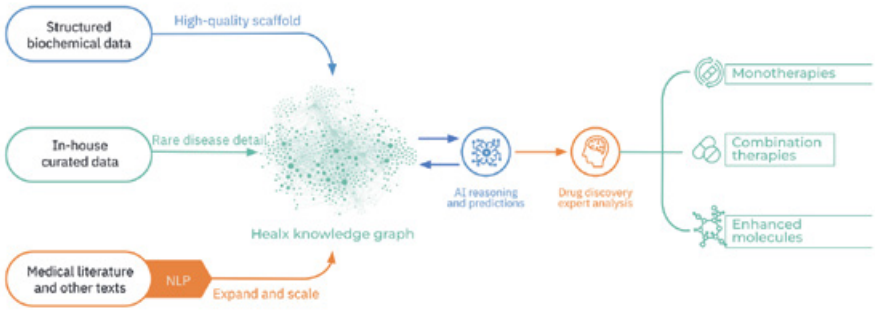

Figure 4. Healx drug discovery platform

Full List of Authors: Simone Manso, Emma Davies, Ivan Angulo-Herrera, Waylan Bessler, Steven Rhodes, Ian Roberts, Dan Mason, Jane Brennan, Alexander Syme, Svetlana Saveljeva, Triin Tammsalu, Emma Tulip, Rita Chaouni, Samantha Boyle, Robert Wilson, Fanny Coulpier, Piotr Topilko, Wade Clapp, Neil Thompson & Dave Brown

Disclosure: Simone Manso, Emma Davies, Ivan Angulo-Herrera, Ian Roberts, Dan Mason, Jane Brennan, Alexander Syme, Svetlana Saveljeva, Triin Tammsalu, Emma Tulip, Rita Chaouni, Samantha Boyle, Robert Wilson, Neil Thompson & Dave Brown are employees of Healx Ltd. Simone Manso is a member of the Board of Directors of the Children's Tumor Foundation and of the Children's Tumor Foundation Europe.

Platform: TEAD Autopalmitoylation Inhibitors Prevent NF2-Deficient Meningioma Growth in an *In Vivo* Skull Convexity Model

Tuesday, June 27, 9:30am – 9:45am

Liyam Laraba, PhD, *University of Plymouth, UK*

Purpose: After showing efficacy *in vitro* and in the *NF2*-deficient Periostin-Cre;*nf2*$^{fl/fl}$ schwannoma mouse model, TEAD autopalmitoylation inhibitors are now being assessed using *in vitro* and *in vivo* meningioma models, both alone and in combination with receptor tyrosine kinase inhibitors.

Methods: We are using orthotopic xenograft models, where Merlin-null human grade I (Ben-Men-1) and grade III (KT21 and NCH93) cell lines are injected into the skull convexity of immunocompromised Nod-Scid Gamma mice. Following tumor establishment, mice are dosed orally with TEAD autopalmitoylation inhibitors (VTs) and tumor growth is monitored by luciferase activity in real time using an IVIS Spectrum imager. Mice are dosed at clinically relevant concentrations with VTs to assess their effect on proliferation and apoptosis, as well as other tumor hallmarks such as angiogenesis and innate immune cell infiltration by immunostaining.

Results: Our previously published work focused on the use of VTs in *NF2*-deficient murine schwannoma. Here, we now demonstrate the efficacy of VTs in meningioma tumors, either alone or in combination with receptor tyrosine kinase inhibitors. Preliminary data shows a potent inhibition of tumour growth in mice injected with KT21-luciferase signal following 28d consecutive treatment with TEAD inhibitors (n=3 vehicle and n=2 drug; **Figure 1**). We also see significant reduction in mitogenic growth of KT21 cells treated with VT by EdU proliferation assay. These results will be repeated in other meningioma cell lines and complemented by western blotting for TEAD transcriptional targets such as CTGF, to show target engagement of VT inhibitors. Immunostaining analysis of skull convexity tumours shows CD31+ and CD68+ cells, demonstrating macrophage and endothelial cell infiltration among the luciferase+ tumor cells.

Conclusions: We are further characterising promising TEAD autopalmitoylation inhibitors in our murine models *NF2*-null meningioma. VT inhibitors are efficacious in reducing proliferation and tumor growth in pre-clinical models of Merlin-null meningioma. We are characterising these tumors histologically to monitor changes to other tumor hallmarks.

Figure 1

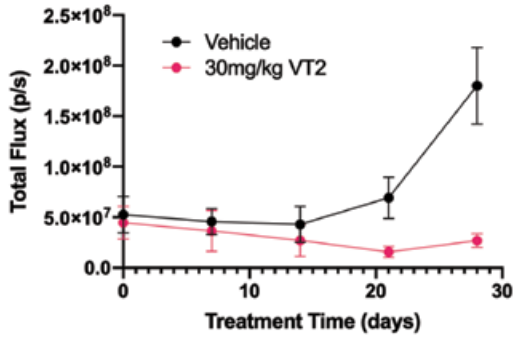

Figure 1: IVIS bioluminescence imaging showing total flux of KT21-luciferase activity in Nod-Scid gamma mice. After 21d of tumor establishment, mice were treated by oral gavage with either vehicle or 30mg/kg TEAD autopalmitoylation inhibitor (VT2) for 28d, VT2 treatment prevented tumor growth (data shown as mean +/- SEM, n=3 for vehicle and n=2 for VT2 treated mice).

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Disclosures: Tracy T. Tang and Leonard Post are employees of Vivace Therapeutics and have equity interest in Vivace Therapeutics. All other authors have declared no conflict of interests.

Funding: Children's Tumor Foundation Young Investigator Award, Brain Tumour Research and Vivace Therapeutics Inc.

Platform: Targeting NF2-Deficient Tumors with VT3989

Tuesday, June 27, 9:45am – 10:00am

Andrew Dorr, MD, *Vivace Therapeutics, San Mateo, CA*

Introduction: Inactivation of NF2, either germline in schwannoma, meningioma and malignant peripheral nerve sheath tumors (MPNST), or somatic in mesothelioma, meningioma and other malignant tumors, results in activation of transcription factors YAP and TAZ. VT3989 was discovered by extensive optimization of hits from a high throughput phenotypic screen for compounds that block YAP-driven gene expression, and shown to block auto-palmitoylation of TEAD and thereby inhibit its function with YAP and TAZ. TEAD palmitoylation inhibitors show efficacy in *in vitro* and *in vivo* models of mesothelioma, schwannoma, and meningioma.

Methods: This first in human trial used a 3 + 3 dose escalation design in solid tumor patients and evaluated VT3989 from 25 to 200 mg QD continuously and 50 to 200 mg intermittently. Pts with refractory solid tumors, ECOG PS 0-1, serum albumin > 2.5, urinary protein creatinine ratio ≤ 0.5 mg/mg and albumin creatinine ratio ≤ 100 mg/g were enrolled. Objectives were to determine the safety, tolerability and recommended Phase 2 dose and schedule (RP2DS) of VT3989 and to evaluate antitumor activity, pharmacokinetics (PK) and correlate *NF2*m and other alterations in tumor and ctDNA with response

Results: 69 patients have been enrolled in part 1 of the phase 1 trial. Tumor types: 44 mesothelioma (29 pleural, 10 peritoneal, 1 pericardial and 1 dual pleural/peritoneal) 13 and 25 other solid tumors including 9 meningioma, 1 schwannoma, 1 MPNST and 4 sarcoma pts. 37 pts had *NF2*m (31 somatic and 6 germline). VT3989 is safe and well tolerated with no dose limiting toxicities. No MTD was defined up to 200 mg QD. The most common AEs are proteinuria, albuminuria and peripheral edema, mainly observed with the continuous schedule. Seven patients (6 refractory mesothelioma and 1 *NF2* mutant sarcoma) achieved partial responses (PRs) with 6 confirmed, and 1 unconfirmed. 3 PRs in mesothelioma patients are ongoing up to 20+ months. Meningioma patients with documented germline or somatic mutations as well as a meningioma patient without a mutation have had minor reductions in tumor size with long-term stable disease up to 14+ months.

Conclusions: In preclinical models and in patients, VT3989 has activity in NF2 deficient tumors at well-tolerated doses. Further clinical study in patients with NF2m tumors, both somatic (mesothelioma, meningioma, and other solid tumors) and germline (meningioma, schwannoma and MPNST), is warranted.

Full List of Authors: F Andrew Dorr, Tracy Tang, Leonard Post

Disclosures: Leonard Post, Ph.D., and Tracy Tang, Ph.D., are employees of Vivace Therapeutics; Andrew Dorr, M.D. is a Consulting Chief Medical Officer for Vivace and has stock options.

Funding: The funding source is Vivace Therapeutics, which is funded by various venture capital organizations.

Platform: NVD-003, an Osteogenic Cell-Based Bone Graft Derived from Autologous Adipose Tissue, Used in the Treatment of Congenital Pseudarthrosis of the Tibia

Tuesday, June 27, 10:00am – 10:15am

Philip K. McClure, MD, FAAOS, *International Center for Limb Lengthening, Rubin Institute for Advanced Orthopedics, Sinai Hospital of Baltimore, Baltimore, MD*

Purpose: NVD-003 returned promising safety and efficacy results in the first-in-human phase I/IIa clinical trial for adult long bone non-union treatment and is now being evaluated for the treatment of pediatric congenital pseudarthrosis of the tibia (CPT) (IND 27221).

CPT is a rare pediatric disease occurring in only 1 out of 150,000 births, which is strongly interrelated with NF1. Around 50% of patients with NF1 have significant musculoskeletal manifestations, the most common being scoliosis and CPT. Because NF1 mutations interfere with osteoblastic differentiation and osteoclastic activity, the management of these orthopedic malformations is challenging.

The current standard-of-care bone procedure for CPT treatment involves autologous bone harvested at the iliac crest. This is associated with significant comorbidities such as pain, anemia, and neuralgia, and is impeded by shortness of available bone substitute material, especially in pediatric patients. Pre-clinical data support the safety and efficacy of NVD-003 independent of the presence of an NF1 mutation.

Methods: This single-arm multi-center study will enroll male and female pediatric patients aged 2 to 8 years who have been diagnosed with Paley type 3 or 4 CPT, with or without NF1. Per trial protocol, the NVD-003 bone graft will be implanted during the grafting surgery at 11 to 13 weeks following the adipose tissue collection intervention. To reduce bias, only the reconstructive Paley cross-union technique and osteosynthesis materials can be used.

While safety is the primary focus of this study, the bone healing potential of NVD-003 will be evaluated through clinical and radiological assessments. Bone formation, union, and remodeling will be assessed with dual-energy computed tomography and plain X-rays at 6 weeks post-grafting surgery, as well as at 3-, 6-, 12- and 24 months post-grafting surgery.

Conclusions: Based on the currently available pre-clinical and first-in-human safety and efficacy results, NVD-003 has the potential to become an alternative for autologous bone and/or other bone graft substitutes and bone-modulating agents (e.g., bone morphogenetic protein-2). Besides its promising safety profile, NVD-003 has demonstrated bone healing capacity in severe pathological non-union indications. The CPT trial, for which a detailed study outline can be retrieved from Clinical trials.gov, is currently open for recruitment (identifier: NCT05693558; study sponsor: Novadip Biosciences SA/NV).

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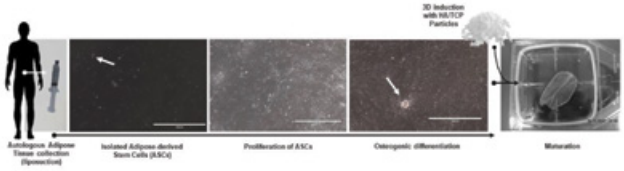

Figure 1: Good manufacturing practice (GMP)-controlled production process. Using stem cells isolated from adipose tissue, proliferated and differentiated to osteogenic cells, complemented with hydroxyapatite/β-tricalcium phosphate (HA/TCP) particles and maturated to an autologous 3D scaffold-free osteogenic bone graft.

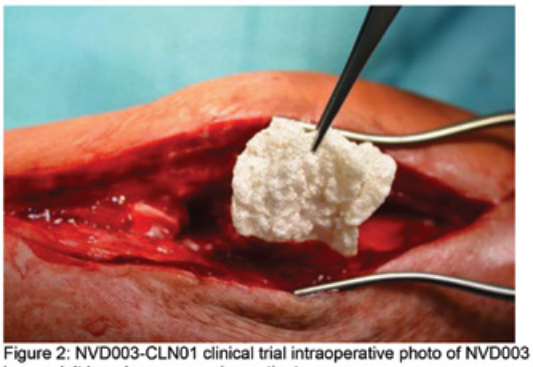

Figure 2: NVD003-CLN01 clinical trial intraoperative photo of NVD003 being implanted in an adult long-bone non-union patient.

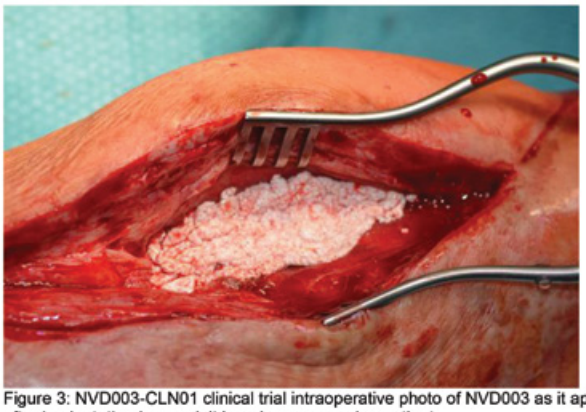

Figure 3: NVD003-CLN01 clinical trial intraoperative photo of NVD003 as it appears after implantation in an adult long-bone non-union patient.

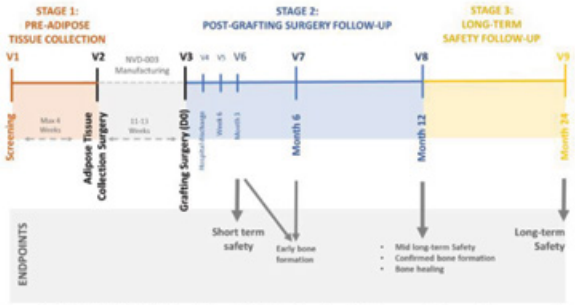

Figure 4. NVD003-CLN02 clinical trial design includes an adipose tissue collection phase, an NVD003 production phase, and short-term and long-term safety follow-up periods. Besides the safety endpoints, short- and long-term efficacy will be explored.

Disclosure of relevant financial relationships: Philip K. McClure is a consultant for DePuy Synthes Companies, Novadip, NuVasive Specialized Orthopedics, Orthofix, and Smith & Nephew. The following organizations supported the institution of Philip K. McClure: DePuy Synthes, NuVasive Specialized Orthopedics, Orthofix, OrthoPediatrics, Paragon 28, Pega Medical, Smith & Nephew, Stryker, Turner Imaging Systems, and WishBone Medical.

Platform: A Population Pharmacokinetic (PopPK) Assessment of the Effect of Food on Selumetinib in Patients with Neurofibromatosis Type 1 (NF1)-Related Plexiform Neurofibromas (PN) and Healthy Volunteers

Tuesday, June 27, 10:15am – 10:30am

Million Arefayene, PhD, *Clinical Pharmacology and Safety Sciences, Alexion, AstraZeneca Rare Disease, Boston, MA*

Purpose: Selumetinib (ARRY-142886, AZD6244) is a MEK1/2 inhibitor approved, in the fasted state, for pediatric patients with NF1 and symptomatic, inoperable PN (EMA, aged ≥3 years; FDA, aged ≥2 years). This analysis aimed to evaluate the effect of food on the pharmacokinetic (PK) parameters of selumetinib and its active metabolite, N-desmethyl selumetinib, using a PopPK assessment composed of a dataset of 15 clinical studies.

Methods: A PopPK model of selumetinib and N-desmethyl selumetinib was developed based on pooled data from 15 clinical studies in adult and pediatric/adolescent subjects with NF1-PN and healthy volunteers. The driver for the impact of food-effect, AUC, was calculated using individual post-hoc estimates from the final PopPK model and compared between fed (high-fat and low-fat) and fasted states. Numerous dose levels were included in the pooled dataset; therefore, dose normalization of AUC was deemed appropriate because the AUC of selumetinib was expected to increase in a dose-dependent manner. The lower bound of the one-sided 90% confidence interval (CI), for AUC_{0-12}, was the pre-specified criteria.

Results: This dataset included 511 subjects who received ≥1 dose of selumetinib and provided ≥1 measurable concentration of selumetinib and N-desmethyl selumetinib post-dose. The PK of selumetinib was described using a two-compartment model with sequential zero- and first-order delayed absorption and first-order elimination. The PK of N-desmethyl selumetinib was described using a one-compartment model and simultaneous analysis with PK measurements of the parent compound. The PopPK analysis showed that the mean AUC (one-sided 90% CI lower bound) of selumetinib, when taken with a low-fat meal, was 77.5% (73.7%), a reduction of 22.5% versus the fasted state. The lower bound of the one-sided 90% CI was >70%, demonstrating a <30% difference between low-fat and fasted states. This is not considered to be a clinically relevant effect on the AUC of selumetinib due to the flat exposure-response relationship within investigated dose range (20–30 mg/m^2). The mean AUC (one-sided 90% CI lower bound) of selumetinib with a high-fat meal was 79.2% (76.2%); a reduction of 20.8% versus the fasted state. The effect of a high-fat meal on the AUC of selumetinib was also deemed not clinically relevant. The effect of food (low and high fat) on the active metabolite, N-desmethyl selumetinib, was similar to selumetinib.

Conclusions: Overall, the effect of a low-fat or high-fat meal did not appear to have a clinically meaningful effect on the AUC of selumetinib, based on PopPK modeling of the pooled dataset.

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Disclosure of relevant financial relationships: PZ, MA, WJP and JM report employment at Alexion, AstraZeneca Rare Diseases. PZ, MA, and WJP also own AstraZeneca stock. TF reports employment at Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc.

Funding: This study was sponsored by Alexion, AstraZeneca Rare Disease as part of an alliance between AstraZeneca and Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD).

Platform: PRG-N-01, a Selective Inhibitor of TβR1-RKIP Interaction, Suppresses Schwannoma Formation in NF2 Mouse Model

Tuesday, June 27, 10:30am – 10:45am

Yeoun-Ho Chung, *Rare Disease R&D Center, PRG S&T Co., Ltd, Republic of Korea*

Background: NF2 is characterized by the growth of multiple benign tumors on the nervous system, for which there is currently no approved targeted treatment. Previously, we reported that Nf18001, a selective inhibitor of TGF-beta receptor 1-mediated RKIP degradation, inhibits tumor growth and promotes schwannoma cell differentiation. Here, we report on the mode of action, *in vitro/vivo* efficacies, comparative efficacy study, ADME, and pharmacokinetic properties of PRG-N-01 (new code name of Nf18001).

Methods: We assessed the interaction between TGF-beta receptor 1 kinase domain or domains of TGF-beta receptor 1, and RKIP using in vitro GST-pull down assays. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was used to compare the binding affinity of PRG-N-01 to TGF-beta receptor 1 kinase domain and RKIP in an *in vitro* binding assay. The *in vivo* efficacy of PRG-N-01 was evaluated in the NF2 mouse model *(Postn-Cre; Nf2$^{f/f}$),* and we assessed schwannoma cell differentiation by western blot analysis. Preclinical studies of PRG-N-01 were also completed.

Results: Using biochemical studies, we characterized the amino acids 230-322 region of TGF-beta receptor 1 as the direct binding domain to RKIP and confirmed PRG-N-0-1 disrupts the direct interaction between TGF-beta receptor 1 (aa 230-322) and RKIP. Long-term oral treatment with PRG-N-01 in NF2 mouse model suppressed tumorigenesis throughout the body, reduced the whorls of Schwann cell proliferation and schwannoma progression in the dorsal root ganglion, and promotes tumor cell differentiation. Unlike Brigatinib, PRG-N-01 selectively exhibited anti-tumor efficacy in NF2-deficient cells. Preclinical study shows PRG-N-01 has promising pharmacological properties, including good stability, permeability, and oral bioavailability, as well as low toxicity and tolerability.

Conclusions: Our study provides preliminary evidence that PRG-N-01 may represent a promising treatment option for NF2, with several unique features that differentiate it from other NF2 therapies currently under investigation. The development of PRG-N-01 as a targeted therapy for NF2 possess significant potential for improving the lives of NF2 patients.

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Platform: Phase 2 Randomized, Double-blind, Placebo-Controlled Study of the Anti-Nerve Growth Factor (NGF) Antibody Tanezumab in Subjects with Moderate to Severe Pain Due to Schwannomatosis

Tuesday, June 27, 10:45am – 11:00am

Scott Plotkin, MD, PhD, *Massachusetts General Hospital*

Introduction: Schwannomatosis (SWN) is a rare neurogenetic condition characterized by multiple schwannomas and severe chronic pain. Increased expression of nerve growth factor (NGF) has been identified in painful schwannomas.

Methods: We conducted a single-institution, phase 2, randomized, double-blind, placebo-controlled trial in which we evaluated the analgesic efficacy and safety of the subcutaneous administration of tanezumab, an anti-NGF antibody. Eligible subjects were 18 year of age or older; had moderate-to-severe SWN-related pain intensity (11-point Numerical Rating Scale (NRS-11) score ≥5 with 0=no pain and 10=worst pain) despite having tried neuropathic pain medications, non-steroidal anti-inflammatory drugs (NSAIDs), and opioids; were able to discontinue use of NSAIDs; and did not have osteoarthritis. The study periods included double-blind treatment with tanezumab 10 mg SQ or placebo (days 1-56) followed by single arm treatment with tanezumab 10 mg SQ (days 57-112), and safety follow-up (days 113-281). The primary endpoint was change in NRS-11 scores between day 1 and day 57 with a clinically meaningful change defined as >2 points. Secondary endpoints included the change in NRS-11 scores at other time points and in PROMIS-Pain Interference T-scores (PROMIS-PI; normative mean=50, SD=10, with higher scores reflecting more interference with activities due to pain) at all time points.

Results: During 21 months of enrollment, 9 subjects were enrolled. The median age was 44 years (range, 33-65) with 7 females. The mean baseline NRS-11 score was 7.6 (SD 2.1). During the double-blind period, four patients were assigned to receive tanezumab and five to receive placebo. The mean change in NRS-11 score was -2.5 (SD 4.5) in the tanezumab group and -0.4 (SD 2.1) in the placebo group. During the single-arm treatment when all participants received tanezumab, the mean change in NRS-11 score was 0 (SD 0) in the initial tanezumab group and was -1.4 (SD 1.9) in the initial placebo group. During the safety follow up period, the mean change in NRS-11 score was 0.8 (SD 1.6). The mean baseline PROMIS-PI T-score was 63.2 (SD 6.6). The mean change in PROMIS-PI score was -6.2 (SD 10.9) in the tanezumab group and 1.1 (SD 2.9) in the placebo group. During the single-arm treatment, the mean change in PROMIS-PI T-score was -0.4 (SD 2.0) in the initial tanezumab group and was -3.3 (SD 4.1) in the initial placebo. During the safety follow up period, the mean change in PROMIS-PI T-score was 2.0 (SD 6.4).

Discussion: Among patients with schwannomatosis and moderate-to-severe pain not well controlled by standard medicines, treatment with tanezumab was associated with decreases in pain intensity and pain interference; although some of these changes reflect clinically meaningful differences per published literature, they were not statistically significant. This study was underpowered due to poor accrual during the pandemic. A larger study of tanezumab in this patient population is warranted to further evaluate this promising treatment for SWN-related pain.

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Funding source: Pfizer and Children's Tumor Foundation

CONSORTIA AND COLLABORATION UPDATES – CME SESSION

NF Clinical Trials Consortium

Tuesday, June 27, 11:15am – 11:40am

Michael Fisher, MD, *Children's Hospital of Philadelphia*

The Neurofibromatosis Clinical Trials Consortium (NFCTC) was established in 2006 to accelerate clinical trials for all forms of neurofibromatosis (NF) by bringing together clinicians, scientists, and institutions with expertise in NF and in conducting clinical trials. The primary goal of the NFCTC is to develop, perform, and expeditiously complete biologically-based clinical trials for children and adults with significant complications of NF1, NF2, and schwannomatosis. To date, the NFCTC has launched 16 clinical trials, has enrolled more than 490 participants, and has 8 trials in development. Updates on the active clinical trials and trials in development will be presented.

An Update on the NF1-OPG Natural History Study

Tuesday, June 27, 11:40am – 12:00pm

Robert Avery, DO, MSCE, *Children's Hospital of Philadelphia*

Numerous diagnostic and management challenges impact children with NF1 optic pathway gliomas (OPGs). This presentation will discuss the NF1-OPG Natural History Study including recent enrollment achievements, lessons learned and future directions.

Genomic Blood-Based Biomarkers to Improve Cancer Detection in NF1: A Collaboration Between NIH and Washington University

Tuesday, June 27, 12:00pm – 12:25pm

Angela C. Hirbe, MD, PhD, *Washington University in St. Louis*

Neurofibromatosis type 1 (NF1) is among the most commonly inherited tumor predisposition syndromes, affecting 1:2500 individuals worldwide. Approximately 1/3 of children with NF1 will develop benign plexiform neurofibromas (PN) and almost half of these patients will have a PN undergo malignant transformation to malignant peripheral nerve sheath tumors (MPNST), a highly aggressive type of sarcoma with a poor prognosis. Currently, there are no predictive biological markers of PN transformation to MPNST in at-risk patients. Adding to the diagnostic challenge, standard clinical cross-sectional imaging is generally not reliable in distinguishing MPNST from its benign PN precursor.

Since MPNST develop from benign PN, the ability to reliably detect malignant transformation is critical to the prompt institution of aggressive management in order to improve survival outcomes. In 2019, our teams at the NIH and Washington University began a collaborative effort to develop a liquid biopsy to improve cancer detection in NF1. We, and others, have shown that several cancer types can be monitored through plasma cell-free DNA (cfDNA) analysis and that tumor-derived cfDNA is typically shorter in size than normal cfDNA. We have also shown that sequenced MPNST tissue harbors chromosomal copy number alterations (CNAs) that are not present in PN, including in cases of MPNST transformation arising from within benign PN lesions. Additionally, we used cfDNA size analysis coupled with cfDNA ultra-low-pass whole genome sequencing (ULP-WGS) to accurately, specifically and noninvasively distinguish between MPNST and PN patients *(Szymanski and Sundby et al, PLOS Medicine)* with 91% specificity and 75% sensitivity. Current work has been aimed at the development of a Cancer Personalized Profiling by deep sequencing (CAPP-Seq) assay as well as integration of cfDNA fragmentomics to enable more sensitive detection and treatment response monitoring of MPNST cfDNA.

Our groups have been able to collect serial samples from 72 PN patients, 23 AN/ANNUBP patients, and 35 MPNST patients. To date, we have performed whole genome sequencing (WGS) of plasma cfDNA from a cohort of healthy controls ($n = 21$), patients with PN ($n = 72$), AN ($n = 23$) and pre-treatment MPNST ($n = 30$). CNA analysis with *in silico* size selection was completed and fragmentomic methods applied. Additionally, we have designed a CAPP-Seq based on the known mutational landscape of MPNST which we are in the process of validating. Current efforts are aimed at integrating all of these data to enhance the sensitivity and specificity of our classifier.

Our work demonstrates the ability to generate a large dataset through collaborative science, and our novel approach has the potential to dramatically improve patient care for both localized and metastatic disease through early cancer detection enabling earlier therapeutic intervention, improved disease monitoring, and mechanistic studies to allow the development of novel therapeutic strategies.

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The Neurofibromatosis Therapeutic Acceleration Program

Tuesday, June 27, 12:25pm – 12:50pm

Jaishri Blakeley, MD, *Johns Hopkins University*

The Neurofibromatosis Therapeutic Acceleration Program (NTAP) is a radically collaborative not-for-profit research accelerator within Johns Hopkins University. It was founded in 2012 to focus research efforts and resources on the most thoughtful and efficient development of therapies for plexiform neurofibromas (pNFs) and cutaneous neurofibromas (cNFs). NTAP accelerates development of effective therapeutics for these NF1 associated tumors via: focusing on therapeutics, fostering collaboration, open and timely sharing of results, streamlining the research process. More than 90 research initiatives ranging from discovery science to clinical trials have been supported by NTAP contributing to some of the key discoveries and therapeutic developments for pNFs and cNFs. Current areas of focus including the Biology and Therapeutic Development for Cutaneous Neurofibromas initiative, clinical trials for cNFs, the Francis S. Collins Scholars Program and an emerging effort to identify and validate biomarkers pertinent to development of therapies for pNFs and cNFs will be presented in the hopes of further expanding idea generation and collaboration.

NF1: Basic Science (The Poster Sessions are a Non-CME Activity)

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Acar	Simge	1	*UBR5,* a Chromosome 8 Gene, Regulates Cell Survival in MPNST
Adams	Evan	3	Dopamine D1 Beta-Arrestin Recruitment is Reduced in Cells Lacking Neurofibromin
Ahmari	Niousha	5	C5aR-Antagonism in Combination with MEK Inhibition Durably Alters the Tumor Micro-Environment and Reveals Ongoing Apoptosis in Plexiform Neurofibroma Cells
Allaway	Robert	7	NF Research Tools Central: A Disease-Specific Knowledgebase of Experimental Tools
Amani	Vladimir	9	Integration of Single-Nuclei RNA-Sequencing and Spatial Transcriptomics to Study the Highly Complex Tumor Microenvironment of NF1-Associated Plexiform Neurofibromas
Banerjee	Jineta	11	Maximizing Utility of Neurofibromatosis Genomic Data Through the NF Data Portal and cBioportal
Bénédetti	Hélène	13	A New Post-Translational Modification of Neurofibromin, SUMOylation, Sheds Light on the Molecular Mechanisms Involved in the Pathogenicity of Different Patient-Specific NF1 Variants
Bhunia	Minu	15	Integrative Multiomic Analysis Reveals NOTCH Signaling is Derepressed by Loss of PRC2 in Malignant Peripheral Nerve Sheath Tumors
Bozaoglu	Kiymet	17	Using Patient-Derived Stem Cells to Understand the Neurobiological Mechanisms of NF1-Related Neurodevelopmental Difficulties
Bradley	William	19	Evaluation of NF1 Interacting Proteins and Global Proteomics in Schwann Cells
Brossier	Nicole	21	Obesogenic Diet Exposure Increases Pediatric Brain Tumor Formation, Partly Through Effects of Maternal Exposure on the Tumor Cell of Origin
Burgess	Breanne	23	AXL-Dependent Signaling Promotes Cancer Phenotypes in NF-1 Associated Malignant Peripheral Nerve Sheath Tumors
Chinnapaka	Somaiah	25	Restoring Neurofibromin Function in Cutaneous Neurofibroma
Church	Cameron	27	snRNA-seq of Human Cutaneous Neurofibromas Before and After Selumetinib Treatment Indicates Decreases in Both MEK and Opioid Pathway Signaling Post-Treatment
Coulpier	Fanny	29	Platform for *In Vivo* Validation of Candidate Drugs Dedicated to Preventive and Curative Treatment of Cutaneous Neurofibromas in NF1
Currie	Graeme	31	Evaluation of the Effects of a Novel MEK Inhibitor PAS-004 in Plexiform Neurofibroma in a Pre-Clinical Mouse Model of Neurofibromatosis Type 1
De Raedt	Thomas	33	Identification of *In Vitro* and *In Vivo* Therapeutic Sensitivities for NF1 Associated High Grade Gliomas
Dischinger	Patrick	35	Impact of Post-Transcriptional Gene Regulation in Neurofibromatosis Type 1-Related Breast Cancer
Dodd	Rebecca	37	PRC2 Loss Drives MPNST Metastasis and Matrix Remodeling
Draper	Garrett	39	Development of a Novel *In Vivo*/*Ex Vivo* CRISPR-Based Mouse Model That Recapitulates Various Stages of NF1-Associated Peripheral Nerve Sheath Tumorigenesis
Durham	Desmond	41	Dopamine D2 Signaling Through Beta-Arrestin is Increased in Cells Lacking Neurofibromin
Fahrenholtz	Cale	43	Silver Nanoparticles Selectively Treat Plexiform Neurofibroma Cells Compared to Patient-Matched Schwann Cells
Fay	Christian	45	Allele-Specific Effects of NF1 Indicate Tumor Immune Response Modulates Onset of ER-Positive Mammary Tumorigenesis in Rats
Fertitta	Laura	47	Cherry Angiomas: A Specific Feature of Neurofibromatosis 1?
Funk	Margaret	49	Preliminary Study Using Deoxycholic Acid and Polidocanol Injections for the Treatment of Cutaneous Neurofibromas
Gel	Bernat	51	A Comprehensive Genomic Definition of NF1-Associated MPNSTs
Gel	Bernat	53	The pNF-ANNUBP-MPNST Progression at Single-Cell Resolution: A Resource for the NF1 Community
Grit	Jamie	55	Targeting Inflammatory Signaling in Cutaneous Neurofibromas
He	Kevin	57	MEK and TYK2 Combination Therapy in NF1-Associated Malignant Peripheral Nerve Sheath Tumors
Hung	Pei-Yu	59	MSU-42011, Alone and in Combination with Selumetinib, Reduces pERK Levels in NF1 Cancer Cells and Decreases *CCL2* Expression in Macrophages

NF1: Basic Science (The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Ioannou	Maria	61	MEK Inhibitor Mirdametinib Augments the Efficacy of Irradiation in *NF1* Deficient High-Grade Glioma Preclinical Models
Kanish	Mirchia	63	Spatial Transcriptomic Analysis of Malignant Peripheral Nerve Sheath Tumors Reveals Transcriptionally Divergent Populations Across a Gradient of Histopathologic Transformation
Kesterson	Robert	65	Recapitulation of NF1 Phenotype in a Humanized Mouse Model for c.1466A>G (p.Tyr489Cys) Patient Mutation
Larsson	Alex	67	Patient-Derived Xenograft-Based Three-Dimensional Microtissue Model of Malignant Peripheral Nerve Sheath Tumors for Precision Oncology
Lopez-Juarez	Alejandro	71	NF1 Neurological Issues: Potential Roles for Myelin in Their Life-Long Progression
Martin	Rose	73	A Cloud-Hosted Software Solution to Enable Data Exploration for Researchers of Varying Computational Skill Levels
Mattingly	Raymond	75	Transcriptomic Profiling of Cutaneous Neurofibromas from NF1 Patients
Mazuelas	Helena	77	Unbalancing cAMP and Ras/MAPK Pathways as a Potential Therapeutic Strategy for Cutaneous Neurofibromas
Moye	Stefanie	79	Econazole Selectively Induces Cell Death in *NF1*-Homozygous Mutant Tumor Cells
Nikrad	Julia	81	Identification of Synthetic Lethality Targets Through Genome-Wide CRISPR Screen in *NF1*- and *NF1*/*SUZ12*-Deficient Human Schwann Cells and MPNST Cell Lines
Onfroy	Audrey	83	NF1 Genetic Background in the Tumor Microenvironment Govern Transcriptomic Landscape of Tumor Cells in Malignant Peripheral Nerve Sheath Tumors
Panken	Daniel	85	Mutant iPSC-Derived Schwann Lineage Cells as a Novel Drug Discovery Platform for Peripheral Nerve Sheath Tumors
Paudel	Siddhi	87	Myelolytic Treatments Can Augment the Therapeutic Benefit of Oncolytic Virus in Malignant Peripheral Nerve Sheath Tumors by Modulating the Tumor Microenvironment
Perrin	Simon	89	Schwann Cells and Skeletal Stem/Progenitor Cells Drive Fibrosis and Congenital Pseudarthrosis of the Tibia in NF1
Plante	Camille	91	Revisiting the *cisNf1*$^{+/-}$*p53*$^{+/-}$ Mice Model
Pulh	Pernelle	93	Decipher the Mechanisms Driving Cutaneous Neurofibromas Development in a Mouse Model of Neurofibromatosis Type I
Pundavela	Jay	95	Contribution of Sterile Inflammation Through STING and T Cells to Plexiform Neurofibroma Initiation and Growth
Rambo	Micah	97	The Impact of Neurofibromatosis Type 1 on Tumor Biomechanics
Raut	Namrata	99	Schwann Cell Calcium and Growth Factor Signaling Modulates Pain in NF1
Richards	Kyle	101	Towards the Identification of the HLA Class I Immunopeptidome of Malignant Peripheral Nerve Sheath Tumors via Mass Spectrometry
Robinson	J. Elliott	103	Molecular and Circuit Mechanisms of Visual Hypersensitivity in Neurofibromatosis Type 1 Model Mice
Sammons	Josh	105	Nonsense Suppression is a Viable Approach for Restoring Full Length Neurofibromin Protein and Function
Scantamburlo	Francesca	107	Taming the Metabolism of Tumor Associated Macrophages to Fight NF1-Related Tumors
Somatilaka	Nipunika	109	STING Inflames NF1 Malignancies for Immunotherapy
Stehn	Christopher	111	Characterizing the Super Enhancer Landscape of PRC2 Inactivation in MPNST Development
Stillwell	Alexis	113	"Mild" Neurofibromatosis Type I Patient Mutation p.M992del in Knock-In Mouse Model Suggests Novel Roles in Skeletal Development
Sundararaghavan	Harini	115	NF Schwann Cell Behavior on Electrically Conductive Neurite Mimics
Suppiah	Suganth	117	SHH Pathway Activation Drives a Neural Crest-Like State in MPNSTs
Tomkinson	Jenna	119	Cell Painting Distinguishes Isogenic Schwann Cells with Different *NF1* Genotypes
Uriarte-Arrazola	Itziar	121	Functional Impact of *NF1, CDKN2A* and *SUZ12* Loss in an iPSC-Based MPNST Model System

NF1: Basic Science (The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Vasudevan	Harish	123	A Functional Genomic Ras GAP Atlas Reveals Distinct Neurofibromin Functions and Druggable Dependencies in the Peripheral Nervous System
Voigt	Ellen	125	New Models to Define Cooperating Events That Drive PNF Transformation into MPNST
Warrier	Akshaya	127	Multiomic Analysis of Mouse and Human Malignant Peripheral Nerve Sheath Tumors (MPNSTs) Identifies G6PD Dysregulation as a Metabolic Vulnerability
Williams	Kyle	129	Study of Nutraceutical Intervention with High Phenolic Extra Virgin Olive Oil and Curcumin for Neurofibromatosis Type 1
Wu	Jianqiang	131	Combining SOS1 and MEK Inhibitors in a Murine Model of Plexiform Neurofibroma Results in Tumor Shrinkage
Wu	Natalie Lai Man	133	Single-Cell Analysis Identifies Clinically Relevant Mesenchymal Stem-Like Cells in MPNST and Distinct Tumor Ecosystem in Benign Schwann Cell-Derived Tumors
Zamora	Matthew	135	Drug Response Evaluation for NF1 Tumors Using High Throughput Screening Analysis and DREA Web Tool
Zhang	Lindy	137	Mechanisms of Immune Escape in NF1-Associated Peripheral Nerve Sheath Tumors
Zhu	Iowis	139	Malignant Peripheral Nerve Sheath Tumors Demonstrate Distinct Patterns of Radiation Response Through Activation of Immunosuppressive Pathways

NF1: Basic Science

UBR5, a Chromosome 8 Gene, Regulates Cell Survival in MPNST

Simge Acar, MD, *Washington University in St. Louis School of Medicine, Division of Medical Oncology*

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant cancer predisposition syndrome that increases the risk of developing plexiform neurofibroma (PN), a benign tumor that occurs in 30-50% of patients with NF1. Malignant peripheral nerve sheath tumors (MPNST) are a rare but aggressive type of cancer that can arise from PN, and they are a leading cause of death for NF1 patients. Currently, surgical resection is the only curative treatment for MPNST, but the tumors often recur, and many are not diagnosed until they have already metastasized. Therefore, more effective therapies are urgently needed, and a deeper understanding of the molecular drivers of MPNST development is required. Previous studies have shown that chromosome 8 gain is present in >80% of MPNST, and this chromosome contains multiple cancer-related genes. One of these genes, *UBR5*, an E3 ubiquitin ligase, is highly expressed in MPNST compared to PN. In this study, we aimed to investigate the role of *UBR5* in MPNST pathogenesis.

Methods: To investigate the role of *UBR5* in MPNST tumorigenesis, *UBR5* knockdown (KD) human (JH-2-002) and murine (JW23.3) MPNST cells were generated using shRNA and tumor proliferation and survival were evaluated *in vitro* and *in vivo*. Bulk RNAseq analysis and WES protein analysis were performed to identify altered gene and protein expression in sh*UBR5* KD MPNST cells.

Results: We demonstrated that *UBR5* KD led to decreased proliferation and increased cell death in both JH-2-002 and JW23.3 MPNST cell lines. In addition, decreased tumor growth was observed *in vivo* in both cell lines. RNAseq analysis revealed increased expression of genes involved in apoptosis in *UBR5* KD cells compared to control cells. Activation of apoptotic pathways was confirmed by cleaved caspase 3 and cleaved PARP-1 expression levels in *UBR5* KD cells in both cell lines. Overall, these findings support that *UBR5* is a Chr8 gene that promotes cell survival in MPNST progression, and targeting the *UBR5* pathway may be a promising therapeutic strategy for MPNST.

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Dopamine D1 Beta-Arrestin Recruitment is Reduced in Cells Lacking Neurofibromin

Evan Adams, BS, *High Point University, Department of Basic Pharmaceutical Sciences, High Point, NC*

Purpose: To test the hypothesis that normal D1 receptor signal transduction is dependent on the presence of neurofibromin.

Methods: HEK293T cells were treated with CRISPR to remove neurofibromin and paired with WT controls. D1 receptor fused to nanoluc or luc8 were transfected with mini-G-proteins (Gs) to assess G-protein recruitment to the receptor, after dosing with dopamine. A repurposed PRESTO-Tango assay was used to assess arrestin recruitment as well as D2-Luc8—Arrestin-GFP BRET. Cells were treated for 45 minutes to maximize beta-arrestin signaling and then western blot was used to assess known downstream pathways and the effects of NF1 loss.

Results: We found that G-protein recruitment was not affected by loss of NF1 in this assay. However, we found that beta-arrestin recruitment was significantly reduced after removal of NF1. The reduced recruitment of beta-arrestin2 was seen in both the transcriptional reporter assay and BRET based assay. When downstream pathways commonly associated with beta-arrestin2 signal transduction were analyzed by western blot we found that phosphorylation of AKT was increased at both the Thr308 and Ser473 residues in the NF1 null cells, indicating an increased level of AKT activity.

Conclusions: We found that loss of neurofibromin did not impact G-protein recruitment to the DRD1 receptor but did significantly affect arrestin recruitment. Beta-arrestin2 recruitment to the D1 receptor was decreased in an NF1 deficient cell model which resulted in different downstream effects than what occurs in the WT setting. These data directly implicate signaling at the receptor level for future investigations into normalizing dopamine signaling and treating some of the cognitive issues observed in NF1 patients.

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C5aR-Antagonism in Combination with MEK Inhibition Durably Alters the Tumor Micro-Environment and Reveals Ongoing Apoptosis in Plexiform Neurofibroma Cells

Niousha Ahmari, PhD, *Cincinnati Children's Hospital*

The formation and growth of tumors is dependent on the interplay between tumor cells and the surrounding tumor microenvironment (TME). The plexiform neurofibroma (pNF) microenvironment is marked by chronic inflammation, myeloid cell expansion, and remodeling of local and systemic immune compartments. Beyond its regulatory role in controlling innate immunity, complement activation has been implicated as a regulator of inflammation in the tumor microenvironment. Using data from human patients and murine models, we discovered an increase in the C5a-C5a receptor (C5aR) system in pNF as characterized by an increase in C5aR1 expressing macrophages, the predominant cell in pNF tumors, which is not normalized by MEK inhibition. We tested the hypothesis that C5aR modulates inflammatory responses in pNF. We found that genetic deletion of C5aR1 suppresses lipopolysaccharide induced proliferation and apoptosis of ex-vivo treated tumor cells. Moreover, both genetic reduction of C5aR1 and therapeutic reduction of C5aR1 activity induced cell death in tumor macrophages and enhanced the engulfment of dying Schwann cells by macrophages. MEK inhibitors shrink most pNFs, but the effect is not durable, and continued administration of MEKi is necessary to prevent tumor regrowth. We found that inhibitors of the MEK1/2 kinases reduce total number of immune cells in pNF, but at the same time enhance C5aR1 protein at the macrophage cell surface suggesting that the combination of MEK inhibitor PD0325901 and the C5aR1/2 inhibitor A8Δ71-73 might alter the effects than MEKi alone. To test this idea, we treated mice for 1 month, and then maintained mice off therapy for one month. Tumors regrew in all groups, but only mice treated with a combination of MEK and C5aR1/2 inhibitors showed altered tissue cellular architecture, expansion of dendritic cells, and increased macrophage MHCII expression. We conclude that C5aRA in combination with MEK inhibition is tolerable and causes durable immunosuppressive effects on the neurofibroma microenvironment.

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NF Research Tools Central: A Disease-Specific Knowledgebase of Experimental Tools

Robert Allaway, PhD, *Sage Bionetworks*

Purpose: This study aims to address the challenges researchers face in identifying available and emerging research tools in the field of neurofibromatosis (NF) research. Our objective is to create an open-access, user-friendly database, designed to facilitate the discovery and utilization of relevant research tools within the NF research community.

Methods: We developed NF Research Tools Central, which encompasses a broad range of NF-related research tools, including animal models, cell lines, genetic reagents, antibodies, and biobanks. The database provides detailed metadata for each tool, such as the name, developer, and tool-specific information, including the type of tumor the model represents. In addition, we incorporated observational data directly contributed by the research community.

Results: NF Research Tools Central and its accompanying web portal (tools.nf.synapse.org) enable users to search, filter, and explore from over 1000 available tools while contributing information about their reliability, biology, usage, and other observations. Expanding the project could potentially assist researchers in discovering and repurposing valuable tools from biologically similar syndromes or pinpointing areas where new tools are needed.

Conclusions: The NF Research Tools Central database represents a valuable resource for the neurofibromatosis community, promoting the discovery, understanding, and utilization of research tools in order to accelerate advancements in the field.

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Funding: This project was funded by the Gilbert Family Foundation. The NF Data Portal, which hosts NF Research Tools Central, is funded by the Gilbert Family Foundation, The Neurofibromatosis Therapeutic Acceleration Program, and the Children's Tumor Foundation.

Integration of Single-Nuclei RNA-Sequencing and Spatial Transcriptomics to Study the Highly Complex Tumor Microenvironment of NF1-Associated Plexiform Neurofibromas

Vladimir Amani, BSc, *University of Colorado Anschutz Medical Campus*

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

Due to the cohesive cellularity of PN, single-cell RNA-sequencing is difficult and may result in a loss of detection of critical cellular subpopulations. Single-nuclei RNA-sequencing (snRNA-seq) is a cutting-edge technology that can be applied to fibrous and bulk frozen tissues, such as NF1-associated PN. This technology was applied retrospectively to 9 frozen PN, 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.

Our snRNA-seq analysis definitively charted the heterogeneous cellular subpopulations in the PN TME, with the predominant fraction being fibroblast-like cells. PN have a remarkable amount of inter-sample homogeneity regarding cellular subpopulation proportions despite being resected from a variety of anatomical locations. PN ST samples showed well-defined, juxtaposed nerve fascicles surrounded by a relatively well-demarcated perineurial fibrous capsule. Between the nerve fascicles is a more prototypical neurofibromatous proliferation. ST analysis identified distinct cellular subpopulations which were annotated using snRNA-seq data that correlated with histological features. Interestingly, there seems to be close interaction between non-myelinating Schwann cells (NMSC) and fibroblasts 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/NRGLN1) receptor-ligand cross-talk was predicted between NMSC and fibroblast subpopulations, respectively. Elevated NRXN1 expression is seen in PN-associated NMSC compared to normal NMSC. This 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 NMSC cells of origin and surrounding cancer associated fibroblasts, potentially driving disease progression.

SnRNA-seq integrated with spatial transcriptomics advances our understanding of the complex cellular heterogeneity of PN. These data identify potential novel communication pathways that may drive disease progression, a finding that could provide translational therapy options for patients with these devastating tumors of childhood and early adulthood.

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Maximizing Utility of Neurofibromatosis Genomic Data Through the NF Data Portal and cBioportal

Jineta Banerjee, PhD, *Sage Bionetworks*

Purpose: Research in rare diseases 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 (NFOSI) has processed genomic and transcriptomic data from the Johns Hopkins NF1 Biospecimen Repository (JHU Biobank) using standardized publicly available pipelines and released on the NF Data Portal and cBioPortal.

Methods: Raw whole exome sequencing (WES) and RNA sequencing (RNA-seq) data generated from human NF1 tumor samples by the JHU Biobank were shared on the NF Data Portal. NF-OSI used Nextflow workflows from the nf-core community (nf-core/sarek v2.7 and nf-core/rnaseq v3.7) to align the data to the most recent human reference genome (GRCh38). Germline and somatic genomic variant calls as well as RNA-seq counts were generated from the Biobank raw data. After quality checks and sample annotations, the processed files were made available on the NF Data Portal. The somatic variant calls from published samples were then formatted and submitted to cBioPortal.

Results: With this effort, NF-OSI released its first consistently processed genomics and transcriptomics dataset on the NF Data Portal, making it easily and rapidly reusable (https://tinyurl.com/biobankdata). 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.

Conclusion: This initiative is a significant step towards large-scale processing of NF data from diverse sources enabling researchers to use harmonized datasets for computational analysis. Continuing the effort, more datasets from multiple additional studies, including 3 WES, 1 whole genome, and 10 RNA-seq datasets, are being processed and will be made available on the NF Data Portal in the near future. A subset of these datasets will be available on cBioPortal for researchers and clinicians to explore and compare with other cancer samples. Overall, this effort empowers the NF research community to explore the genomic profiles of NF samples from multiple studies without extensive computational resources.

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A New Post-Translational Modification of Neurofibromin, SUMOylation, Sheds Light on the Molecular Mechanisms Involved in the Pathogenicity of Different Patient-Specific NF1 Variants

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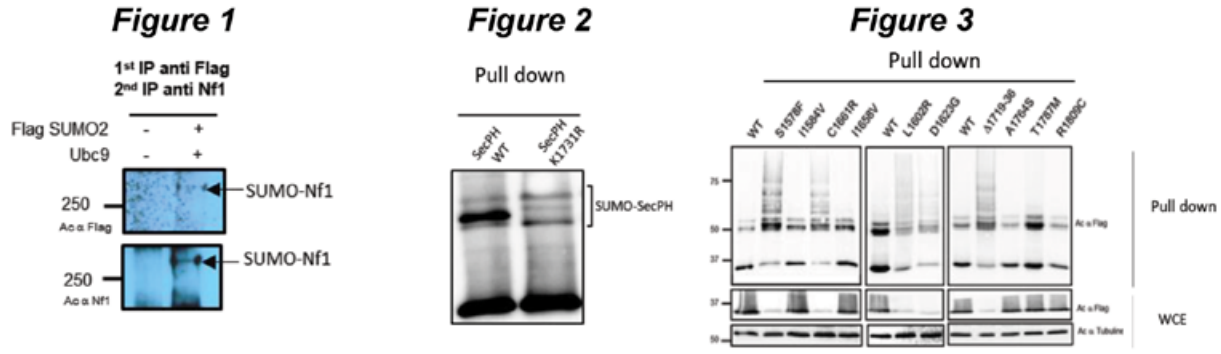
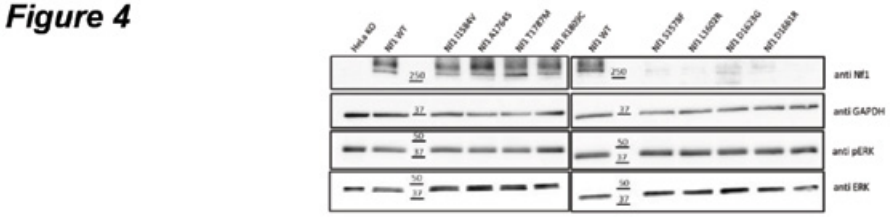
Neurofibromin (Nf1), the protein responsible for neurofibromatosis type1 disease, is a large, multi-domain protein encoded by the tumor-suppressor gene *NF1*. Nf1 is thought to be multifunctional and its best described function is its Ras-GTPase activity, carried out by its GAP-related domain (GRD). Nf1 is also connected to diverse signalling pathways through its SecPH domain which interacts with lipids and different protein partners. Nf1 has been identified as highly regulated by post-translational modifications (PTM), particularly by phosphorylation and ubiquitylation.

In a previous work our team showed that Nf1 colocalizes with PML (promyelocytic leukemia) nuclear bodies (PML-NBs) in the nucleus which are SUMOylation hotspots. These observations suggested that Nf1 might be modified by SUMOylation.

In this work, we demonstrate that Nf1 isoform 1 and SecPH are substrates of the SUMO pathway (**Fig. 1:** Endogenous Nf1 is SUMOylated and **Fig. 2:** left, SUMOylation profile of SecPH)



Study of this SUMOylation profile in ten patient-specific NF1 variants localized in SecPH allowed us to define unsuspected classes of variants. One variant, K1731R affects the SUMOylation of a highly conserved and surface exposed residue, demonstrating the importance of SecPH SUMOylation in a yet unidentified function of Nf1 (**Fig. 2,** right). The atypical SecPH SUMOylation pattern of five other variants (**Fig. 3,** *S1578F, C1661R, L1602R, D1623G, Δ1719-36*) is associated to SecPH folding defects, increased ubiquitylation and proteasomal degradation. This unfolding and unstability extends to the entire NF1 variants (**Fig. 4** Characterization of different pathogenic Nf1 variants) unraveling the molecular mechanism responsible of pathogenicity for this class of mutants.



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Integrative Multiomic Analysis Reveals NOTCH Signaling is Derepressed by Loss of PRC2 in Malignant Peripheral Nerve Sheath Tumors

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Neurofibromatosis Type 1 syndrome (NF1) is a cancer predisposition syndrome caused by inheritance of one loss of function allele of the *NF1* gene. NF1 patients can develop malignant peripheral nerve sheath tumors (MPNST). MPNSTs develop after somatic loss of the wild-type *NF1* allele, resulting in increased Ras-GTP activated signaling[1]. This malignant transformation is still not completely understood, but loss of *TP53* or *CDKN2A/2B* function and the polycomb repressor complex 2 (PRC2) are common events during the transition to MPNST[2–4]. 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 loss of PRC2 has direct and indirect effects on gene expression resulting in MPNSTs. PRC2 loss may result in altered topologically associated domains, which can affect access of promoters by distal enhancers. Altered gene expression leads to deregulation of cell differentiation and proliferation controls, promoting the transition to MPNSTs. **The purpose of this study is to identify epigenomic vulnerabilities and potential drivers of MPNSTs using multi-omics to elucidate more effective treatments. We have engineered *NF1*-deficient human Schwann cells 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 H3K27 acetylation. SUZ12-deficient cells also become hypersensitive to histone deacetylase inhibitors. **RNA sequencing has revealed many differentially expressed genes when *SUZ12* and *NF1* are lost in our engineered cell lines. We identified 149 differentially expressed genes that are common to MPNSTs and engineered cell lines. 824 genes are common between our engineered cell lines where 686 of these are derepressed when *SUZ12* or *EED* are lost with *NF1*.** We also identified an increase in differentially expressed genes when PRC2 is lost with *NF1* versus *NF1* loss alone. Comparing these to genes expressed in MPNST patient samples, we have identified potential drivers of MPNST generation. Pathway enrichment analysis on differentially expressed genes indicates many upregulated cancer related pathways when PRC2 is lost. **We found NOTCH and Sonic Hedgehog signaling are common to all comparisons. NOTCH signaling has been implicated in Schwann cell development. NOTCH pathway members may be worthy candidates for drug targeting.** We confirmed these findings with Western blot, transient cell line models, and drug assays. Proteomics data will also be compared to the findings.

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Using Patient-Derived Stem Cells to Understand the Neurobiological Mechanisms of NF1-Related Neurodevelopmental Difficulties

Kiymet Bozaoglu, PhD, *Murdoch Children's Research Institute, University of Melbourne, Parkville, Australia*

Neurodevelopmental difficulties such as attention deficit hyperactivity disorder and autism spectrum disorder (ASD) are common in children with neurofibromatosis type 1 (NF1). Over the past 20 years, various genetically engineered Nf1 mouse models have been generated to investigate the molecular and neurobiological mechanisms disrupted by variants in *NF1.* Despite promising data from these models, translational precision-based clinical trials in humans have not identified a successful treatment and clinical practice remains focused on general symptom management, rather than targeting NF1-specific pathways. There is a need to better understand NF1-related drivers of neurodevelopmental symptoms in children with NF1 and identify new therapeutic candidates.

As part of a longitudinal study, we have recruited over 180 children and adolescents with NF1 who have undergone extensive clinical phenotyping to classify their neurodevelopment profile. In addition, we have performed genetic studies to identify the mutation/s that may be contributing to the disorder. We have selected 6 patients with NF1 and 6 patients with NF1 and co-occurring ASD to assess whether there are any functional attributes that differ between the 2 groups. Peripheral blood mononuclear cells from these 12 individuals have undergone stem cell reprograming and gene editing using CRISPR Cas9 technology. Edited samples serve as an isogenic control. Patient-derived neuronal stem cell models using the lentiviral based NGN2 induction protocol have been developed to model structural and functional deficits that may occur in these cell lines. With this model we will identify disease causing mechanisms using live cell imaging, histology, neural activity assays and biochemical assays.

These data will provide us with a better understanding of how *NF1* variants affect brain development and function and lead to neurodevelopmental symptoms in children with NF1. Validation of these preclinical models of NF1 will progress our understanding of the pathological processes in humans, advance future clinical trial design, and ultimately, the clinical care of our patients.

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Evaluation of NF1 Interacting Proteins and Global Proteomics in Schwann Cells

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We investigated proteomes from immortalized human Schwann cells (ipNF97.4) with (+/+) and without (-/-) neurofibromin to evaluate potential targets in NF1 deficient cells. We identified 1,745 total proteins common to -/- and +/+ cells, and we find 72 proteins significantly up-regulated and 76 proteins significantly down-regulated in NF1 deficient cells. Ingenuity Pathway Analysis (IPA) identified multiple altered pathways including "oxidative phosphorylation, "fatty acid alpha and beta oxidation," and "mitochondrial dysfunction". We identified potential biomarkers from altered pathways including decreased proteins in -/- compared to +/+ cells (NADH dehydrogenase, acyl-CoA synthetase long chain family member 1, and Pyruvate dehydrogenase E1 component subunit beta) and increased proteins in -/- compared to +/+ cells (Calpain-2 catalytic subunit and CREB-binding protein). We then evaluated how restoration of NF1 expression would impact the proteome by stably transfecting NF1 -/- cells with a strep-tagged WT or variant NF1 cDNA, and again analyzed the global proteome. We utilized variants with known genotype-phenotype associations; R1809C and delM992 associated with mild phenotypes and R1276Q and G848R associated with severe phenotypes. Principle component analysis indicates that cells expressing exogenous NF1 cDNAs are most alike while cells that do not express cDNA cluster together; this is despite differences in NF1 genotype. Upon IPA analysis of WT cDNA and mutant cDNA transfected cells we identified metabolic differences pertaining to "oxidative phosphorylation," "mitochondrial dysfunction"," fatty acid oxidation," "TCA cycle," and "glycolysis" in the mutant cDNA expressing cells compared to WT cDNA expressing cells. We then utilized the strep-tag encoded into the WT and variant cDNAs to affinity purify neurofibromin and any additional protein-protein interactors (PPIs). The PPIs were identified through affinity purification mass spectrometry. To eliminate non-specific interactions, the data sets from NF1 -/- Schwann cells were compared to a prior dataset isolated from HEK293 NF1-/- cells stably transfected with the same strep-tagged mNF1 cDNA. We identified 28 PPIs present in both sets. Finally, we examined differences in affinity to WT NF1 compared to the four NF1 variants in the Schwann cells. We observed 40S ribosomal protein S28 purifies significantly less with the delM992 variant compared to WT NF1, and General vesicular transport factor p115 purifies significantly more with the delM992 NF1 variant. We observed 3 proteins that purify more with WT NF1 compared to the R1809C mutation (Eukaryotic translation initiation factor 2 subunit 1, Nicotinamide phosphoribosyltransferase, and Zinc finger CCCH-type antiviral protein 1), and 2 proteins that purify more with the R1809C NF1 mutation compared to WT NF1 (GTP-binding protein SAR1a and Small glutamine-rich tetratricopeptide repeat-containing protein alpha). Thus, loss of NF1 can alter the overall proteome of Schwann cells particularly in relation to oxidative phosphorylation and mitochondrial dysfunction. These proteins and pathways may serve as biomarkers for NF1 deficiency. Further, NF1 variants alter the proteins that potentially bind to NF1 which may alter downstream signaling pathways.

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Obesogenic Diet Exposure Increases Pediatric Brain Tumor Formation, Partly Through Effects of Maternal Exposure on the Tumor Cell of Origin

Nicole M. Brossier, MD, PhD, *Washington University in St. Louis*

Abstract: Pediatric low-grade glioma incidence has been rising in the U.S. over the last 20 years, concurrent with a rising rate of obesity in both the adult and pediatric populations. Recently, children of obese mothers have been demonstrated to have increased rates of several tumors, 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. High-fat diet exposure has been previously demonstrated to promote proliferation and glial differentiation of neuroglial progenitor cells (NPCs) around the third ventricular zone (TVZ), suggesting that *in utero* exposure to an obesogenic diet might affect the formation of pediatric tumors derived from these cells, such as neurofibromatosis type 1 (NF1)-related optic pathway glioma (OPG). Based on these data, we hypothesized that maternal obesogenic exposure would increase *Nf1*-OPG formation through intrinsic effects on the tumor cell of origin.

Methods: We utilized a series of *Nf1*-heterozygous and *Nf1*-OPG mouse models for these experiments. We exposed dams and offspring to an obesogenic high-fat, high-sucrose diet to mimic dietary conditions prevalent in the U.S., with control chow-exposed animals as a comparison. Fetal brains from mothers exposed to different dietary conditions were analysed at E19. For tumor development studies, offspring were continued on their respective maternal diets and optic nerves analyzed for tumor formation at 6w-3mo.

Results: We demonstrated that progeny from obese dams exposed to an obesogenic diet demonstrated increased proliferation and glial differentiation of WT and *Nf1*-heterozygous TVZ NPCs *in vivo*. We found that this effect was due to diet rather than weight, as progeny of non-obese dams exposed to this diet only during gestation demonstrated a similar phenotype, and offspring of overweight dams switched to control chow at mating did not. We then assessed how obesogenic diet exposure affected tumor formation. We determined that this exposure increases glioma penetrance in two low-penetrance models of *Nf1*-OPG. Finally, we demonstrated that obesogenic diet exposure resulted in earlier tumor onset in a high-penetrance *Nf1*-OPG model.

Conclusion: Taken together, these findings demonstrate that obesogenic diet exposure increases pediatric glioma formation in this region of the brain, and suggest that this might occur in part through effects on the tumor cell of origin while still *in utero.* It may also have implications for clinical prognosis, as earlier age of tumor onset has been negatively associated with visual outcomes in NF1-OPG.

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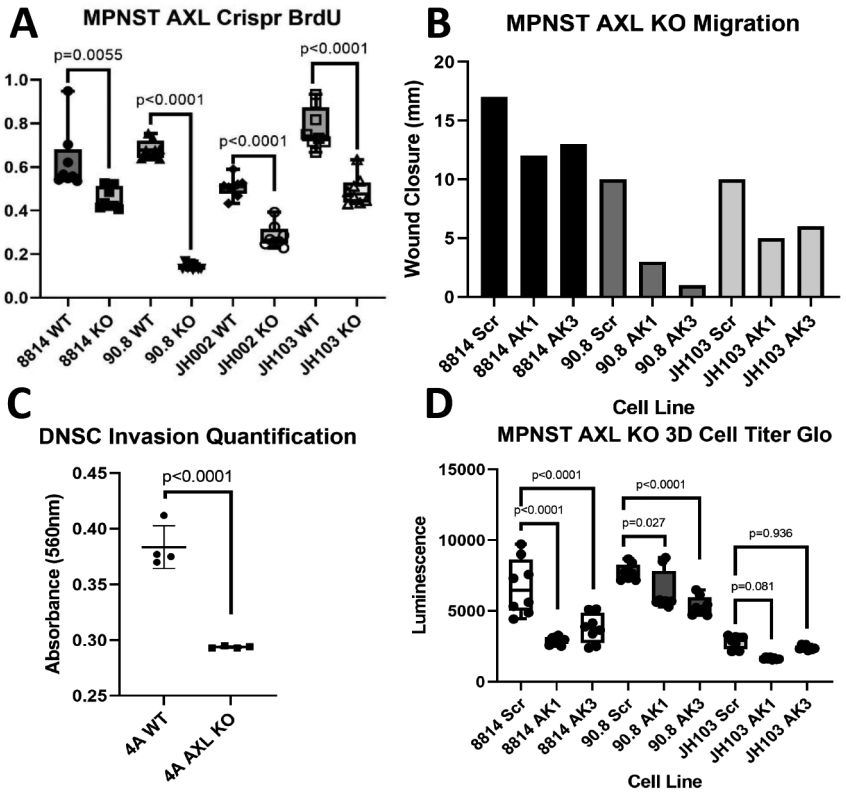
AXL-Dependent Signaling Promotes Cancer Phenotypes in NF-1 Associated Malignant Peripheral Nerve Sheath Tumors

Breanne Burgess, *Indiana University School of Medicine*

Emerging data implicates a critical role of the AXL receptor tyrosine kinase in NF1 tumorigenesis. Cabozantinib, a multi-RTK inhibitor that targets AXL, demonstrated activity in murine plexiform neurofibroma (PNF) and adults with symptomatic, inoperable PNF in a multi-institutional phase 2 trial[1]. Kinome analysis by multiplexed inhibitor bead coupled with mass spectrometry (MIB/MS) revealed AXL to be among the top kinases inhibited by cabozantinib in murine PNF. Cabozantinib delayed the malignant transformation of a subset of PNFs and premalignant atypical neurofibromas (ANFs) that arise following engraftment of primary *Nf1-Cdkn2a* mutant Schwann cell precursors[2, 3] into the sciatic nerve of recipient mice *in vivo.* Immunohistochemistry performed on a cohort of 35 human NF1 tumors revealed increased staining of AXL in PNF, ANF, and MPNST versus normal sciatic nerve. Further, there is an increased ratio of active, phosphorylated AXL to total AXL in ANF and PNF versus PNF. Informed by these data, we hypothesize that AXL-dependent signaling modulates cancer phenotypes in NF1-associated MPNST tumorigenesis. To test this hypothesis, we engineered human MPNST cell lines with CRISPR-Cas9 guided knockout of AXL (AKO) or scramble vector (WT). Across four MPNST cell lines, AKO showed decreased proliferation compared to WT via Brdu ELISA assay (Figure A). Forty-eight hours after introduction of a wound to WT or AKO monolayers, AKO cells showed decreased migratory capacity compared to WT (Figure B). Invasion into a basement membrane was reduced in both human AKO cells and in *Nf1-Cdkn2a* deficient Schwann cell precursors engineered with genetic AXL (Axl) loss (Figure C). Finally, AKO cells and *Nf1-Cdkn2a-Axl* deficient Schwann cells formed fewer viable 3D spheroids in serum-free, ultra-low attachment media conditions compared to WT controls, quantified by 3D CellTiter Glo (Figure D). These data provide evidence that AXL-dependent signaling modulates proliferative, migrative, invasive, and cancer stemness phenotypes in *in vitro* models of NF1-associated MPNST. These studies address the need to describe mechanisms of tumorigenesis in MPNST and identify tractable targets to treat this aggressive form of sarcoma.



Additional Authors: Angus, S. P., Davis, C., Mang, H. Bessler, W. Lu, Q. Jiang, L. Li, X., Rhodes, S. R., Clapp, D. W.

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Restoring Neurofibromin Function in Cutaneous Neurofibroma

Somaiah Chinnapaka, PhD, *Department of Dermatology, University of Texas Southwestern Medical Center*

Purpose: Neurofibromatosis is one of the most common autosomal dominant disorders, affecting approximately 1 in 3,500 live births. NF1 is caused by loss/mutation of the tumor suppressor gene neurofibromin (*NF1*), which encodes a RAS-GAP. Most patients with NF1 develop cutaneous neurofibromas (cNFs), benign tumors in the dermis that are often reported as the most burdensome NF1 symptom. Unfortunately, there are limited treatment options available for cNF and new therapeutic approaches are sorely needed. One possible approach would be to restore neurofibromin activity using gene therapy. As neurofibromin is a large protein, we are testing this approach using adeno-associated virus (AAV) delivery of the RAS-GAP domain of *NF1*.

Methods: We first tested AAV serotype transduction efficiency to target neurofibroma cells of origin. We used a) In vitro: *Nf1*-null dorsal root ganglion/nerve root neurosphere cells (DNSCs), skin neurosphere cells from *Hoxb7-Cre;Nf1$^{f/f}$* mice (a murine neurofibroma model), and human cNF; b) In vivo: *Hoxb7-Cre;Nf1$^{f/f}$;tdTomato* (pre-cNF and post cNF development via intra-dermal injection). We analyzed transduction efficiency by measuring AAV-GFP expression with flow cytometry and real-time PCR. Confocal microscopy was used to determine the colocalization of GFP (AAV) with tdTomato (cells of tumor origin in *Hoxb7-Cre;Nf1$^{f/f}$* mice). We then tested AAVDJ-NF1-GRD-GFP for transduction efficiency and effect on tumor growth in two neurofibroma models: our *Hoxb7-Cre;Nf1$^{f/f}$;tdTomato* mice and immunocompromised allograft mice.

Results: Flow cytometry and real-time PCR analysis revealed that the AAVDJ-GFP serotype exhibited a higher percentage of transduction compared to other serotypes in *Nf1*-null DNSCs, skin neurosphere cells, and human cNF cells. AAVDJ-GFP also co-localized with tumor cells of origin in *Hoxb7-Cre;Nf1$^{f/f}$;tdTomato* mice. AAVDJ-NF1-GRD-GFP injected into sciatic nerve tumors generated by implantation of *Nf1*-null DNSCs demonstrated colocalization of GFP with tdTomato+ *Nf1*-null cells, as well as decreased tumor cell number and lower phospho-ERK and collagen 1 expression compared to AAVDJ-GFP control. In a different set of experiments, we found that implantation of DNSCs with doxycycline-inducible *Nf1* shRNA into sciatic nerves increased Schwann cell number, phospho-ERK levels, and extracellular matrix deposition when the mice were administered doxycycline water. We will next test whether restoring *Nf1* expression by turning off *Nf1* knockdown can rescue the tumorigenic phenotype.

Conclusion: We found that: 1) AAV can effectively target neurofibroma cells of origin both in vitro and in vivo; and 2) AAV-NF1-GRD can attenuate tumor progression in a sciatic nerve transplantation model of neurofibroma.

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snRNA-seq of Human Cutaneous Neurofibromas Before and After Selumetinib Treatment Indicates Decreases in Both MEK and Opioid Pathway Signaling Post-Treatment

Cameron Church, *The University of Alabama at Birmingham*

Neurofibromatosis Type 1 (NF1) is a neurogenetic disorder characterized by a variety of clinical features, including the development of benign skin lesions called cutaneous neurofibromas (cNFs) that occur due to biallelic loss of NF1 expression in Schwann cell lineages. These cNFs significantly impact quality of life. The only approved therapies for NF1 involve downstream inactivation of Ras signaling through the MEK inhibitor selumetinib. The purpose of this study is to analyze the transcriptome of cells in the cNF tumor microenvironment before and after selumetinib treatment to identify cell types that contribute to response and potentially reveal biomarkers or novel drug targets that would allow for enhanced patient care. For this study, we obtained biopsy sets of tumors both pre- and post- selumetinib treatment from the same individual as part of a clinical trial of selumetinib treatment of cutaneous neurofibromas. We were able to collect sets from four separate individuals (4 treated and 4 untreated tumors; including 2 males and 2 females). Biopsies were snap frozen and stored for later use. Tumors were then processed for snRNA sequencing by isolating nuclei and utilization of 10X Genomics reagents. After quality filtering, we sequenced a total of 5,844 nuclei and identified 30,442 genes in the untreated group and sequenced 5,701 nuclei and identified 30,127 genes in the selumetinib treated group. In efforts to understand the composition of the microenvironment, we identified populations of Schwann cells, fibroblasts, pericytes, myeloid cells, melanocytes, keratinocytes, and two populations of endothelial cells and calculated their proportions in both groups. While we anticipated that cell proportions might change, we did not identify any one cell population that changed significantly, likely due to an inherent level of variability between tumors. Further, we anticipated that selumetinib might specifically target Schwann cells for apoptosis; however, instead of decreasing in proportion following treatment, Schwann cells doubled in proportion. Further, myeloid cells tripled in proportions after treatment. We also evaluated differential gene expression based on drug treatment. We identified the top differentially regulated genes in each cell type. As Schwann cells are thought to be the most clinically relevant, we identified the top upregulated (MGAT4C, PCSK2, NKAIN2, LSAMP, ADAM23, ADAMTSL1, CADM2, GRIK2, AC092691.1, OPCML) and downregulated genes (LOXL3, ITGB8, F13A1, KCNMA1, COL14A1, ITIH5, IL1RAP, PLCG2, PMEPA1, KCNS3). Ingenuity Pathway Analysis on the differentially expressed genes identified pathways that differ after treatment. As anticipated, we identified a significant decrease in ERK/MAPK signaling in all cells including Schwann cells but most specifically in myeloid cells. We noted an upward trend in autophagy signaling in fibroblasts, pericytes, and myeloid cells, suggesting cell death after treatment. Interestingly, there was a significant decrease in opioid signaling in myeloid and endothelial cells. We also see a downward trend in opioid signaling in Schwann cells and fibroblasts. Decreases in opioid signaling may provide clues as to why individuals with plexiform NFs experience clinically significant decreases in pain post-selumetinib treatment and may provide new pain targets for patients.

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Platform for *In Vivo* Validation of Candidate Drugs Dedicated to Preventive and Curative Treatment of Cutaneous Neurofibromas in NF1

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Cutaneous neurofibromas (cNF) are benign nerve sheath tumors emerging around puberty in patients with neurofibromatosis type I (NF1). Despite their high penetrance and often large number, cNFs pathophysiology is only partly elucidated and no effective therapeutics exist for their prevention and cure. This is mostly due to the lack of animal models recapitulating development of cNFs. Our team has developed genetically engineered mouse model *(Nf1-KO)* simultaneously targeting *Nf1* loss and permanent expression of fluorescent reporter Tomato into boundary cap cells, whose derivatives correspond to Schwann cells (SC) in the nerve roots and nerve endings from skin. *Nf1-KO* mice develop bona fide cNFs from one-year.

Temporal analysis of cNFs development in this model and NF1 patients reveals 3 successive steps: initiation, progression and stabilization, all characterized by changes in the cellular proportions, composition and activities. Interestingly, proliferative and MAPK activities of tumor SC was only reported during initiation and progression periods revealing that the majority of tumor SC are quiescent in the mature cNFs *(see poster by P. Pulh).*

We observed that in this model, skin trauma accelerates development and increase the number of cNFs. This allows us to conceive so called “inducible model of cNFs” with nearly 100% penetrance and to elaborate two two experimental protocols, called preventive and curative, all suitable for conducting preclinical validations of candidate drugs. Since the tumor SC express Tomato that is easily detected by epifluorescent microscopy we taken advantage of such marker for the monitoring, counting and semi-quantitative evaluation of cNF before and after treatment with candidate drugs. Natural history of cNFs reveals dynamic evolution in cellular composition and activities during initiation and progression steps. This concern morphological and molecular alterations of tumor cells and of their cellular and acellular microenvironment. To address such complexity, we conceived a pipeline for the qualitative and quantitative analysis of the efficacy of candidate drugs to prevent or shrink cNFs. For quantitative evaluation, cNFs and healthy-looking adjacent skin are collected, dissociated and analyzed by FACS using panel of markers of each cellular component. For qualitative exploration, samples are processed for IHC with the same panel of markers complemented with markers of proliferation, apoptosis, MAPK pathway activity and fibrosis. Overall, we consider that such complete cellular and molecular analysis of each component of cNFs is necessary to precisely define the global efficacy of the candidate drug.

We have already tested 7 drugs alone and in combination targeting different elements of the RAS pathway with some showing promising results in curative paradigm. Interestingly, we discovered that the MEKinh binimetinib had no effect on quiescent tumor SC from mature cNFs (curative protocol) but efficiently prevent proliferation of tumor SC in growing cNFs (preventive protocol) up to 6 months after the end of the treatment.

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Evaluation of the Effects of a Novel MEK Inhibitor PAS-004 in Plexiform Neurofibroma in a Pre-Clinical Mouse Model of Neurofibromatosis Type 1

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Purpose: A genetically engineered mouse model (GEMM) of plexiform neurofibroma (pNF) that utilizes a Cre-mediated allele excision to create a SC specific *Nf1* null mouse has been shown to drive reporter gene expression in SCs progenitors beginning at E11 (1). These mice develop pNFs that closely phenocopy human tumors by 4 months of age with 100% penetrance. We sought to investigate the efficacy of a novel allosteric MEK1/2 inhibitor, PAS-004 in reducing existing tumor burden, as a single agent in this NF1-associated pNF mouse model.

Methods: In this pilot study, we tested PAS-004 tolerability in the GEMM of pNF and also evaluated preliminary biological efficacy. The maximum tolerated dose of selumetinib, 10mg/Kg BID was administered in parallel serving as a positive control. Both compounds were administered as single-agents to N=6 mice/group. PAS-004 was administered at a dose of 10mg/kg QD. Treatment began at 4 months of age and continued for 12 weeks or until death.

Results: As shown in Figure 1, both, selumetinib and PAS-004, significantly reduced DRG size compared to vehicle-treated mice (p=0.0048 and 0.0123, respectively). Target inhibition was confirmed by Western blot of pERK1/2 at 4 and 24 hours after last dose. To examine number, morphology, and cellular content of the tumors in detail, paraffin sections were prepared and stained with hematoxylin and eosin (H&E) and Masson's trichrome.

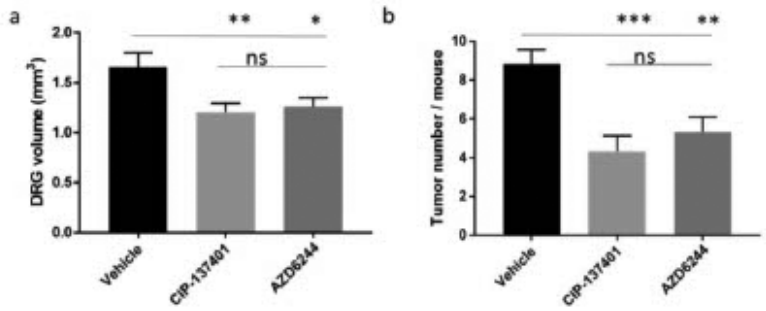

Figure 1: PAS-004 reduces Tumor Burden

PAS-004 and selumetinib showed similar toxicity profiles. The change in body mass over the 12-week study was variable, but not significant (p=0.0931) At sacrifice, peripheral nerve trees were collected, and tumor numbers and histology were examined and compared to animals receiving vehicle control.

Conclusion: PAS-004 is a macrocydic MEK-kinase inhibitor with excellent safety and pharmacokinetics profile. It demonstrates efficacy in reducing tumor burden in NF1-associated pNF mice when administered QD. The longer half-life (7.7h vs 2.6h AZD6244) could potentially enhance MEK/ERK signaling inhibition and allow for greater intervals between dosing.

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Identification of *In Vitro* and *In Vivo* Therapeutic Sensitivities for NF1 Associated High Grade Gliomas

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Neurofibromatosis Type 1 (NF1) patients develop an array of benign and malignant tumors, of which Malignant Peripheral Nerve Sheath Tumors (MPNST) and High Grade Gliomas (HGG) have a dismal prognosis. About 15–20% of individuals with NF1 develop brain tumors and one third of these occur outside of the optic pathway. These non-optic pathway gliomas are more likely to progress to malignancy, especially in adults. Despite their low frequency, high grade gliomas have a disproportional effect on the morbidity of NF1 patients. *In vitro* drug combination screens have not been performed on NF1-associated HGG, hindering our ability to develop informed clinical trials. Here we present the first *in vitro* drug combination screen (21 compounds alone or in combination with MEK or PI3K inhibitors) on the only human NF1 patient derived HGG cell line available and on three mouse glioma cell lines derived from the NF1-P53 genetically engineered mouse model, which sporadically develop HGG. These mouse glioma cell lines were never exposed to serum, grow as spheres and express markers that are consistent with an Oligodendrocyte Precursor Cell (OPC) lineage origin. Importantly, even though the true cell of origin for HGG remains elusive, they are thought to arise from the OPC lineage. We evaluated drug sensitivities of the three murine glioma cell lines in a 3D spheroid growth assay, which more accurately reflects drug sensitivities in vivo. Excitingly, we identified six compounds targeting HDACs, BRD4, CHEK1, BMI-1, CDK1/2/5/9, and the proteasome that potently induced cell death in our NF1-associated HGG. Moreover, several of these inhibitors work synergistically with either MEK or PI3K inhibitors. We are currently evaluating these drug combinations in our mouse preclinical models. Our initial results show that intracranial administration of the HDAC inhibitor Panobinostat indeed enhances survival by about 1 week (from 22 days post treatment start in controls to 28 days in Panobinostat treated mice, $p=0.03$). Of note, none of the HDAC inhibitors evaluated had a good blood brain barrier penetration, therefore we opted for intracranial dosing through a cannula, which is reminiscent of drug dosing through an Ommaya reservoir in patients. We were able to show potent target inhibition in our pharmacodynamic studies. Currently, we are evaluating the efficacy of a MEK and BMI-1 inhibitor combination therapy. Our results form the basis for further pre-clinical evaluation of promising targets, with an eventual hope to translate these to the clinic.

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Impact of Post-Transcriptional Gene Regulation in Neurofibromatosis Type 1-Related Breast Cancer

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Mutations in the *NF1* gene frequently appear in breast cancer metastatic sites suggesting that loss of *NF1* drives epithelial to mesenchymal transition to support clonal expansion of cancer cells. A critical link between NF1 and ER-alpha in regulation of ER-alpha signaling has recently been established. However, recent novel findings show ER-alpha harbors additional functions beyond its canonical ER signaling and can act as an RNA binding protein (RBP) to influence cell fitness through post-transcriptional gene regulation. These studies have sparked motivation to investigate mechanisms in which neurofibromin regulates ER-alpha's post-transcriptional gene regulation and how disruption of this neurofibromin-ER-alpha interaction can contribute to metastasis and endocrine resistance. To study this, we first performed a cox proportional-hazard model using the TCGA breast cancer dataset to investigate alternative splicing of NF1 transcripts and identify specific NF1 isoforms that contribute to decreased breast cancer survival. Additionally, using CRIPSR, we generated a *NF1*-deficient, ER positive breast cancer cell line (MCF7 cells) to further investigate ER-alpha post-transcriptional gene regulation activity. We performed RNA pulldown assays along with bioinformatic analysis of differential splicing events of RNA sequencing data from *NF1*-deficient MCF7 cells and TCGA breast cancer dataset. Our findings demonstrate that NF1 transcript isoforms can be used to predict breast cancer prognosis. Concurrently, *NF1*-deficient breast cancers harbor increased ER-alpha RNA binding and splicing burden. With these data we expose a new mechanism of therapeutic resistance and unexplored therapeutic vulnerabilities for *NF1*-deficient breast cancers.

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PRC2 Loss Drives MPNST Metastasis and Matrix Remodeling

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The histone methyltransferase PRC2 is a major epigenetic regulator that plays a complex role in cancer. Dysregulation of PRC2 activity is linked to poor prognosis, metastatic disease, and chemotherapy resistance across multiple cancers. Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive sarcomas with frequent loss-of-function mutations in PRC2 that are associated with poor outcome. In this study, we use a combination of *in vitro* metastasis assays, 3D collagen studies, and orthotopic mouse models to examine mechanisms of metastasis in multiple paired, isogenic MPNST cells. This use of isogenic cell series allows for a direct comparison between *Suz12* and *Eed* loss in the same cells, in addition to examining these events in both *Cdkn2a*-null and *p53*-null genetic contexts. By combining these approaches with time-lapse microscopy and analysis of patient samples, we have determined a critical role for PRC2 loss in driving MPNST metastasis and remodeling of the extracellular matrix (ECM). Our data show that PRC2 deletion increases collagen-dependent invasion *in vitro* and elevates lung metastases in orthotopic mouse models. Clinical sample analysis determines that PRC2 loss correlates with metastatic disease, increased fibrosis, and decreased survival in patients with MPNSTs. Furthermore, we demonstrate that PRC2 loss in MPNSTs induces expression of MMP and LOX family matrix-remodeling enzymes that drive metastatic phenotypes. This deeper understanding of PRC2-dependent metastatic events has a high potential to improve the clinical management of MPNSTs and has broad implications for PRC2 function across multiple cancers.

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Development of a Novel *In Vivo*/*Ex Vivo* CRISPR-Based Mouse Model That Recapitulates Various Stages of NF1-Associated Peripheral Nerve Sheath Tumorigenesis

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Our aim was to develop a novel, murine, syngeneic, immunoproficient model of multiple stages of neurofibromatosis type 1 (NF1)-associated peripheral nerve sheath tumor development. Several genetically engineered mouse models exist that recapitulate various tumor phenotypes of NF1 syndrome. These models have relied on temporal and/or tissue specific loss of NF1 and other tumor suppressor genes throughout specific stages in the Schwann cell lineage. Development of these models and investigation of their phenotypes can take months or years, hindering large scale *in vivo* drug studies. Recently, murine models have employed *in vivo* CRISPR-Cas9 mediated knockout of tumor suppressor genes associated with malignant peripheral nerve sheath tumor (MPNST) formation. This approach allows for a more rapid interrogation of various MPNST genetic alterations through Cas9 gene editing techniques. Motivated by the success of these and other Cas9-mouse knockout models, we created a mouse model of the following genotype: *Dhh-Cre ; $Nf1^{flox/\Delta}$; Rosa26-Lox-STOP-Lox-Cas9-IRES-eGFP.* These 'quad' mice harbor functionally $Nf1^{+/-}$ somatic cells and $Nf1^{-/-}$ Cas9-expressing GFP-positive Schwann cells. Adeno associated virus (AAV)-mediated single guide RNA delivery into the sciatic nerve of these animals was unsuccessful in disrupting target gene function. However, fluorescence activated cell sorting (FACS) of GFP+ primary Schwann cells from adult quad peripheral nerves yielded $Nf1^{-/-}$ Schwann cells that expressed Cas9 and S100b. Targeted *in vitro* Cas9 knockout of *Cdkn2a/b* alone or in combination with *Suz12* achieved 70-95% editing at each locus. Implantation of 1 million $Nf1^{-/-}$, $Nf1^{-/-}$ + $Cdkn2a/b^{-/-}$, or $Nf1^{-/-}$ + $Cdkn2a/b^{-/-}$ + $Suz12^{-/-}$ murine Schwann cells into the sciatic nerve pocket of NRG mice led to aggressive tumor growth with all animals requiring sacrifice by 50 days post injection. Both $Nf1^{-/-}$ + $Cdkn2a/b^{-/-}$, and $Nf1^{-/-}$ + $Cdkn2a/b^{-/-}$ + $Suz12^{-/-}$ murine Schwann cells could also form large tumors requiring sacrifice in immunocompetent recipient quad animals. Additional efforts are ongoing to characterize harvested tumors from both immunodeficient and immunocompetent recipient animals. Future studies utilizing this model could interrogate drug sensitivities present in atypical neurofibroma-like tumors lacking *Nf1* and *Cdkn2a/b* and potential immunotherapeutic intervention in PRC2 deficient tumors, all in the NF1 patient-like context of an immune proficient $Nf1^{+/-}$ microenvironment.

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Dopamine D2 Signaling Through Beta-Arrestin is Increased in Cells Lacking Neurofibromin

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Purpose: To test the hypothesis that normal D2 receptor signal transduction is dependent on the presence of neurofibromin.

Methods: HEK293T cells were treated with CRISPR to remove neurofibromin and paired with WT controls. D2 receptor fused to nanoluc or luc8 were transfected with mini-G-proteins to assess G-protein recruitment to the receptor after dosing with dopamine. A repurposed PRESTO-Tango assay was used to assess arrestin recruitment as well as D2-Luc8—Arrestin-GFP BRET. Cells were treated for 45 minutes to maximize beta-arrestin signaling and then western blot was used to assess known downstream pathways and the effects of NF1 loss.

Results: We found that G-protein recruitment was only modestly affected by the loss of NF1 in this assay. However, we found that beta-arrestin recruitment was significantly increased after removal of NF1. When downstream pathways commonly associated with arrestin signal transduction were analyzed by western blot, we found a number of interesting results: pERK and pCREB experienced a greater relative reduction in the amount observed in the NF1 KO cells vs WT; while pAKT Thr308 and pAK Ser473 both displayed large relative increased in their detected amounts. NF1 knock out was confirmed via western blot and equal transfection amounts were accounted for by detection of the flag-tag on the D2 receptor.

Conclusions: Loss of neurofibromin has direct implications for signaling transduction of the D2 GPCR. The findings of this study complement known data from previous studies about cAMP reserves and beta-gamma roles in G-protein dependent signaling. Beta-arrestin recruitment to the D2 receptor is increased in an NF1 deficient cell model which results in different downstream effects than what occurs in the WT setting. These data directly implicate signaling at the receptor level is significant for future investigations into normalizing these and treating some of the cognitive issues observed in NF1 patients.

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Silver Nanoparticles Selectively Treat Plexiform Neurofibroma Cells Compared to Patient-Matched Schwann Cells

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Neurofibromatosis Type 1 (NF1) is one of the most common neurogenic disorders defined by the heterozygous loss of function of the neurofibromin gene. NF1 patients suffer from a range of symptoms, with one the most problematic being the development of neurofibromas which can be debilitating or deadly. Plexiform neurofibroma (pNF) are benign tumors that develop in 30-50% of NF1 patients and are treated with surgery if accessible followed by MEK inhibitor selumetinib. Neurofibromin is a tumor suppressor gene responsible for suppressing RAS activity. Loss of functional neurofibromin expression inappropriately sustains RAS activity and subsequently increases the formation of intracellular reactive oxygen species (ROS). We and other groups have demonstrated that systemically administrable silver nanoparticles (AgNPs) are cytotoxic to a variety of cancers including NF1-associated malignant peripheral nerve sheath tumors. We further showed that AgNP-mediated cytotoxicity is dependent upon intracellular ROS which induces rapid ionization (activation) of Ag^0 to cytotoxic Ag^+. Therefore, AgNPs represent a rational cancer-selective therapy for neurofibromin-deficient pNFs. **The purpose of this study** is to evaluate preclinical efficacy of AgNP as a potential treatment for pNFs using patient-matched *in vitro* cell models. Here we show that AgNPs are ~7-fold more cytotoxic to neurofibromin-null pNF cells (ipNF95.11.bC) relative to patient-matched Schwann cells (ipNF95.11.C) which harbor one functional copy of neurofibromin. We found AgNPs have improved cancer-selectivity relative to MEK inhibitor selumetinib. Remarkably, restoration of functional neurofibromin in pNF cells decreased sensitivity to AgNP. On the contrary, when neurofibromin expression was reduced in NF1 patient-matched Schwann cells we found that sensitivity to AgNP was increased. This change is unique to AgNP as there was no noted effect of gene dose on sensitivity to selumetinib in either cell line. We then fluorescently labeled ipNF95.11.bC (RFP) and ipNF95.11C (GFP) and grew both as a co-culture *in vitro* model system. We found that AgNP could selectively remove the ipNF95.11.bC-RFP at doses which allowed ipNF95.11-GFP to remain viable. In summary, our work provides evidence that AgNPs are a safe medicine with an anticipated wide therapeutic window for the treatment pNF in NF1 patients addressing clinical need.

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Allele-Specific Effects of NF1 Indicate Tumor Immune Response Modulates Onset of ER-Positive Mammary Tumorigenesis in Rats

Christian Fay, BS, *The University of Alabama at Birmingham*

The purpose of this study is to analyze the transcriptome of rat mammary tumors that arise from Nf1 deficiency as both germline and somatic loss of NF1 can result in breast cancer. Furthermore, loss of *NF1* is also a prognostic indicator for increased cancer risk at an earlier age, poorer outcomes, and therapeutic resistance. We have created novel heterozygous Nf1 KI and KO rat models including pathogenic patient missense allele c.3827G>A, p.R1276Q (knockin or KI), associated in humans with spinal NF1 and malignancy, as well as a 14 base pair deletion c.3661_3674del, p.P1220fs*1223 (knockout or KO) model. Both show robust ER+ BC phenotypes. Phenotypic differences between our models indicate that the allelic variant dictates onset of disease with the KO having earlier onset and more numerous tumors develop in comparison to KI. This difference may be due to complete loss of all NF1 functions (KO) versus loss of Ras-GTPase stimulating activity with the KI mutation. We utilized mammary tumors from 7 KI and 5 KO rats and normal mammary tissue from 5 WT pooled animal samples to conduct snRNA-sequencing. We identified B cells, T cells, 2 populations of myeloid cells, two subsets of epithelial cells, myocytes, adipocytes, myoepithelial cells, endothelial cells, and fibroblasts. Ingenuity pathway analysis (IPA) on the differentially expressed genes among cell types and genotypes elucidated pathways that differ between genotypes. Of interest, we identified a significant de-activation of the “oxytocin signaling”, “aldosterone,” and “wound healing” pathways in a subset of epithelial cells in NF1 deficient samples (KI and KO combined) compared to WT samples. When comparing the KI and KO tumors to each other, IPA analysis found very minimal differences between the cancerous epithelial cell populations. However, when examining myeloid and T cell populations between the KI and KO tumors we find drastic differences in pathways. We observed that the KI tumor myeloid cells have predicted activation of pathways related to “phagosome formation”, “production of ROS”, “alternative macrophage activation, and “Fcy receptor mediated phagocytosis in macrophages and monocytes” compared to KO tumor myeloid cells. Additionally, in the T cell population we observed predicted activation of “Th1”, “Th2”, and “T cell receptor signaling” in the KI compared to KO tumor T cells. Our analysis suggests that the tumors from KI rats may have a relatively immunologically “hotter” environment than the relatively “cooler” KO tumors. The immunological response may contribute to a delay in tumor onset in KI tumors compared to KO tumors.

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Cherry Angiomas: A Specific Feature of Neurofibromatosis 1?

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Cherry angiomas (CA) are the most frequent benign vascular skin tumors in the general population, with a prevalence increasing with age. Their association with Neurofibromatosis type 1 (NF1) is classically described. Recently, we confirmed the higher prevalence of CA in a prospective study comparing 102 individuals with NF1 and 157 controls: 48% versus 18%. The OR was 4.26 (97.5%CI[2.23-8.12]; p<0,0001) and 10.79 after age-adjustment (97.5%CI[5.25-23.92]; p<0,0001).

The aim of our study was to decipher the molecular mechanisms driving the development of CA in NF1.

Six CA from 6 patients and 2 CA from to 2 controls, confirmed histologically, were analysed using a panel of 23 genes involved in overgrowth syndromes (Figure 1) and sequencing the *NF1* gene. In individuals with NF1, constitutional genetic testing was performed.

In all individuals with NF1, the presence of a heterozygous pathogenic *NF1* variant was confirmed in white blood cells, and a second hit in the *NF1* gene was systematically present (N=6, 100%) in their CA. In addition, a somatic third molecular event was found in the GNAQ gene (N=2, 33%)(Table 1). The variant allele frequencies (VAF) of the *NF1* and *GNAQ* mutations were similar. No somatic mutation in the *NF1* gene was found in controls but a mutation in the *GNA11* gene in 1 out of 2 (50%).

Our results suggest a specific pathophysiology of CA in NF1, resulting from a second hit in *NF1.* CA are known to result from dysregulation of *GNAQ, GNA11* and *GNA14* genes related pathways. The similarity between the resulted VAF of the *NF1* versus *GNAQ* mutations suggests a common cellular origin. Further ongoing analyses should better identify this particular cell type which seems to not be Schwann cells. In addition, a larger panel is currently characterizing more accurately the prevalence and type of a third molecular event.

Figure 1: List of the 23 genes included in the "Overgrowth syndromes" panel

« Overgrowth syndroms » Panel :
AKT1, AKT2, AKT3, BRAF, EPHB4, GNA11, GNA14, GNAQ, HRAS, KRAS, KRIT1, MAP2K1, MAP3K3, MTOR, NRAS, PIK3CA, PIK3R1, PIK3R2, PTEN, RASA1, TEK, TSC1 et *TSC2*

Table 1: Results of the molecular analyses of cherry angiomas from individuals living with NF1 (N=6) and controls (N=2). A panel of 23 genes involved in overgrowth syndromes was used and the *NF1* gene was sequenced. The mean variant allele frequency (VAF) of the constitutive *NF1* mutation was of 0.043.

Somatic mutations / Individuals	NF1 (mean VAF = 0.043)	GNAQ	GNA11
Individuals with NF1			
1	c.6709C>T (2.5%)	p.Q209H (3%)	-
2	c.4950C>A	-	-
3	c.6756_6756+1delinsAC	-	-
4	c.1008G>A (7%)	p.Q209H (8%)	-
5	c.5750-1G>A	-	-
6	c.7782_7783delinsTT	-	-
Controls			
1	-	-	p.Q209H (5%)
2	-	-	-

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Preliminary Study Using Deoxycholic Acid and Polidocanol Injections for the Treatment of Cutaneous Neurofibromas

Margaret Funk, MS, *Department of Dermatology, Harvard Medical School; Wellman Center for Photomedicine, Massachusetts General Hospital*

Purpose: We investigated the effects of two injectable, FDA-approved surfactants deoxycholate and polidocanol in excised cutaneous neurofibromas (cNF) to explore efficacy and potential for future clinical application.[1&2]

Methods: 30 surgically excised cNF were included. The Massachusetts General Hospital IRB approved this study. To prevent deterioration, 26 lesions were frozen and stored in a -80ºC freezer; 4 lesions were stored in a -20ºC freezer as removal occurred 24 hours before surfactant injection.

All cNF were defrosted and heated to 37ºC in saline to simulate body temperature. Lesions were either injected with sterile 1% deoxycholate solution (Kybella©, Allergan-AbbVie) or sterile 1% polidocanol solution (Asclera, Merz). All tumors were injected at a uniform depth of 2.5mm with a volume of 0.1cc, 0.2cc, or 0.4cc. Tumors were then placed in normal saline for one hour. The experiments were repeated 6 times at 0.2cc for statistical analysis. After one hour, the specimens were bisected, placing half in formalin solution and the other half in optimal cutting temperature compound before sectioning every 20um on a cryostat. The slides of both halves were stained with hematoxylin and eosin (H&E).The frozen sections were also stained with nitroblue tetrazolium chloride (NBTC), which assesses cell viability as a function of redox potential. All slides were scanned and the major and minor axes of cNF showing loss of cell structure or NBTC staining measured. Percentage of damage for injection with 0.2cc was evaluated with two-tailed independent t-tests. A p-value of 0.05 or less was considered statistically significant.

Results: Significant tumor damage was observed when cNF were treated with 0.2cc of deoxycholate and 1% polidocanol (Figures 2-3, arrows mark edge of necrotic regions). The average area of damage was 12.22mm from injection with deoxycholate and 24.01mm from injection with 1% polidocanol. The average percent of damage in untreated control cNF was smaller than cNF injected with deoxycholate (5% vs 39%, $p<0.02$) and smaller than cNF injected with 1% polidocanol (5% vs 68%, $p<0.02$) (Figure 4, individual data points shown with mean ± 95% CI).

Conclusion: Treatment of *ex vivo* cNF with deoxycholate and 1% polidocanol caused significant loss of cNF cell structure and NBTC staining. Local injection of these agents holds promise as an off-label treatment for cNF that is potentially rapid, well-tolerated and less morbid than surgical excision or other destructive modalities. In a separate ongoing clinical trial, we found deoxycholate to be effective and well tolerated for treatment of small cNF in adults and aim to compare deoxycholate and polidocanol local injection treatments in future clinical trials.

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References:
1. Blakeley, J. O. *et al. Neurology* 91, S1–S4 (2018)
2. Page, P. Z. *et al. Am. J. Med. Genet. Part A* 140A, 1893–1898 (2006)

Funding: This study is funded by the Neurofibromatosis Therapeutic Acceleration Program (NTAP) at Johns Hopkins University.

Figure 1. cNF untreated

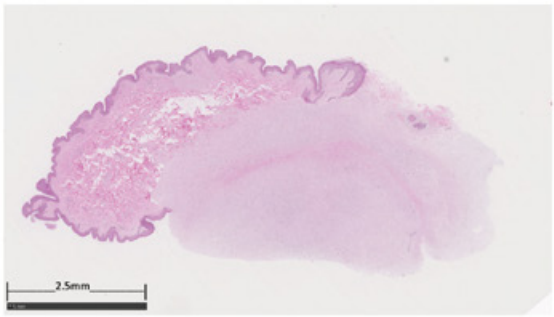


Figure 2. cNF treated with 0.2cc of deoxycholate solution

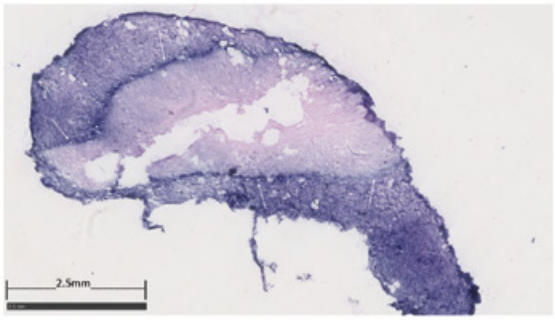


Figure 3. cNF treated with 0.2cc of 1% polidocanol solution

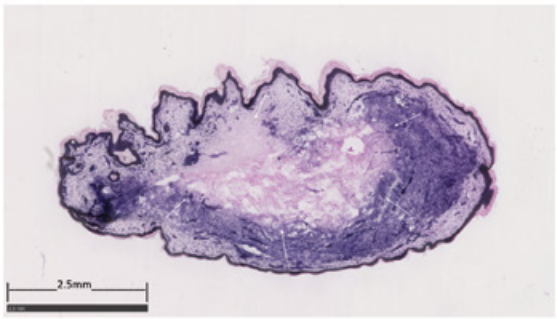
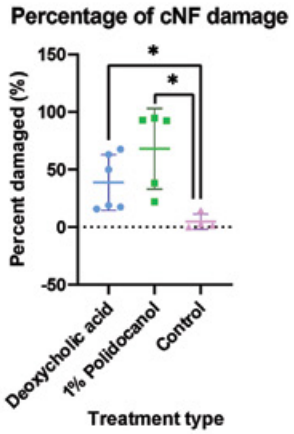


Figure 4.



A Comprehensive Genomic Definition of NF1-Associated MPNSTs

Bernat Gel, PhD, *Germans Trias i Pujol Research Institute (IGTP)*

Malignant peripheral nerve sheath tumors (MPNSTs) are soft tissue sarcomas that arise from the peripheral nervous system. MPNSTs have a bad prognosis and constitute the first cause of mortality in the Neurofibromatosis type 1 (NF1) context. The diagnosis of MPNSTs can be challenging, especially outside the NF1 context since these are rare tumors and there are multiple tumor entities with overlapping histological criteria. A careful genomic analysis of widely used MPNST cell lines helped us uncover potentially misdiagnosed MPNSTs and provided new types of useful genomic information to complement histological MPNST diagnostics.

We present here the genomic and transcriptomic analysis (WGS, WES, RNA-seq) of 19 tumors diagnosed as MPNST. To assess for heterogeneity concerning tumor identities regardless of any genomic information, we classified these samples according to the expression of transcription factors (TF), since these genes are good markers of cell identity and their short length is less prone to biases due to RNA quality. We used as a reference the TF expression of genuine MPNST cell lines and other similar but misdiagnosed cell lines, and we also considered the percentage of tumoral tissue in each tumor. A principal component analysis (PCA) broadly grouped the tumors into two main clusters. C1 grouped 14 tumors (NF1-related and sporadic tumors) together with genuine MPNST cell lines, whereas C2 grouped 5 tumors clustered with the other non-MPNST cell lines. The next step was to genomically characterize these two clusters.

Tumors in C1 exhibited a high recurrence in their genomic characteristics. Their genome was basically gained and represented by even copy numbers, highly compatible with a genome doubling event. Besides, there were chromosomal regions exhibiting a striking recurrence in copy-neutral LOH that we hypothesize might be genomic regions lost before a tetraploidization event. We also detected the accumulation of structural variants inactivating *CDKN2A.* In fact, and contrary to C2, almost all tumors in C1 exhibited the inactivation of *NF1, CDKN2A,* and PRC2 tumor suppressor genes, and lacked fusion genes and activating oncogenic mutations. C1 tumors had a low mutation burden and the absence of distinctive mutational signatures. C2 was clearly enriched in tumors with completely different genomic characteristics.

We have been able to provide a genomic definition of a classic MPNST and generated a classifier that uses genomic information for aiding in their correct diagnostics. We are currently validating our findings in a larger set of already published MPNST genomic data.

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The pNF-ANNUBP-MPNST Progression at Single-Cell Resolution: A Resource for the NF1 Community

Bernat Gel, PhD, *Germans Trias i Pujol Research Institute*

Single-cell technologies have revolutionized the study of tumors, providing an unprecedented view into the diversity of cell types and states that can be found inside them. These technologies have evolved rapidly, from the original single-cell RNA-seq to the expansion to epigenomics and more recently spatial transcriptomics, bringing together tissue structure and single-cell gene expression, constituting a key component for unraveling tumor biology and complexity.

In the neurofibromatosis type 1 (NF1) context, there are still few single-cell datasets regarding NF1-related tumors. Particularly, there has been no systematic effort to comprehensively characterize at the single cell level the human NF1-associated peripheral nervous system (PNS) tumors and their path to progression, from a benign plexiform neurofibroma (pNF), to an atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) and finally a malignant peripheral nerve sheath tumor (MPNST).

We present the first multi-modal single-cell catalog of NF1-associated PNS tumors. We have characterized a set of 4 PNF, 4 ANNUBP and 4 MPNST together with healthy nerves as controls using multiple complementary single-cell techniques: scRNA-seq for a comprehensive broad transcriptomic view of thousands of cells per tumor, SMART-seq for a complete transcriptome of ~100 cells, ATAC-seq to explore the chromatin landscape of the exact same cells for which scRNA-seq data is available, DNA-seq for copy-number analysis and cell-surface markers combined and finally, spatial transcriptomics to bring tissue structure to the mix. We have also performed histological analysis of the same tumors. The observed proportion of the different cell types by histology matches those estimated from single-cell data, validating both analyses and tumor dissociation procedures.

The multiple data types included in this dataset can be analyzed together and this is allowing us to start studying the changes in cell composition through the MPNST progression; linking the epigenetic differences to changes in gene expression; studying copy-number heterogeneity in MPNST and the genome of atypical cells in ANNUBP; and searching for the cell-of-origin of these tumors, among other aspects.

In summary, we have analyzed more 130K single cells from tumors and healthy nerves using five different technologies to provide a complete and systematic view of the cellular diversity and complexity of NF1-related PNS tumors. All this data will be freely available to the broad NF1 research community through the NF Data Portal to serve as the go-to resource for any project investigating the cellular composition, origin and progression of pNFs, ANNUBPs and MPNST.

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Targeting Inflammatory Signaling in Cutaneous Neurofibromas

Jamie Grit, PhD, *Van Andel Research Institute*

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. We identified reciprocal prostaglandin (COX2) and leukotriene (5-lipoxygenase) signaling in CNF PDEs and show that that *ex vivo* treatment with a corticosteroid reduces expression of COX2 and other inflammatory mediators. 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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MEK and TYK2 Combination Therapy in NF1-Associated Malignant Peripheral Nerve Sheath Tumors

Kevin He, *Washington University in St. Louis*

Purpose: Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas that develop from benign plexiform neurofibromas (PN) in individuals with the Neurofibromatosis Type 1 (NF1) cancer predisposition syndrome. Currently, there are no predictive biological markers of transformation from PN to MPNST and no effective therapies for MPNST. The Hirbe lab previously identified TYK2 as a gene mutated in a subset of MPNST and showed that Tyk2/TYK2 knockdown of in murine and human MPNST cells significantly increased cell death. Furthermore, these Tyk2 knockdown cells demonstrated decreased growth in subcutaneous and metastasis models. Our objectives are to determine whether TYK2 is a diagnostic and prognostic biomarker for MPNST, determine TYK2 mechanisms in MPNST, and evaluate combined TYK2 and MEK inhibition as a treatment strategy for MPNST.

Methods: Immunohistochemistry (IHC) for TYK2 was performed on MPNST, PN, ANNUBP and correlated with patient information from EPIC to evaluate TYK2 as a diagnostic and prognostic biomarker. MPNST cell-lines were treated with deucravacitinib (TYK2 inhibitor) in combination with mirdametinib (MEK inhibitor), and the HSA system and SynergyFinder 2.0 were used to determine synergy. IncuCyte live cell imaging assays were used to analyze cell confluence and apoptosis and calculate IC50 values. TYK2 protein levels and phosphorylation were assessed by the simple western WES western blot system. RNA-seq and qPCR arrays were used to assess TYK2 inhibition on downstream pathways.

Results: Immunohistochemistry revealed elevated TYK2 protein levels in MPNST vs. benign PN and a weak correlation with patient survival. Interestingly, high expression was seen in all ANNUBP examined. RNA seq and qPCR array showed upregulation of the MEK/ERK pathway and decreased expression of cell cycle, mitotic, and glycolysis pathways. MEK inhibitors synergized with TYK2 inhibitors in increasing MPNST cell apoptosis and decreasing proliferation.

Conclusion: These findings support the development of a deucravacitinib and mirdametinib phase I clinical trial of in NF1-associated MPNST. Furthermore, while TYK2 expression is associated with MPNST, expression is not specific enough for diagnosis or prognostic implications.

Funding: This work was funded by a New Investigator Award through the Neurofibromatosis Research Program (NFRP) from the Department of Defense Office of the Congressionally Directed Medical Research Programs (CDMRP; W81XWH-20–1-0148 to A.C. Hirbe), the St. Louis Men's Group Against Cancer (to A.C. Hirbe), The Doris Duke Charitable Foundation (to A.C. Hirbe), and the Neurofibromatosis Therapeutic Acceleration Program (NTAP; to C.A. Pratilas). Mirdametinib for this work was provided by SpringWorks Therapeutics, Inc. under an investigator-initiated research agreement with Washington University.

MSU-42011, Alone and in Combination with Selumetinib, Reduces pERK Levels in NF1 Cancer Cells and Decreases *CCL2* Expression in Macrophages

Pei-Yu Hung, MS, *Department of Physiology, Michigan State University, East Lansing, MI*

Neurofibromatosis type 1 (NF1) is a common genetic disease that predisposes approximately 50% of affected individuals to develop plexiform neurofibromas (PNFs), which can progress to highly aggressive malignant peripheral nerve sheath tumors (MPNSTs) in approximately 10% of patients. NF1 is caused by mutations in the tumor suppressor gene *NF1,* which encodes for neurofibromin, a negative regulator of RAS activity. Selumetinib, a specific inhibitor of MEK1/2, is the only FDA-approved drug for NF1-associated PNFs. However, the anti-tumor effects of selumetinib are limited in MPNSTs and have dose-limiting side effects. Deficiency of the *NF1* gene not only promotes tumorigenesis but also has broad effects on the immune cells and cytokine signaling driven by hyperactive RAS signaling. Because macrophages account for almost half of cells in NF1 lesions and their infiltration correlates with disease progression, we hypothesized that targeting tumor-promoting immune cells could be an alternative approach for treating NF1. The novel retinoid X receptor (RXR) agonist MSU-42011 reduces tumor growth in experimental Kras-driven cancers by decreasing pERK expression, reducing tumor-promoting immune cells like CD206+ macrophages and regulatory T cells, and increasing activated cytotoxic T cells. Here, we tested MSU-42011 and selumetinib, either alone or in combination, to evaluate their efficacy against NF1 cancer cells and macrophages using monoculture and conditioned media (CM) treatments. In human PNF cells, treatment with 200 nM MSU-40211, 50 nM selumetinib, and the combination for 3 hours reduced pERK protein levels by approximately 40%, 70% and 90%, respectively vs. untreated controls. Treatment for 72 hours with selumetinib and the combination reduced the viability of PNF cells in a dose-dependent manner. Moreover, CM from human and mouse PNF cells increased monocyte chemoattractant *CCL2* (C-C motif chemokine ligand 2) mRNA expression in human THP1 monocytes/macrophages and bone marrow derived macrophages (BMDM). Notably, MSU-42011 and selumetinib alone inhibited *CCL2* mRNA expression in THP1 macrophages and BMDM stimulated with CM from human and mouse PNF cells, respectively, and the inhibition of *CCL2* mRNA expression was greatest with combination treatment. Taken together, our data suggest that MSU-42011, alone and in combination with selumetinib, should be tested in relevant preclinical mouse models of NF1.

Figures:

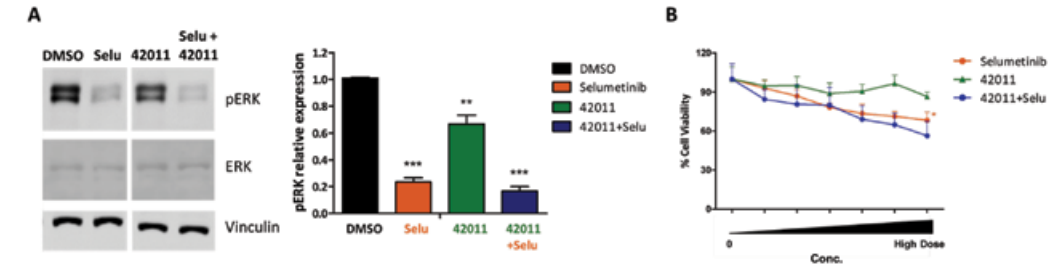

Fig 1. Combination treatment with MSU-42011 and selumetinib inhibited pERK levels and modestly reduced cell viability in human PNF cells.
(A) Human PNF cells (ipNF95.6) were treated with 50 nM selumetinib (Selu), 200 nM MSU-42011, or the combination for 3 hrs. The level of pERK was evaluated by western blotting. (B) Human PNF cells (ipNF95.6) were treated with an increasing concentration of drugs (0-1000 nM selumetinib, 0-2000 nM MSU-42011, or the combination) for 72 hrs. The cell viability was measured by a MTT assay. Data represent means ± standard deviations of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with vehicle treatment.

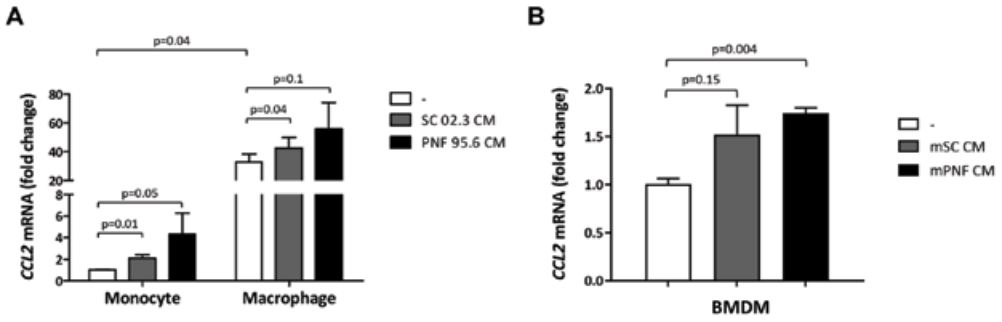

Fig 2. CM from human and mouse PNF cells induced higher *CCL2* mRNA expression in THP1 cells and BMDM than CM from Schwann cells.
(A) THP1 monocytes or THP1 macrophages pre-differentiated by 50 ng/ml PMA for 3 days were treated with conditioned medium (CM) from human normal Schwann cells (ipn02.3 2λ) and human PNF cells (ipNF95.6) for 24 hrs. Left panel: undifferentiated THP1 monocytes. Right panel: PMA-differentiated THP1 macrophages. (B) Bone marrow derived macrophages (BMDM) pre-differentiated by 20 ng/ml M-CSF for 5 days were treated with CM from mouse normal Schwann cells ($NF1^{f/f}$ Cre -) and mouse PNF cells ($NF1^{f/f}$ Cre +) for 24 hrs. *CCL2* mRNA expression was evaluated by qPCR. Data represent means ± standard deviations of n=3 or 1 experiments.

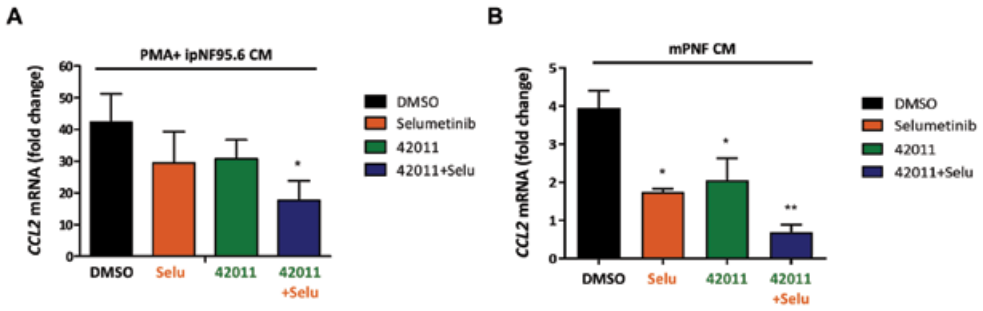

Fig 3. Combination treatment with MSU-42011 and selumetinib decreased *CCL2* mRNA expression in THP1 cells and BMDM stimulated with CM from human and mouse PNF cells.
(A) THP1 monocytes were differentiated into macrophages with 50 ng/ml PMA for 3 days, followed by treatment with CM from human PNF cells (ipNF95.6) and drugs (50 nM selumetinib, 200 nM MSU-42011, or the combination) for an additional 24 hrs. (B) BMDM were differentiated into macrophages with 20 ng/ml M-CSF for 5 days, followed by treatment with CM from mouse PNF cells ($NF1^{f/f}$ Cre +) and drugs (50 nM selumetinib, 200 nM MSU-42011, or the combination) for an additional 24 hrs. *CCL2* mRNA expression was evaluated by qPCR and normalized to the DMSO control without CM treatment (not shown). Data represent means ± standard deviations of n=3 or 1 experiments. * $p < 0.05$, ** $p < 0.01$ compared with vehicle treatment.

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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 e named inventor on the patent applications and a founding scientist of Akeila Bio.

MEK Inhibitor Mirdametinib Augments the Efficacy of Irradiation in *NF1* Deficient High-Grade Glioma Preclinical Models

Maria I. Ioannou, MD, *Department of Neurology and Oncology Johns Hopkins University School of Medicine, Baltimore, MD*

Purpose: Individuals with NF1, an autosomal dominant neurogenetic and tumor predisposition syndrome, are susceptible to developing low-grade glioma and, less commonly, high-grade glioma. These gliomas harbor bi-allelic loss of the neurofibromin gene *(NF1),* which may present a therapeutic window of opportunity, given the established efficacy of MEK inhibitors (MEKi) in plexiform neurofibromas and some low-grade glioma. We hypothesized that combining the MEKi, mirdametinib, with standard-of-care cytotoxic agents such as temozolomide (oral chemotherapy) or radiation could potentially improve efficacy in high-grade glioma.

Methods: We utilized seven human-derived glioma neurosphere lines with varied *NF1* status (intact or functionally deficient) and evaluated sensitivity to mirdametinib alone or in combination with radiotherapy, temozolomide, or both in preclinical models. Cell growth was quantified by counting a nuclear-red tag using a real-time live cell imaging system, or by colony formation in soft agar. Homologous recombination (HR) efficacy was assessed by the transcription of pathway genes *(RAD51, BRCA2)* using qPCR and confirmed by quantifying RAD51 foci by immunofluorescence (IF) and immunoblot. NSG mice were implanted with subcutaneous xenografts from *NF1*-deficient (JHH-136, JHH-520) or *NF1*-intact (HSR-GBM1) patient-derived neurosphere lines, and treated with vehicle, mirdametinib (1.5 mg/kg/daily) via oral gavage, irradiation (fractioned 2 Gy for 3 days) or their combination. Tumor volume was measured twice weekly.

Results: *NF1*-deficient neurospheres were sensitive to combined mirdametinib with irradiation as evidenced by synergistic inhibition of cell growth and colony formation in soft agar, and increased cell death (measured by Annexin V and sub-G1 population). *NF1*-intact neurospheres were not similarly sensitive, despite effective ERK inhibition, nor were any of these lines sensitive to temozolomide combined with mirdametinib. In *NF1*-deficient neurospheres, mirdametinib was associated with decreased transcription of HR genes, as well as decreased RAD51 foci. Heterotopic xenograft models confirmed synergistic growth inhibition on exposure to combined mirdametinib with irradiation in *NF1*-deficient glioma xenografts, but not those with intact *NF1.* In sensitive models, benefits were observed at least three weeks beyond the completion of treatment, including sustained pathway inhibition on immunoblot and decreased Ki-67 on immunohistochemistry.

Conclusions: Our findings demonstrate combination activity between mirdametinib and irradiation in *NF1*-deficient glioma models, both *in vitro* and *in vivo*. These observations may have clinical implications for patients with high-grade glioma that harbor *NF1* alterations.

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Disclosure of relevant financial relationships: Mirdametinib, a MEK inhibitor, and funding for this study were provided by SpringWorks Therapeutics, Inc. under an investigator-initiated research agreement with Johns Hopkins University.

Spatial Transcriptomic Analysis of Malignant Peripheral Nerve Sheath Tumors Reveals Transcriptionally Divergent Populations Across a Gradient of Histopathologic Transformation

Mirchia Kanish

Purpose: Malignant peripheral nerve sheath tumors (MPNSTs) arise from plexiform neurofibromas in patients with neurofibromatosis type-1 (NF-1), suggesting a spectrum of tumor evolution from low to high grade within a single lesion. Here, we perform spatial gene expression profiling to correlate histologic observations with transcriptomic programs in order to identify mechanisms underlying malignant transformation of NF-1 associated peripheral nervous system tumors.

Methods: Sixteen MPNSTs, including a subset that showed neighboring low-grade and high-grade areas, were retrospectively identified. Spatial transcriptomic profiling was performed using the Visium Spatial assay. Data were processed using SpaceRanger (v2.0.1), visualized using the Loupe Browser (v6.3.0), and analyzed in Seurat.

Results: Spatial gene expression profiling of 16 MPNSTs yielded 51,811 unique transcriptomes distributed across 25 clusters, of which 15 were enriched for tumor cells and 10 were enriched for non-tumor microenvironment cells identified through a combination of histologic evaluation, unsupervised cell type assignment, and marker gene expression. The 10 microenvironment-rich transcriptomes were comprised of 3 endothelial cell clusters, 3 Schwann cell clusters, and 1 perineurium cluster. Of the 15 tumor-dominant clusters, 4 were seen solely in low-grade (histologic neurofibroma) areas with 1 conserved cluster shared across 3 separate tumors and 3 patient-specific clusters. The remaining 11 clusters were exclusively in the high-grade (histologic MPNST) areas. Transcriptomic clusters in low-grade areas were highlighted by marker genes enriched for regulation of mononuclear cell migration and cell-cell adhesion. In contrast, transcriptomic clusters in high-grade areas lacked Schwann cell differentiation markers and contained marker genes enriched for upregulation of Ras/Raf/MEK/ERK target genes, negative regulation of extrinsic apoptotic signaling, and extracellular matrix organization. Pseudotime trajectories with low-grade regions set as t0 revealed cellular and transcriptomic heterogeneity in cell cycle, migration, and adhesion programs within histopathologically homogeneous regions. Finally, analysis of 3 tumors capturing the contiguous transformation zone between low-grade neurofibromatous and high-grade MPNST histopathologic regions revealed the presence of a distinct niche marked by increased cell cycle rates, and upregulation of genes involving extracellular matrix remodeling (THBS1, SERPINE1) and regulation of leukocyte chemotaxis (CCL2, CXCL8).

Conclusions: Spatial transcriptomic analysis revealed distinct populations in low-grade, transformation zone, and high-grade regions in MPNSTs highlighted by expected alterations in Schwann cell differentiation, cell cycle, and Ras signaling as well as unexpected differences in immune cell chemotaxis and cell-adhesion even within histologically similar regions, underscoring the value of spatial profiling to identify mechanisms of tumor heterogeneity and malignant transformation.

Funding: Francis Collins Scholar Award, NTAP

Recapitulation of NF1 Phenotype in a Humanized Mouse Model for c.1466A>G (p.Tyr489Cys) Patient Mutation

Robert A. Kesterson, PhD, *Department of Cancer Precision Medicine, Pennington Biomedical Research Center*

The relatively frequent c.1466A>G (p.Tyr489Cys) NF1 missense variant leads to a new "cryptic" donor splicing site being created in Exon 13 far upstream of the normal splice signal, thereby inducing partial exon skipping and a subsequent 62bp deletion in the mRNA leading to a truncated neurofibromin protein. The Tyr489Cys variant is pathogenic with patients displaying the full spectrum of NF1 phenotypes. We previously developed a cDNA expression system to show that if a full length missense mRNA was generated (i.e. mouse p.Tyr489Cys cDNA), the mutant protein was able to maintain Ras-inhibitory functions. This indicates that suppression of the cryptic splice site will produce functional neurofibromin. We also have shown that phosphorodiamidate morpholino oligomers (PMOs) can mask the cryptic splice site on the RNA level, thus restoring normal splicing of the variant allele in human induced pluripotent stem cells (iPSCs) with restoration of neurofibromin functionality through GTP-Ras and pERK/ERK testing.

As a final step in establishing pre-clinical modeling for precision therapeutics to treat c.1466A>G (p.Tyr489Cys) NF1 missense alleles, we now report the creation of a humanized mouse model in which mouse exon 13 has been replace with human exon 13 harboring the c.1466A>G variant. Animals heterozygous for the variant ($Nf1^{+/humEX13Tyr489Cys}$) are viable with no discernable phenotype, whereas homozygosity leads to embryonic lethality. To model human disease, we used a HoxB7-cre conditional knockout model to target the deletion of a "floxed" NF1 allele in the context of the variant allele (i.e. $Nf1^{F/humEX13Tyr489Cys}$ mice or HoxB7-$Nf1^{Y489C}$). The HoxB7-$Nf1^{Y489C}$ mice display both plexiform and cutaneous neurofibromas as well as hyperpigmented skin. Preliminary analyses indicate that 13 of 19 (68%) animals display cutaneous neurofibromas (average age of appearance 7 months) with 10 of 16 (63%) animals displaying plexiform tumors (average age of paralysis onset 9 months).

The $Nf1^{F/humEX13Tyr489Cys}$ mouse model will provide essential preclinical data testing the efficacy of antisense oligonucleotide therapies to treat and/or prevent NF1 disease in patients with constitutional c.1466A>G (p.Tyr489Cys) NF1 missense mutations.

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Patient-Derived Xenograft-Based Three-Dimensional Microtissue Model of Malignant Peripheral Nerve Sheath Tumors for Precision Oncology

Alex T. Larsson, BS, *Department of Pediatrics, Masonic Cancer Center, University of Minnesota, Minneapolis*

Purpose: Using *ex vivo* human tumor samples in an extracellular matrix-rich environment, our study addressed the challenges posed by mutational heterogeneity and DNA aneuploidy in developing effective therapies for MPNST in a medium-throughput manner.

Methods: Pre-clinical platforms that rely on immortalized cell lines or genetically engineered mouse models are often not capable of representing the full heterogeneity of genomic alterations present in patient tumors. To address this problem, we developed a patient-derived xenograft (PDX)-based *ex vivo* 3D microtissue platform that maintains MPNST tumor heterogeneity and allows for rapid and cost-effective drug response studies. After implanting and passaging human patient samples in NOD-Rag1^{null} IL2rg^{null} mice, we removed tumors for complete cellular dissociation and assembly into 3D microtissues composed of collagen and Matrigel. We exposed both our individual PDX 3D microtissues and associated *in vivo* PDX models to three drugs - trabectedin, mirdametinib and olaparib. In addition to single agent exposure, we also combined either mirdametinib or olaparib with trabectedin to look for enhanced drug effects. We also performed RNA sequencing on all of the PDX samples.

Results: The PDX tumor models we used successfully reflected the genomic diversity of MPNST and accurately recapitulated the genetic signature of corresponding parental tumors. We identified common genetic alterations in our 13 PDX-MPNST pairs, including germline and somatic *NF1* mutations, MPNST-associated mutations in the PRC2 genes, *SUZ12* and *EED,* as well as genetic variants of *CDKN2A.* We identified trabectedin as a potent single agent against all microtissues tested. Enhanced drug effects emerged when trabectedin was combined with either mirdametinib or olaparib. We found that response to drugs in *ex vivo* microtissues was similar to the response seen *in vivo* with mice harboring PDX. RNA sequencing of PDX showed RAS and NOTCH signaling pathways enriched in those samples that were better able to assemble into viable microtissues than those that could not.

Conclusion: In addition to cost savings, reducing experimental study time and animal sacrifice, we found that our *ex vivo* 3D microtissue platform has the potential to streamline therapeutic options for patients compared to a traditional PDX system. Three-dimensional microtissues developed from PDX lines are an ideal platform to study drug response as they closely mimic the natural tumor microenvironment. We posit that our 3D microtissue system can be modified by adding signaling factors or other cell types to systematically test variables that enhance drug response and could be translated to several other tumor types.

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NF1 Neurological Issues: Potential Roles for Myelin in Their Life-Long Progression

Alejandro Lopez-Juarez, PhD, *Department of Health and Biomedical Sciences, The University of Texas Rio Grande Valley*

Purpose of the study: Most NF1 patients present with variable neurological issues during their life including learning deficits, delayed motor skills, attention deficit, and increased risk for depression and dementia. Interestingly, NF1 patients also present with multiple abnormalities in the brain WM and myelin; although proposed decades ago, myelin-learning links remain obscure in NF1. The overall purpose of our studies is to provide robust experimental evidence for the impact of *Nf1* mutation on myelin and associated behavior and learning.

Methods: Adult male and female mice with *Nf1* mutation in either the germline or specifically in myelin producing cells we tested in a voluntary/myelin-regulated motor learning test, the Complex Wheel (CW; a running wheel with irregularly spaced rungs). Five running parameters were analyzed and compared between WT (control) and hemizygous or homozygous *Nf1* mutant mice.

Results: Adult mice carrying germline *Nf1* mutation (*Nf1*+/- mice) showed progressively abnormal CW learning curves throughout their lifespan. This phenotype was firstly caused by compromised activity levels without affecting capability to run. To test the specific contribution of abnormal mature myelin to learning phenotypes, mice with conditionally induced *Nf1* mutation in mature myelin-producing cells (using the tamoxifen inducible system *PlpCreEr;Nf1flox*) were subjected to the CW; mutant mice showed subnormal learning curves with gender and gene-dose-dependent regulation.

Conclusion: Our results shed light onto the roles of myelin in the control of behavior and learning, particularly on the regulation of a voluntary fine motor skill learning task, potentially relevant for attention deficit and motivation/depression issues in NF1.

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A Cloud-Hosted Software Solution to Enable Data Exploration for Researchers of Varying Computational Skill Levels

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While innovations in 'omics and bioinformatics have proliferated in recent years, harnessing the potential of these technologies to drive breakthroughs in our understanding and treatment of disease often is contingent upon researchers' ability to navigate tools and technologies that require specific computational skills. Here, we present results from a case study in which a purpose-built, cloud-hosted software solution, Manifold Science Cloud, was implemented to enable researchers of varying technical abilities to derive insights from data using data catalogs, dashboards, and one-click computational environments.

Manifold Science Cloud is a suite of purpose-built software applications designed to support end-to-end scientific research workflows, from cohort study participant enrollment and management, through data management and exploration, to data analysis and reporting of findings in research publications. In our case study, we are implementing the Catalog and Analytics applications to enable data analysts and research scientists to more efficiently navigate and derive insights from neurofibromatosis study data using interactive dashboards and charts. With the aim of understanding impact on ability of researchers to answer scientific questions using available data without support from more technical team members, we are tracking metrics related to researchers' use of the Science Cloud solution relative to previously employed solutions: Time taken to address scientific questions using available data and improvements to researchers' abilities to "self-serve" answers to exploratory data questions using "no-code" solutions such as dashboards.

Our findings indicate that improvements to data centralization, discoverability, and accessibility enabled by use of the Catalog and Analytics applications have streamlined and expedited workflows for researchers, enabling those without strong computational backgrounds to derive insights from data with significantly less overhead. Moving forward, we will increase the access and usability of this data by combining this functionality into the broader NF data ecosystem and facilitating analyses of data hosted on Synapse and the NF Data Portal. The cohesive efforts of the Manifold Science Cloud and Synapse teams will enable researchers of all computational abilities a seamless path to exploring data.

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Transcriptomic Profiling of Cutaneous Neurofibromas from NF1 Patients

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Neurofibromas are a cardinal feature of neurofibromatosis type 1 (NF1), which results from inherited or spontaneous mutations in the neurofibromin gene *(Nf1)*. These benign tumors are very complex at the cellular level. They are driven by Schwann cells (SCs) that have undergone loss-of-heterozygosity for *Nf1* (1, 2), but also depend on a microenvironment of multiple cell types that are haploinsufficient for *Nf1* (3, 4). Dermal or cutaneous neurofibromas (cNFs) occur in most NF1 patients. In contrast to plexiform neurofibromas, cNFs do not bear a significant risk of malignant transformation (5). We do not yet know how to prevent their development and treatment options are limited. These tumors are, however, a principal concern for many NF1 patients. They typically increase in number and size over time, are often associated with chronic pain and itch, may produce cosmetic disfigurement, and frequently impose psychosocial burdens (6).

Our goal is to identify cell populations and sub-populations within cNF tissue samples to develop a better understanding of these complex tumors and make progress toward identifying effective treatments. Using fresh-frozen cNF samples from the NF1 biospecimens repository at Johns Hopkins University, we have explored the feasibility of single-cell sequencing. Single-cell techniques with fresh-frozen tissue samples are challenging, especially in the context of fibrous tissue which requires extended dissociation for several hours. Although it does not provide single cell resolution (3-10 cells per 55 micrometer diameter spot), spatial transcriptomics provides context for the relationship between cell types and does not require tissue dissociation. We have successfully completed in-situ transcriptomics analysis of 16 cNF samples from different patients using the Visium 10x Genomics platform. Bioinformatic analyses of these combined results are expected to reveal significant new information on the cellular microenvironment of cNF.

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Unbalancing cAMP and Ras/MAPK Pathways as a Potential Therapeutic Strategy for Cutaneous Neurofibromas

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The development of multiple cutaneous neurofibromas (cNFs) constitute 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. To completely understand cNF formation, the role of cell-cell interactions needs to be better characterized. We identified a transcriptional signature due to the interaction of human cNF-derived *NF1*(-/-) SCs and *NF1*(+/-) fibroblasts (FB), being the proton-sensing G protein-coupled receptor GPR68 one of the upregulated genes that caught our attention (Mazuelas et al. 2022 b.).

Activation of Gpr68 using Ogerin, increased cAMP levels and decreased SC proliferation and viability in both cNF-derived SC cultures and SC-FB co-cultures. Contrarily, Ogerin did not affect cNF-FBs and was neither toxic for *NF1*(+/-) skin-derived fibroblasts. Since a coordinated activation of cAMP and Ras/MAPK pathways is required for balancing SC proliferation vs differentiation and neurofibromin has been implicated in both pathways, we analyzed the functional impact resulting from unbalancing both pathways by Selumetinib and Ogerin co-treatment. The coordinated activation of cAMP and inhibition of Ras/MAPK pathways resulted in SC myelinization, decreased cell viability, and increased proportion of cells in early and late apoptosis in cNF-derived SC cultures. The use of cAMP analogues replacing Ogerin in co-treatment experiments using SC cultures generated the same response. Furthermore, co-treatment also increased cell death in a 3D iPSC-based neurofibromasphere model (Mazuelas et al. 2022 a.) composed of differentiating SCs and cNF-derived FBs. We are currently exploring additional activators and inhibitors of the cAMP pathway in combination to MEK inhibitors in cNF-derived SC cultures and in 3D neurofibromaspheres and assessing their physiological impact.

In conclusion, we identified a potential therapeutic approach for treating cNFs resulting from the simultaneous activation of the cAMP pathway and inhibition of the Ras/MAPK pathway that drives SC differentiation and cell death in 2D and 3D systems.

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Econazole Selectively Induces Cell Death in *NF1*-Homozygous Mutant Tumor Cells

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Purpose: Cutaneous neurofibromas (cNFs) are benign peripheral nerve sheath tumors that develop in a majority of patients with Neurofibromatosis Type 1 (NF1). However, the therapeutic options for cNF are limited, reflecting the need for the development of novel medical treatments to target tumor growth. In this study, we aimed to identify drugs that could be repurposed for cNF treatment.

Methods: We screened a library of 2,570 FDA-approved drugs using human induced pluripotent stem cells (hiPSCs) from NF1 patients *(NF1+/-).* To generate *NF1-/-* hiPSCs, we used CRISPR/Cas9 to engineer an *NF1* mutation in the second allele to mimic loss of heterozygosity. *NF1+/-* and *NF1-/-* hiPSCs were differentiated into Schwann cell precursor cells (SCPs) and these cells were used to screen a drug library to assess for selective growth inhibition of *NF1-/-* hiPSC-SCP but not *NF1+/-* hiPSC-SCP cells. We also measured apoptosis and cell proliferation and confirmed our findings with mouse Schwann cell precursors. Positive medication from our *in vitro* drug screen was subsequently tested either alone or in combination with MEK inhibitor, and vehicle treatment using two *ex vivo* human neurofibroma assays. We assessed for apoptosis in cNF tumor tissue and adjacent non-tumor skin at various time points.

Results: We identified a clinically approved medication, the antifungal drug econazole, in our drug library screen: Econazole selectively 1) inhibited proliferation and 2) induced apoptosis in *NF1-/-* hiPSC-SCPs and mouse SCPs. In our *ex vivo* model system, we found that topical econazole cream selectively induced apoptosis in human neurofibroma tumor tissue and the effect was significantly greater than with vehicle treatment. There was no evidence of cell death in adjacent non-tumor tissue with econazole treatment. Mechanistically, we found that econazole treatment does not downregulate phospho-ERK levels, indicating that econazole likely acts downstream of or independently from the MAPK signaling pathway to selectively induce apoptosis in *NF1-/-* tumor cells.

Conclusions: In sum, using a human stem cell-based disease model, we have identified the anti-fungal cream econazole as a potential therapeutic drug with selective anti-tumor activity in NF1-/- neurofibroma cells by efficiently inducing apoptosis in the tumor tissue. This study nominates econazole as a potential treatment for cNF and supports further testing in clinical trials.

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

Julia Nikrad, *University of Minnesota*

Purpose: Synthetic lethality (SL) occurs when simultaneous alterations in two or more genes lead to the loss of viability of the cell, while the same alterations in either gene alone do not result in the loss of viability. This phenomenon can produce vulnerabilities that can be therapeutically exploited to selectively kill tumor cells, greatly expanding the current range of potential targets for anti-cancer drugs. Our hypothesis is that *NF1*-null or *NF1*/*SUZ12*-null Schwann cells may be selectively vulnerable to the loss of function of another gene or genes that are not essential to isogenic *NF1*-proficient cells. To test this, we have performed a genome-wide CRISPR/Cas9 knockout screen for SL in *NF1*- and *NF1*/*SUZ12*-deficient human Schwann cells and in several malignant peripheral nerve sheath tumor (MPNST) cell lines. *NF1*/*SUZ12* knock out in engineered Schwann cells mimics plexiform neurofibroma-like cells and also PRC2 deficient MPNST. The **purpose** of this study is to identify new SL genes in *NF1*-deficient and *NF1*/*SUZ12*-deficient cells to drive selective cell death in peripheral nerve sheath tumors associated with NF1 syndrome.

Methods: We utilized a CRISPR/Cas9-mediated gene knockout approach to generate *NF1* and *NF1*/*SUZ12* mutant isogenic cell lines, starting with two independently derived immortalized human Schwann cells. Subsequently, the cells and established MPNST cell lines were engineered to stably express Cas9 nuclease. We performed genome-wide CRISPR screens for synthetic lethality in *NF1*- and *NF1*/*SUZ12*-deficient human Schwann cells and MPNST cell lines, in a high-throughput, pooled format. We collected cells at two time points after negative selection and analyzed guide frequencies to identify potential SL targets. Additionally, we developed a medium-throughput synthetic interaction validation pipeline for candidate guides.

Results: Our CRISPR screens identified a number of potential SL candidate genes unique to each genotype, as well as some found in both the *NF1*- and *NF1*/*SUZ12*-deficient contexts. Pathway analysis revealed that these SL genes belong to many pathways associated with ribosome biogenesis, RNA splicing and processing (*NF1*-null) and DNA replication, chromosomal organization, and cell cycle (*NF1*/*SUZ12*-null). We are currently validating the identified targets through the synthetic interaction validation pipeline.

Conclusion: Genome-wide CRISPR screens for SL are a promising approach to identify novel targets for targeting non-druggable cancer mutations, by exploiting second-site targets that may be druggable. Our study identified potential SL genes and pathways in *NF1*-deficient and *NF1*/*SUZ12*-deficient cells, which could be further explored for selective cancer therapy.

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NF1 Genetic Background in the Tumor Microenvironment Govern Transcriptomic Landscape of Tumor Cells in Malignant Peripheral Nerve Sheath Tumors

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Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas for which there is no effective treatment to date. They usually arise in the context of the genetic disease neurofibromatosis type I (NF1) or sporadically. While in both types of MPNSTs, the tumor Schwann cells exhibit biallelic loss of NF1, their cellular microenvironment is NF1 heterozygous (NF1+/-) and wild-type (wt), respectively.

In this study, we address the impact of NF1 heterozygosity in the tumor microenvironment on tumor cell progression in NF1-induced and sporadic MPNSTs. We designed and characterized an Nf1 mouse model with simultaneous biallelic loss of Nf1 and Tomato reporter expression in boundary cap cells and their derivatives (Radomska et al., 2019). Mutants were generated on an NF1 heterozygous (Prss56-Nf1KO, NF1+/-) and wt (Prss56-Nf1KO) genetic background. Both types of mutants develop cutaneous and plexiform neurofibromas. Interestingly, in about 70% of the mutants, pNFs evolve spontaneously, between 7 and 12 months, into true MPNSTs.

We performed extensive scRNAseq analysis (10x Genomics) of 16 Prss56-Nf1KO and 5 Prss56-Nf1KO, NF1+/- tumors. We annotated 10 distinct cell populations, including tumor (Tom+) cells, various immune populations, fibroblasts, and endothelial cells in each dataset. Analyses of the proportions and transcriptomic signatures of each cell population in relation to the tumor genetic background led us to the following observations: (i) among tumor cells, we distinguished two distinct subpopulations, either with a glial or with a mesenchymal profile, without cells sharing both signatures, suggesting a rapid glial-to-mesenchymal switch, (ii) while on NF1+/- background, both types of tumor cells (glial and mesenchymal) co-exist in the same tumor; on wt background, virtually all tumor cells are either glial or mesenchymal, in the same sample, (iii) differential expression analysis reveal that tumor cells on NF1+/- background over-express a panel of pro-fibrotic genes, compared to wt background. Such pro-fibrotic profile may be related to over-activation of pro-inflammatory signaling observed pathways-related genes in NF1+/- versus wt micro-environment cells.

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Mutant iPSC-Derived Schwann Lineage Cells as a Novel Drug Discovery Platform for Peripheral Nerve Sheath Tumors

Daniel Panken, BS, *University of Minnesota-Twin Cities*

Neurofibromatosis Type I (NF1) is an autosomal dominant tumor predisposition syndrome characterized by a germline mutation that inactivates one allele of the RAS- GAP, *NF1.* Loss of the remaining wild-type *NF1* allele leads to complete inactivation of neurofibromin function and hyperactive RAS pathway signaling. *NF1* deficient Schwann cells have the potential to form benign plexiform neurofibromas (PNFs) and further mutation in both *CDKN2A* and *CDKN2B* can lead to more proliferative premalignant atypical neurofibromas (ANFs). Furthermore, inactivation of the polycomb repressive complex 2 (PRC2) through loss of function mutations at *SUZ12* or *EED* loci can promote transformation to malignant peripheral nerve sheath tumors (MPNSTs). These highly recurrent mutations can be recapitulated and studied in induced pluripotent stem cell (iPSC) models to better understand tumor development and discover treatments for ANFs and MPNSTs. Using a predefined differentiation protocol, iPSCs from three individual donors have been successfully differentiated into Schwann cell precursors (iSCPs) and Schwann Cells (iSCs). Using CRISPR/Cas9, isogenic iPSC clones were generated with either loss of *NF1* alone or in combination with *CDKN2A/B* loss. These mutant iPSCs showed a perturbed ability to differentiate to iSCPs by qRT-PCR analysis. The resulting mutant iSCPs were subjected to high throughput drug screening and demonstrated resistance to single agent MEKi or CDKi compared to wild-type iSCPs. Wild type iSCPs were also edited using CRISPR/Cas9 ribonucleoproteins to knockout *NF1* and *CDKN2A/B* to understand whether the timing of genetic mutations is relevant to drug response and tumor development. Further differentiation of mutant iSCPs to iSC and additional gene knockouts in wild type iSCs are underway. Ongoing *in vitro* and *in vivo* studies are in-progress to compare how the presence and timing of these and other genetic alterations affect tumorigenicity and drug sensitivities in this new model.

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Disclosure Statement: D.A.L. is the co-founder and co-owner of several biotechnology companies, including NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immusoft, Inc.), B-MoGen Biotechnologies, Inc. (recently acquired by Biotechne Corporation), and Luminary Therapeutics, Inc. D.A.L. holds equity in, serves as an advisor to Styx Biotechnologies and as a Senior Scientific Advisor for and a Board of Director member for Recombinetics, a genome editing company. D.A.L. consults for Genentech, Inc., which is funding some of his research. The business of all these companies is unrelated to the contents of this research.

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Myelolytic Treatments Can Augment the Therapeutic Benefit of Oncolytic Virus in Malignant Peripheral Nerve Sheath Tumors by Modulating the Tumor Microenvironment

Siddhi N. Paudel, *The Ohio State University*

The unprecedented response of the FDA-approved oncolytic virus Talimogene Laherparepvec (T-VEC) in melanoma patients marks a breakthrough in translating immune-based strategies to the clinic, but the immunosuppressive microenvironment remains a key challenge for advancing its use against other solid tumors. While myeloid cells are known to orchestrate tumor progression and immune suppression in Malignant Peripheral Nerve Sheath Tumors (MPNST), their role(s) in influencing the therapeutic response of immune-based strategies like oncolytic virotherapy remains elusive. We hypothesized that inhibiting the activities of myeloid cells or depleting them could potentiate oncolytic virotherapy and enhance the antitumor immune response against MPNST. To test this hypothesis, we implanted murine MPNST cell lines subcutaneously into immunocompetent syngeneic mice. We then treated these mice with three intra-tumoral doses of oncolytic herpes simplex virus and compared the relative frequency of immune cells present in the tumor microenvironment using flow cytometry. The frequency of T cells (both CD4 and CD8) increased significantly after treatment with the virus by day 11 after virus injection. Following these promising results, we examined the relative therapeutic efficacy of T-VEC against MPNST tumors established in immunocompetent mice. While we found T-VEC to be marginally effective as a monotherapy, combining it with the myelolytic therapies pexidartinib or trabectedin resulted in a statistically significant increase in survival in immunocompetent C57BL/6 or Balb/c tumor models respectively. Subsequent flow cytometry analysis of mice treated with a combination of T-VEC and trabectedin revealed a decrease in myeloid-derived suppressor cells and increase in CD8/Treg ratio in the combination treatment groups compared to the virus alone. Our results suggest that a rational combination of myelolytic therapies can leverage the therapeutic benefit of immune-based strategies in MPNST.

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Schwann Cells and Skeletal Stem/Progenitor Cells Drive Fibrosis and Congenital Pseudarthrosis of the Tibia in NF1

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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 (cutaneous and plexiform neurofibromas, NFBs), skin hyperpigmentation (café-au-lait macules, CALMs) 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 fractures 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 (Radomska et al.).

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 required for bone repair. *NF1* biallelic inactivation was detected in skeletal stem/progenitor cells (SSPCs) in pathological periosteum and these SSPCs exhibit a pro-fibrotic phenotype. Analyses also revealed an increase proportion of pERK+ Schwann cells (SCs) in pathological periosteum suggesting that SCs are also affected in CPT. 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. We demonstrate that TGFβ inhibition prevents pseudarthrosis in *Prss56-Nf1 KO* mice. Overall, our study deciphers the mechanisms of CPT and shows that CPT is caused by boundary cap-derived SCs and SSPCs. Thus, CPT shares common origin and mechanisms with other NF1 manifestations, including NFBs, suggesting new therapeutic strategies targeting SC-derived pro-fibrotic factors, including TGFβ, for CPT and other NF1 symptoms.

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Revisiting the cis*Nf1*$^{+/-}$*p53*$^{+/-}$ Mice Model

Camille Plante

NF1 patients frequently develop a benign tumor in peripheral nerve plexuses called plexiform neurofibroma. In the past two decades, tissue-specific *Nf1* knockout mice models were developed using commercially available tissue-specific Cre recombinase and the *Nf1* flox mice to mimic neurofibroma development. However, these models develop para-spinal neurofibroma, recapitulating a rare type of neurofibroma found in NF1 patients. The cis*Nf1*$^{+/-}$*p53*$^{+/-}$ mice model developed a malignant version of neurofibroma called malignant peripheral nerve sheath tumor (MPNST) within 3 to 6 months but intriguingly without apparent benign precursor lesion. Here, we revisited the cis*Nf1*$^{+/-}$*p53*$^{+/-}$ model (n=61) and discovered that about 20% display clinical signs [kyphosis (hunched posture) and ambulation difficulties] similar to *Nf1* tissue-specific knockout mice models. However, systematic histological analysis could not explain the clinical signs although we noticed lesions reminiscent of a neurofibroma in a peripheral nerve, a cutaneous neurofibroma, and para-spinal neurofibroma on rare occasion in cis*Nf1*$^{+/-}$*p53*$^{+/-}$ mice. We also observed that 10% of the mice developed a malignant peripheral nerve sheath tumor (MPNST) spontaneously, coinciding with their earring tag identification, suggesting that injury-induce MPNST development. Therefore, we intentionally injured the sciatic nerves of cis*Nf1*$^{+/-}$*p53*$^{+/-}$ mice. Strikingly, half of the sciatic nerves from cis*Nf1*$^{+/-}$*p53*$^{+/-}$ mice developed plexiform neurofibroma within 1-6 months when intentionally injured (n=18). Thus, we provided a procedure to turn the widely used cis*Nf1*$^{+/-}$*p53*$^{+/-}$ sarcoma model into a model recapitulating human plexiform neurofibroma.

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Decipher the Mechanisms Driving Cutaneous Neurofibromas Development in a Mouse Model of Neurofibromatosis Type I

Pernelle Pulh, PhD Student, *Mondor Institute for Biomedical Research, Créteil, France*

Cutaneous neurofibromas (cNFs) are benign skin tumors present in all patients with Neurofibromatosis type I (NF1). They represent a major cosmetic burden and there is no treatment to prevent or treat their development. In this context, the laboratory designed an NF1 mouse model (*Prss56*$^{Cre/+}$*, R26*$^{Tom/+}$*, Nf1*$^{fl/fl}$*; Nf1*-KO) in which boundary cap cells and their derivatives are mutated for the *Nf1* gene and express the *Tomato* reporter gene, allowing survey of mutant cells through their fluorescence. Mutant animals spontaneously develop cNFs whose cellular composition recapitulates that of cNFs from NF1 patients[1]. Moreover, we have shown that skin trauma accelerates the growth of these tumor in this model. This observation has led us to conceive an inducible mouse model (*Nf1*-KOi) in which cNFs growth is triggered by bites of housed-grouped males. Such "induced" cNFs successfully recapitulate spontaneously developing tumors, characterized by an accumulation of Schwann cells (SCs), fibroblasts, immune cells, axons, and collagen in the dermis similarly to previously described human cNFs[2,3].

The goal of this study is to decipher cellular and molecular mechanisms driving development of cNFs using *Nf1*-KOi model. First, immunohistochemical analyses were performed to establish the kinetics of cellular changes taking place in *Nf1*-KOi mouse skins at different postnatal timepoints and three stages of cNFs development were identified: initiation, progression, and stabilization. Interestingly, mutant SCs proliferative activity was only transient and limited to the initiation and progression phases while most SCs from stabilized tumors appear quiescent with undetectable MAPK activity[4]. Across all stages, cNFs present defects in innervation, characterized by a dense network of defasciculated nerves, a phenotype that we also observed in cNFs from NF1 patients.

To characterize molecular changes occurring during cNFs growth, we aim to perform single-cell RNA sequencing (scRNAseq) analyses (10x Genomics) at each stage. To do so, we evaluated the efficacy of various skin dissociation protocols on scRNAseq data encompassing cellular composition and transcriptomic signature of specific cell types. Importantly, we observed that while short (1h30) incomplete dissociation of the tumor can lead to under-representation or even omission of some tumor cell populations, prolonged treatment (18h) in turn leads to significant modification of tumoral signature mainly associated to crosstalk between tumor cells and their microenvironment.

Preliminary scRNAseq analyses of mature cNFs (stabilization phase) suggest minor immune system modifications at this stage and point to profibrotic activity of tumor SCs and of a particular dermal fibroblast population compared to adjacent healthy-looking skin.

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Contribution of Sterile Inflammation Through STING and T Cells to Plexiform Neurofibroma Initiation and Growth

Jay Pundavela, PhD, *Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center*

Aim: We sought to define pathways promoting a proinflammatory environment and lymphocyte recruitment in plexiform neurofibromas (PNF).

Background: PNFs are peripheral nerve tumours prevalent in patients with Neurofibromatosis type 1 (NF1). Immune cells including macrophages, dendritic cells (DC) and T cells are present in the PNF microenvironment, but what drives CXCL10 cytokine driven CXCR3+ DC/T cell recruitment is unclear. Here, we identified the contribution of stimulator of interferon genes (STING) pathway to inflammation and DC/T cell infiltration for PNF formation.

Methods: To test the role of STING, a selective inhibitor of active STING (H-151) was administered in Nf1f/f; DhhCre mice, and we generated neurofibroma mice harbouring a missense mutation of the transmembrane protein 173 locus that resulted in the absence of STING protein (Tmem173gt/gt;Nf1f/f;DhhCre).

Results: Immunohistochemistry of human (Fig. 1) and murine pNF tissues show the activation of STING pathway indicated by the phosphorylation of STING (pSTING) and TBK-1 (pTBK-1) compared to normal nerves. Colabeling shows pSTING+ cells localized with CD45+ cells. A 60-day treatment of Nf1f/f; DhhCre mice with H-151 reduced pSTING, pTBK-1, a 2-fold decrease in plasma CXCL10/IP10, reduction in tumour number/size, and CD3+ T cells compared to the vehicle-treated mice. MRI imaging of Tmem173gt/gt;Nf1f/f; DhhCre mice show reduced circulatory T cells and tumours. In addition, CD8 T cells were necessary for neurofibroma formation.

Conclusions: We conclude that STING activation contributes to inflammation, and CD8 T cell lymphocyte recruitment is necessary for PNF formation.

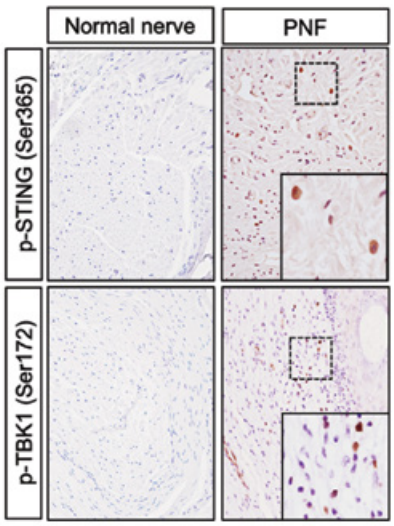

Figure 1. Activation of STING pathway in human PNF.

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The Impact of Neurofibromatosis Type 1 on Tumor Biomechanics

Micah Rambo, *Rensselaer Polytechnic Institute*

Purpose: To identify cancer biomarkers for NF1 patients, we initiated biomechanical studies characterizing healthy and tumor tissues from mouse models of PNF and MPNST.

Introduction: Altered cell and tissue mechanics are linked to tumor initiation, growth, and metastasis.[1] Previous work suggests the likelihood of biomechanical change in NF1 tissues through altered fibroblast contractility and extracellular matrix deposition.[2] Despite this, the biomechanics of NF1 tumors have not been studied. Here, we identify the impacts of NF1 on tumor tissue biomechanics.

Materials and Methods: PNFs were generated spontaneously in the dorsal root ganglion (DRG) of *Nf1*$^{Fl/-}$; *Krox20*$^{Cre/+}$ mice.[3] MPNSTs were generated using a CRISPR-Cas9 injection in the sciatic nerve (SN) for NF1 and wildtype (WT) mice.[4] Tumor progression was monitored, and mice were humanely euthanized at humane or tumors endpoints (1 cm). Tumors and control tissues were excised and biopsied for mechanical testing on an atomic force microscope (AFM). Using a cantilever fit with a spherical microbead tip on the AFM, we generate force vs indentation curves that are fit with the Hertz contact model to find the elastic indentation modulus of the tissues. All mouse work was performed in accordance with protocols approved by the RPI IACUC and under the supervision of a resident veterinarian. Histological sections were stained following standard immunofluorescence or hematoxylin and eosin (H&E) protocols.

Results and Discussion: We characterized the mechanical properties of healthy DRG and SN. Our AFM measurements of the tissues revealed a similar stiffness and distribution between the tissues. Initial mechanical data from an NF1 tumor and WT tumor were taken, revealing the NF1 tumor had a significantly greater stiffness than both healthy environments and the WT tumor. Our initial findings also showed the NF1 tumor displayed varying stiffness based on tumor region. The NF1 tumor was softest at the tumor core and the stiffest region at the tumor edge. Combining these measurements with H&E images we will generate stiffness and structure profiles for all tumors and tissue types and elucidate the effects of NF1.

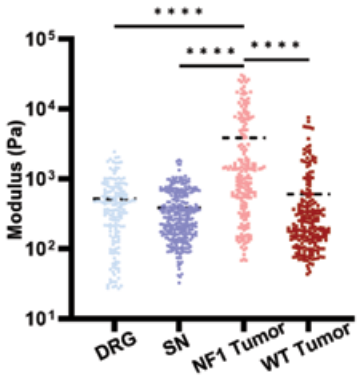

***Figure**. Distributions of measured elastic moduli for all tissue types tested.*

Conclusions: Initial results showed the NF1 tumor displayed greater stiffness compared to WT tissues and tumors, suggesting the NF1 genotype may play an important role in tumor biomechanics. This work plays a crucial role in understanding the mechanism driving the mechanobiology of tumor formation in NF1 as we elucidate the cause of malignant transformation of PNF to MPNSTs.

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Schwann Cell Calcium and Growth Factor Signaling Modulates Pain in NF1

Namrata G.R. Raut, PhD, *Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

Purpose: In the current study, we wanted to determine whether targeting growth factors or calcium signaling in Schwann cells (SCs) could blunt pain-like behaviors in a preclinical model of neurofibromatosis 1 (NF1).

Methods: We performed evoked and 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$^{f/f}$) and littermate controls to analyze the role of SC in pain development. We then performed similar assessments in animals wildtype for *Nf1* or in the DhhCre;Nf1$^{f/f}$ mice with chemogenetic (designer receptors exclusively activated by designer drugs: DREADD) manipulation of SC calcium activity. We finally used glial cell line-derived neurotrophic factor (GDNF) -targeting antibodies to assess the effect of growth factors on mechanical hypersensitivity.

Results: We found that *Nf1* deletion in SCs produces mechanical hypersensitivity (**Fig.1:** *Deletion of Nf1 in SCs induces behavioral mechanical hypersensitivity compared to controls; *p<0.05 vs. control, t test. (F). *p<0.03, #p<0.1 vs. controls 1-way ANOVA. Mean ± SEM; n=20 control; n=12 mutant*). Behavioral hypersensitivity in the SC specific *Nf1* mutants, correlated with sensitization of myelinated A-fiber nociceptors (**Fig. 2:** *Ex vivo recording of individual high threshold mechanoreceptors (HTMR) show significant mechanical hyper-responsiveness in mice with SC specific deletion of Nf1 (DhhCre;Nf1$^{f/f}$) vs controls; (WT C57, n= 9; Nf1$^{f/f}$, n=9, mutant, n=7; **p<0.08 vs. Nf1$^{f/f}$, 1-way ANOVA with Tukey's post hoc; mean ± SEM; Total no. of cells, WT C57, n=45; Nf1$^{f/f}$, n=57, mutant, n=37*)) and unmyelinated polymodal C-fibers (CPM) to mechanical stimuli (not shown) compared to controls using *ex vivo* recording. We then found that chemo-genetic activation of SC calcium activity was able to effectively induce mechanical hypersensitivity at the behavioral level in wildtype mice (not shown), while inhibiting calcium using DREADDs (hM4Di) in DhhCre;Nf1$^{f/f}$ mice reduced hypersensitivity in a separate behavioral choice assay (**Fig. 3:** **p value<0.05 vs controls (RM ANOVA, Tukey's post hoc); *p<0.05 vs. control, before compound21 injection but no difference observed after 7 days of compound 21 injection i.p.; n=16 control, n=7 mutant*). Similarly, systemic targeting of GDNF using antibodies (**Fig. 4**) showed a reduction in mechanical hypersensitivity in the DhhCre;Nf1$^{f/f}$ mouse ((*p = 0.009 vs controls, Two-way ANOVA, with Tukey Post-hoc; Data shown as mean ± SEM *n=19 control, n=8 mutant*).

Conclusions: Data has shown a role for SC in the onset of pain due to NF1. This study has begun to elucidate that calcium and growth factor signaling may serve as therapeutic treatment strategies to ameliorate pain in NF1 patients.

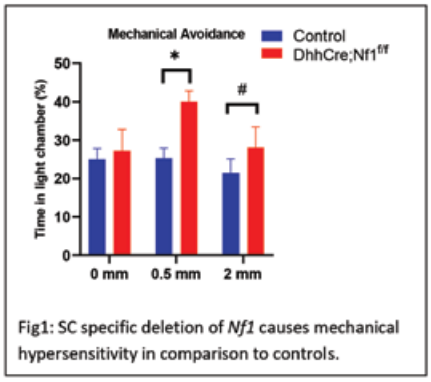

Fig1: SC specific deletion of *Nf1* causes mechanical hypersensitivity in comparison to controls.

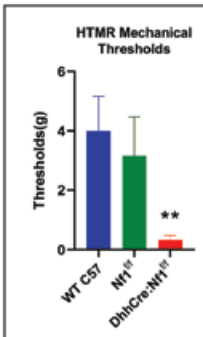

Fig. 2: *Ex vivo* recording of individual high threshold mechanoreceptors (HTMR) show significant reduction in their mechanical thresholds in mice with SC specific deletion of Nf1 (Dhh;Nf1$^{f/f}$) vs controls (WT C57 and Nf1$^{f/f}$).

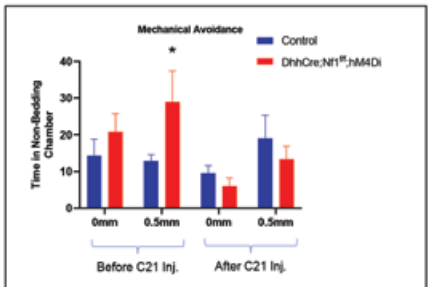

Fig 3: Chemogenetic inhibition of SCs using C21 suppresses mechanical hypersensitivity in DhhCre;Nf1$^{f/f}$;hM4Di mice.

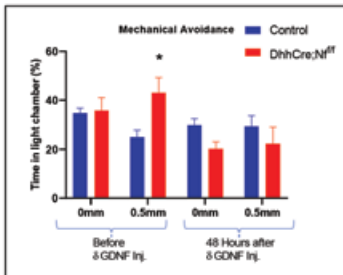

Fig 4: 48hrs after being injected (i.v.) with GDNF targeting antibody, mechanical avoidance is reduced to control levels in DhhCre;Nf1$^{f/f}$ mice.

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Towards the Identification of the HLA Class I Immunopeptidome of Malignant Peripheral Nerve Sheath Tumors via Mass Spectrometry

Kyle Richards, *Masonic Cancer Center, Department of Pediatrics, University of Minnesota*

Purpose: Most malignant nerve sheath tumors (MPNST) are HLA class I positive and some have been reported to respond to immune checkpoint blockade. This suggests that prophylactic or treatment focused vaccination, or T cell receptor transgenic T cells, may be beneficial for MPNST. This is a novel study of immunopeptidomics and customized bioinformatics to characterize antigen presentation by MPNSTs.

Methods: The B cell line Raji, and MPNST cell lines are being used to isolate neoantigens. We've found that MPNST express HLA class II along with MHC class I proteins. We purified both HLA complexes by immunoprecipitation. Peptides were purified from complexes utilizing C18 resin and molecular weight cut off filters. Analysis and identification of peptide epitopes was performed using an Orbitrap Fusion LC-MS system as well as an Orbitrap Eclipse LC-FAIMS-MS system. MS/MS spectra were matched to the human Uniprot reference proteome plus contaminants and novel neoantigen peptide sequences predicted from a custom transcriptome pipeline. The FragPipe software and tools in the Galaxy for proteomics (Galaxy-P) platform were used for peptide spectrum match (PSM) generation and verification.

Results: Optimization of immunoprecipitation and LC-MS/MS conditions were done using Raji cells, which is HLA class I high and grows to high density. We successfully identified thousands of HLA class I peptides per experiment. Peptides isolated match the predicted length of class I bound peptides, centering around 9 amino acids.

Our bioinformatic workflow predicts peptide binding to MHC complexes based on HLA genotyping. The tools used for generating PSMs from the MS/MS data delineate antigens arising from processing of reference protein sequences and from neoantigens arising from non-normal mRNA processing. The FragPipe suite of tools offers efficient PSM generation for non-tryptic antigen peptides, while Galaxy-P tools offer a means to verify confidence in these matches, including confirmation of neoantigens with novel sequences differing from those found in the reference proteome. Ongoing experiments on MPNST lines will be described.

Conclusions: Currently, we are working on optimizing conditions to fully solubilize both HLA class I and II complexes from MPNST cells, followed by serial enrichment of the two different complexes. Tools for label free quantitative analysis of peptidomic data will provide a means to compare epitope presentation under different conditions. As the analytical workflow is optimized, a future goal will be to add neoantigen candidates to the analysis of MPNST LC-MS/MS data for their detection, followed by further investigation of these detected candidates for use in designing prophylactic vaccines.

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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 visual processing abnormalities. Additionally, children with NF1 have high rates of attention deficit/hyperactivity disorder (ADHD), in which difficulties with attentional orientation are associated with a 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 previously discovered that *Nf1* haploinsufficiency causes hypersensitivity to salient visual stimuli, including a looming disc that promotes rapid escape to an available shelter by simulating predator approach from above. NF1 model mice also displayed increased dopamine release in the ventral striatum in response to visual stimuli, which correlated with deficits in attentional control. Here, we present evidence that these phenotypes are driven by hyperactive activation of mitogen-activated protein kinase (MAPK) signaling, as mice containing a gain-of-function mutation in the *Mapk1* gene that codes for ERK2 is sufficient to recapitulate behavioral and neurophysiological abnormalities observed in $Nf1^{+/-}$ mice. In order to identify neural circuit mechanisms contributing to visual hypersensitivity in NF1, we have electrophysiologically characterized retinal ganglion cell firing responses to light stimuli in intact retinas from $Nf1^{+/-}$ and wildtype littermate mice. We have also employed viral vector-based tracing methods to determine how *Nf1* haploinsufficiency contributes to retinal and visual system connectivity. Unpublished results of these studies will be presented, which will provide a comprehensive update on our efforts to identify molecular and circuit-level mechanisms contributing to the cognitive symptoms of NF1.

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Nonsense Suppression is a Viable Approach for Restoring Full Length Neurofibromin Protein and Function

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20% of Neurofibromatosis 1 (NF1) patients carry a germline nonsense mutation in the *NF1* gene, which encodes the protein neurofibromin. This class of mutation generates a premature termination codon (PTC) in the transcribed mRNA that typically prevents expression of a full-length protein. Suppressing translation termination at the resulting PTC, a process called readthrough, can rescue partial levels of full-length, functional protein expression. In this study, we optimize conditions for readthrough of *NF1* PTCs and examine small molecules for the ability to rescue neurofibromin expression and function *via* readthrough. The readthrough compounds tested include eRF1 degraders that deplete the eRF1 termination factor, eRF3 degraders that deplete the eRF3 termination factor, an aminoglycoside that induces ribosomal misreading, and an aminoglycoside enhancer that stimulates aminoglycoside-mediated readthrough *via* an undefined mechanism.

We assessed the ability of these compounds to increase neurofibromin expression and function in two immortalized Schwann cell lines (obtained from Dr. Margaret Wallace, U. Florida). The i28cNF cell line is compound heterozygous for the *NF1* nonsense mutations S1078X/S1053X. The ipn02.8 cell line carries wild-type NF1 and served as a positive control. Western blots were performed to quantify levels of neurofibromin expression; neurofibromin function was assessed by quantifying levels of Ras-GTP and phosphorylated ERK (p-ERK) protein. Low levels of full-length neurofibromin were restored by both eRF1 and eRF3 degraders; neurofibromin abundance was further increased with combination treatments. Reductions in the p-ERK to total ERK ratio, indicating restoration of functional neurofibromin, was observed in the PTC-containing Schwann cells treated with the eRF1 degrader, the eRF3 degrader, and low concentrations of aminoglycosides. Additionally, an acute CAGGs- CreERT2(*Nf1*$^{Flox/R816X}$) NF1 mouse model treated with an aminoglycoside showed a significant phenotypic improvement (in this model, a 30-50% extension in life span) compared to controls. Further *in vivo* studies are ongoing.

In summary, we have identified several readthrough compounds that can rescue partial neurofibromin expression and function by suppressing translation termination at *NF1* nonsense mutations. Using this nonsense suppression strategy, we were able to prolong the life span of NF1 mice expressing a nonsense mutation. This suggests that nonsense suppression could potentially be a new and effective treatment for NF1 in patients who carry a PTC.

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Taming the Metabolism of Tumor Associated Macrophages to Fight NF1-Related Tumors

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Neurofibromatosis type 1 (NF1) is a genetic syndrome caused by germline loss-of-function mutations in the *Nf1* gene encoding the Ras-GAP Neurofibromin. A fraction of NF1 patients develops malignant peripheral nerve sheath tumors (MPNSTs), aggressive sarcomas that are unresponsive to conventional treatments. MPNSTs are the leading cause of death among NF1 patients, making effective treatment strategies for MPNSTs an urgent need. In our previous research, we demonstrated that MPNST cells rely on the mitochondrial molecular chaperone TRAP1, a key metabolic regulator, for their neoplastic growth and we have designed a selective TRAP1 inhibitor that can decrease their tumorigenic properties[1,2].

MPNSTs grow in a complex tumor microenvironment, whereby they interact with a number of cell types, including tumor associated macrophages (TAMs). TAMs are emerging as important players in favoring the growth of many cancers, especially in advanced stages. Yet, nothing is known on the functions played by TAMs in MPNST microenvironment, and *the molecular crosstalk between MPNST cells and TAMs has not been characterized.*

Purpose of the study: The aim of the project is to investigate the cross-talk between MPNST cells and macrophages to identify both factors that drive macrophage polarization towards a pro-neoplastic, TAM-like phenotype, and TAM-derived metabolites that sustain the neoplastic growth of MPNST cells. This study could uncover potential clinical strategies for MPNST treatment.

Methods: We use *in vitro* models of cell crosstalk mechanisms (co-cultures, conditioned media) employing MPNSTs and bone marrow derived macrophages (BMDM). Through WB, ELISA and qPCR assays we study the ability of MPNST cells to drive the acquisition of a pro-neoplastic, TAM-like phenotype. Using Boyden chamber and Matrigel growth assays we investigate how TAM-like cells co-cultured with MPNST cells tune their invasion/migration and tumorigenic growth.

Results: Our results indicate that MPNST cells induce a significant transition of BMDM toward a phenotype characterized by a robust upregulation of ARG1 (Arginase 1), ARG2 (Arginase 2), CD206 (Mannose Receptor) and GLUL (glutamine synthetase) and a profile of induced VEGF-A (Vascular Endothelial Growth Factor), MGL1 (Macrophage Galactose-C type Lectin 1) and HIF1alpha (Hypoxia inducible factor 1alpha) expression. Such anti-inflammatory, M2-like, TAMs sustain *in vitro* MPNST 3D growth and migration, and endothelial cell angiogenesis. Ablation of TRAP1 expression hampers these pro-tumoral functions of TAMs.

Conclusion: Our findings suggest a new crosstalk between MPNSTs and TAMs, in which macrophages exposed to conditioned media of MPNST cells acquire a M2, TAM-like phenotype that in turn enhances the malignant features of the MPNST cells in a TRAP1-regulated way. This inter-cellular signaling could be crucial in facilitating MPNST growth and invasiveness, and could be reversed by TRAP1 inhibition.

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STING Inflames NF1 Malignancies for Immunotherapy

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Neurofibromatosis Type 1 (NF1) is caused by mutations in the *NF1* gene that encodes neurofibromin, a RAS GTPase-Activating Protein. NF1 patients develop benign tumors of which ~10% progress to Malignant Peripheral Nerve Sheath Tumors (MPNST). Attempts to inhibit RAS-signaling to treat MPNST have not worked in clinical trials. Surgical removal is the only cure for MPNST, which is challenging due to large tumor size and/or proximity to nerves. MPNSTs often recur, metastasize, and respond poorly to chemo- and radiotherapy. As such, MPNSTs are the leading cause of death among NF1 patients, and effective treatment strategies for MPNSTs are a dire need.

Immune Checkpoint Blockade (ICB) is an approach to treat inoperable, undruggable cancers. Immune checkpoint proteins (ICPs) terminate normal immune responses to prevent collateral tissue damage. Cancer cells hijack this mechanism and express ICPs to avoid immune detection. ICB disrupts this process, enhancing antitumor immunity. Monoclonal antibodies (mABs) that disrupt the interaction between Programmed Cell Death Receptor 1 (PD-1) and its ligand Programmed Cell Death Ligand 1 (PD-L1) are a widely used ICB therapy. While a T cell-enriched hot tumor microenvironment (TME) is required for successful ICB, MPNSTs lack a T cell-inflamed TME and respond poorly to ICB therapy. Activation of the intracellular protein "stimulator of interferon genes" (STING) enhances antitumor immunity via induction of a pro-inflammatory cytokines and chemokines, thereby increasing T cell infiltration into the TME.

Purpose: To reprogram the MPNST tumor immune microenvironment by activating the STING pathway, turning "cold" MPNSTs into "hot" tumors amenable to ICB therapy.

Methods: We treated mouse MPNSTs with STING agonist or STING agonist + anti-PD-1/ anti-PD-L1 monoclonal antibodies. Tumor size was measured throughout treatment. When mice were sacrificed, tumor tissue was analyzed for STING pathway activation, T cell infiltration, and the presence of cell proliferation and apoptosis markers. MPNST allograft tumors in athymic mice were controls. In parallel, human MPNST xenografts in immunocompetent mice were treated and analyzed similarly.

Results: STING pathway activation and increased T cell presence was observed in STING agonist-treated MPNSTs compared to vehicle-treated. MPNSTs treated with STING agonist alone or STING agonist + PD-1/PD-L1 mAbs showed less tumor growth relative to vehicle. Importantly, STING agonist + PD-1/ PD-L1 mAb-treated tumors showed significantly increased cell death and unchanged cell proliferation. Tumors in athymic mice continued to grow despite treatment suggesting the antitumor effects seen in immunocompetent mice were mediated by T cells. Interestingly, STING agonist + anti-PD-1 treatment resulted in significantly faster xenograft tumor shrinkage compared to STING agonist only or vehicle treatments.

Conclusions: Our data indicate that cold MPNSTs lacking T cells can be turned into hot T cell-rich tumors by activating STING signaling. This reprogramming of the TME made MPNSTs susceptible to immune destruction by ICB therapy. Therefore, these studies support this novel treatment strategy for MPNST, an aggressive and deadly tumor for which no molecular therapy currently exists.

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Characterizing the Super Enhancer Landscape of PRC2 Inactivation in MPNST Development

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Plexiform neurofibromas have a high risk of transforming into malignant peripheral nerve sheath tumors (MPNST) through the sequential deletion of *CDKN2A/B* followed by biallelic loss of *SUZ12* or *EED,* constituting the inactivation of the polycomb repressor complex 2 (PRC2). PRC2 is a transcriptional repressor complex involved in the silencing of many developmental and differentiation genes. Loss of this complex leads to a depletion in trimethylation marks on Histone H3 Lysine 27 (H3K27me3) and the subsequent gain of acetylation marks on the same lysine residue (H3K27Ac). However, the full range of epigenetic consequences of these events are currently not well characterized, especially with respect to MPNST formation and development. Recent studies have highlighted the importance of super enhancers (SE) in maintaining a tumorigenic transcriptional landscape, but no such studies have characterized these newly discovered regulatory elements in the MPNST context. In order to contribute to this effort, the Largaespada lab has CRISPR-engineered human Schwann cell (SC) lines to harbor deletions in *NF1* and either *SUZ12* or *EED* and assayed the H3K27ac landscape of these cells in addition to patient-derived MPNST cell lines. Utilizing the ranked ordering of super enhancers (ROSE) algorithm, we have identified common SE within a set of four MPNST cell lines, and in engineered PRC2-deficient SC lines. Surprisingly, each MPNST cell line has many unique SEs, with a subset seen in 3/4 or 4/4 lines. These also show some overlap with SEs that form after PRC2 loss in human NF1-deficient SCs. We also characterized these SEs by assessing the expression levels of their associated genes and contextualized these genomic regions with various epigenetic marks such as DNA methylation and other histone post-translational modifications like H3K27me3. These efforts provide a roadmap of the SE landscape of MPNSTs and as such allow for insights into how the loss of PRC2 creates a gene regulatory landscape provoking NF1-related MPNST development and maintenance. Finally, this work provides a basis for further investigation into the regulatory landscape such as through the 3D genome or other histone marks and transcription factors at the super enhancer interface.

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"Mild" Neurofibromatosis Type I Patient Mutation p.M992del in Knock-In Mouse Model Suggests Novel Roles in Skeletal Development

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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 (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. Animals were assessed for growth parameters, body composition and bone density (uCT) and bone structure (X-Ray). Histological analysis of growth plates and cranial sutures has also been performed. Embryos from intercrossed heterozygous mice ($Nf1^{M992del/+}$) were analyzed at E19.5.

Results: 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. 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. Bone analyses show 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). 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 strain-dependent genetic background plays a role in embryonic lethality.

Additional Authors: Laura Lambert, Kelley Bradley, Hu Ke, Erik Westin, Min Chen, Amjad Javed, CJ Haycraft, Kai Jiao, Peter Panizzi, Bruce R Korf, Maria Johnson, Jeremy Foote, Deeann Wallis, Robert A. Kesterson

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NF Schwann Cell Behavior on Electrically Conductive Neurite Mimics

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Plexiform Neurofibromas are complex tumors that include a driving, deranged Schwann cell (SC) component and support cells in the perineural environment[1]. This study focuses on signal transduction pathways between neurons, SC and support cells (fibroblasts and endothelial cells) that drive this aberrant proliferation/invasiveness lead to neurofibromas. We have developed a nanofiber-based system that provides a tunable "neurite mimic" to investigate cell behavior in the presence of a neural component.

Neurite mimic: Hyaluronic acid (HA) nanofibers incorporated with multi-walled carbon nanotubes (CNT)[2] have been developed to serve as the "neurite mimic". Scaffolds were stimulated using a custom-built stimulation chamber. Immortalized human normal (wild-type; WT-SC, ipn 0.28) and NF1 PN patient-derived SCs (NF-SC, ipNF95.11b) were evaluated for morphology, cell proliferation, growth factor release.

Effects of electrical stimulation on cell aggressive/invasive phenotypes and growth: NF-SCs grown on HA-CNT exhibit elongated cell body associated with aggressive/invasive phenotype. Cells were electrically stimulated at 100-200 mV/mm for 30 mins. WT-SCs showed significant increase in aggressive phenotypes and proliferation compared to unstimulated cells. WT-SCs grew faster than NF-SCs on all neurite mimics including HA and HA-CNT, and 3D hydrogels. Co-cultures are being conducted with human fibroblasts and endothelial cells, which we hypothesize can increase proliferation of SCs.

Effects of mechanics on cell aggressive/invasive phenotypes and growth: Cell phenotypes and growth were evaluated on neurite mimics with two different mechanics (1-5 MPa) or on 3D hydrogel (40 kPa). Increase in cell elongation, proliferation, and migration of both WT-SC and NF-SC were detected in the highest mechanics. These results suggest matrix mechanics in tumor tissues affect cell invasive phenotypes, growth, and migration.

Effects of electrical stimulation on growth factor release: Growth factor secretion from SCs with electrical stimulation was evaluated using an ELISA. NF-SCs and WT-SCs that were stimulated at 100 mV/mm showed increased nerve growth factor (NGF) secretion compared to controls. Cells grown on higher mechanics scaffolds showed higher NGF secretion.

In conclusion, we found opposing effects of neurite mimics and electrical stimulation on cell behavior: WT-SC, but not NF-SC, grown on HA-CNT and/or electrical stimulation has increase in aggressive/invasive phenotypes, proliferation, migration, and NGF secretion. Similar results in gene expressions specific to SC maturation and myelination are expected. Moreover, SCs grown in higher mechanics shows increase in aggressive/invasive phenotype and proliferation. Ongoing work is in evaluating SC behavior in co-cultures as we develop *in vitro* models of NF1 neurofibromas.

Full List of Authors: Judy Senanayake, Christine Hampton, Kyungmin Ji, Harini G. Sundararaghavan, Raymond R. Mattingly

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SHH Pathway Activation Drives a Neural Crest-Like State in MPNSTs

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Introduction: Neurofibromatosis Type 1 (NF1) is hereditary tumor predisposition syndrome that results in the development of innumerable nerve sheath tumors that fall within the spectrum of benign, premalignant and malignant lesions. The molecular mechanism that drives malignant transformation has yet to be fully understood. Our previous work has demonstrated that SHH pathway activation drives malignant transformation in a subset of malignant peripheral nerve sheath tumors (MPNSTs). Here, we demonstrate that SHH pathway activation induces a neural crest cell-like state in a subset of MPNSTs.

Methods: We performed single nuclear RNA sequencing on 6 tumors (5 MPNSTS and 1 atypical neurofibroma). Data was generated using the 10X Chromium plaltform and analysed using the Suerat 3.0 pipeline. Cellular trajectories were inferred by generating of relative pseudotime through a linear transformation relative to cells with lowest and highest pseudotimes with Moncole2. We performed *in vitro* experiments to assess the correlation between SHH pathway activation and neural crest signatures. We inhibited SHH pathway with sonidegib, a SMO inhibitor, in MPNST cell lines and performed RT-PCR to assess markers of neural crest cell signatures. In addition, we assessed cellular viability with treatment.

Results: A total of 43, 365 cells were analyzed, with 30, 518 cells and 12, 847 cells characterized ad neoplastic and non-neoplastic cells, respectively. Notably, we observed that cells that express SMO at high levels, with SHH pathway activation, express neural crest cell makers. These cell populations are negative for S100B, but overexpress TWIST1 (**Figure 1**). Single cell trajectory analysis further demonstrates a pseudotemporal continuum from atypical neurofibromas (Schwann cells), MPNSTs (Schwann cell precursor cells) and other MPNSTs (neural crest cells), which supports that these tumors fall along the developmental trajectory of neural crest cell lineage. SHH pathway activity was high in S462TY MPNST cell line and low in STS-26T MPNST cell line (**Figure 2**). S462TY also demonstrated prominent elevation in expression of neural crest cell markers TWIST1, SOX9, SNAI2, OTX2, PAX4 and PAX6 (**Figure 3**). We found that treatment with sonidegib treatment attenuates the expression of neural crest cell signatures. Finally, inhibiting SHH pathway activation reduced cellular viability in MPNST cell lines.

Conclusion: SHH pathway activation drives dedifferentiation into a neural crest cell-like state in a subset of MPNSTs. We confirm that inhibiting SHH pathway activity in a subset of MPNSTS prevent growth and malignant progression, providing a rational for future clinical trials.

Figure 1:

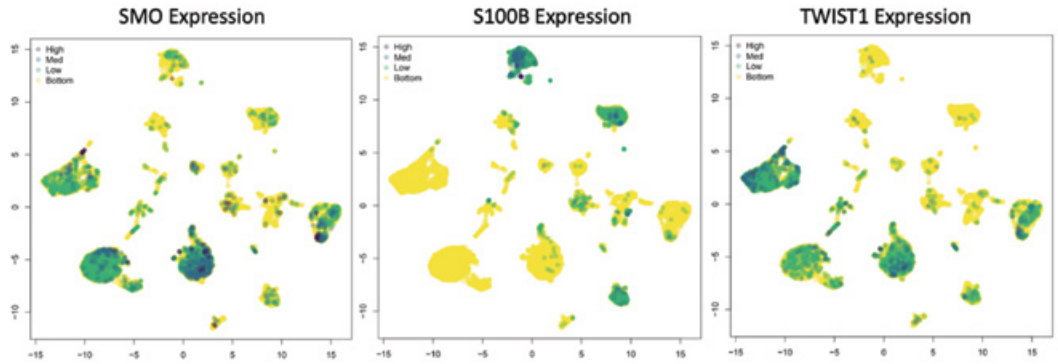


Figure 2:

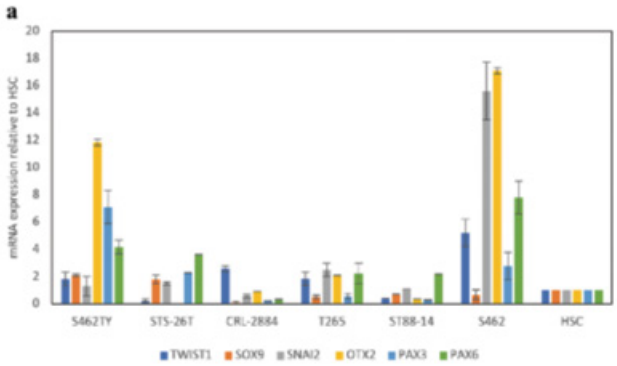
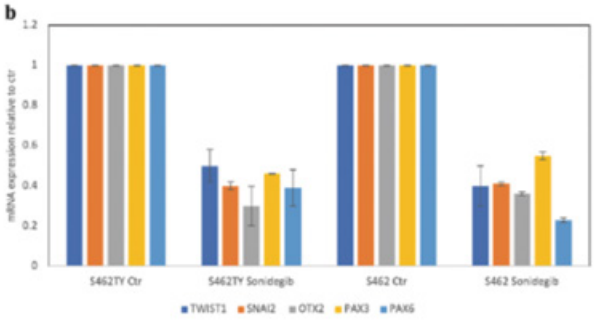


Figure 3:



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Funding: The US Department of Defense, Francis Collins Scholar Program through Neurofibromatosis Therapeutic Accelerator Program and the Canadian Institute of Health Research grants.

Cell Painting Distinguishes Isogenic Schwann Cells with Different *NF1* Genotypes

Jenna Tomkinson, BS, *University of Colorado Anschutz Medical Campus*

Purpose: We aim to discover a cell morphology biomarker specific to *NF1* genotype in Schwann cells. In the future, we will use this biomarker to find drugs that upregulate neurofibromin in *NF1* deficient Schwann cell environments, which we hypothesize will address many systemic NF1 complications.

Methods: We applied a modified Cell Painting assay, which marks three cellular structures (endoplasmic reticulum (ER), actin, and nuclei), to two isogenic Schwann cell lines (one *NF1* wild-type [$NF1^{+/+}$] and the other *NF1* null [$NF1^{-/-}$]). We collected fluorescence microscopy images and performed an image-based analysis pipeline to extract about 2,000 morphology features per single cell (**Figure 1**). We applied fixed effect linear modeling, adjusting for relevant covariates, to identify single-cell features that significantly distinguished *NF1* genotypes. We performed this analysis in two separate plates to confirm consistency. We also performed a power analysis to confirm sufficient power to identify statistically significant differences in *NF1* genotype.

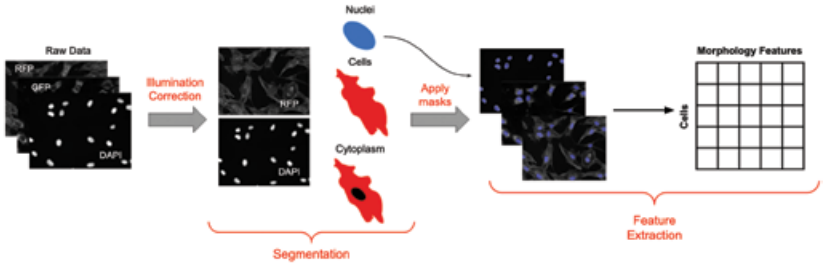

Figure 1. Image-based analysis pipeline. We used CellProfiler for illumination correction, segmentation, and feature extraction.

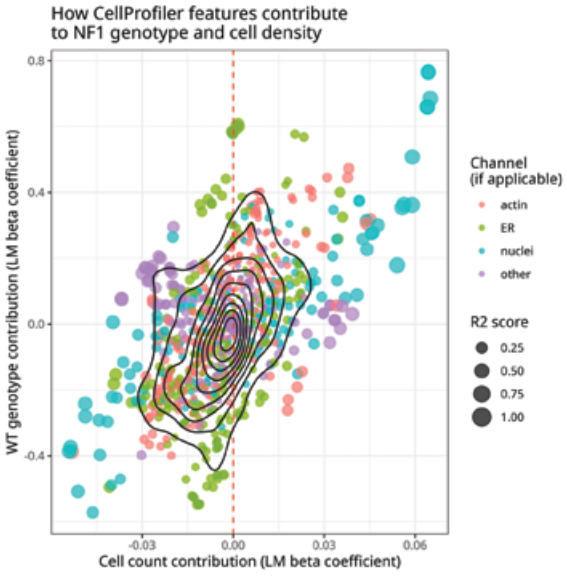

Figure 2. Fixed effect linear modeling adjusting for cell count. We identify many differentially altered organelle morphology features between NF1 genotypes in Schwann cells.

Results summary: The Schwann cells with different *NF1* genotypes showed differential growth patterns, which we were able to adjust for in our linear model. We observed many single-cell morphology features that are significantly different between *NF1* genotypes (**Figure 2**). The most significant features were based on ER morphology and included texture-based measurements that indicated significantly more ER intensity heterogeneity in *NF1* Null cells (more ER splotches in *NF1* deficient environments). We also observed differences in actin morphology, while nuclei were most impacted by cell density. Single-cell morphology feature differences were significantly correlated across plates (Pearson's $R=0.41$, $p<1e10^{-10}$).

Conclusions: Cell Painting provides a rich landscape of organelle-specific differences between Schwann cells of different *NF1* genotypes, which were consistent across plates. Importantly, these subtle differences could not be detected by eye, requiring advanced computer vision algorithms to detect. Specifically, we may be observing differential ER-related stress pathway activation in response to differing levels of neurofibromin production in NF1 deficient Schwann cells. In the immediate future, we will test the ability of our biomarker to detect intermediate levels of neurofibromin using *NF1*-targeted shRNAs to reduce neurofibromin. Our long-term vision is to use this biomarker to prioritize drugs that specifically reverse *NF1*-deficiency causing *NF1*-deficient Schwann cells to structurally resemble Schwann cells with a healthy *NF1* genotype.

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Disclosure of relevant financial relationships: JT is a full-time employee of The University of Colorado and has no other relevant financial relationship to disclose. MMH and HS are employees of INFixion Bioscience. GPW is a full-time Assistant Professor at The University of Colorado and is on scientific advisory board and holds stock options of iNFixion.

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Functional Impact of *NF1, CDKN2A* and *SUZ12* Loss in an iPSC-Based MPNST Model System

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Malignant Peripheral Nerve Sheath Tumor (MPNST) is an aggressive soft tissue sarcoma that can develop both sporadically and in the context of Neurofibromatosis type 1 (NF1). MPNST cells bear hyperploid and highly rearranged genomes. Most MPNSTs arising in NF1 patients carry the complete inactivation of the *NF1, CDKN2A* and *SUZ12*/*EED* (PRC2) tumor suppressor genes (TSGs). In this work we aimed to understand the function of these TSGs and the biological implications of their loss in the progression from a plexiform neurofibroma (PNF) to an MPNST.

We used the Ribonucleic Protein (RNP)-based CRISPR-Cas9 genome editing system to knockout *CDKN2A* and *SUZ12* genes, in a step-wise manner, in *NF1*(-/-) edited iPSCs, and characterized the resulting physiological states. We first generated *NF1*(-/-) *CDKN2A* p16INK4A(-/-) and *NF1*(-/-) *CDKN2A* p14ARF-p16INK4A(-/-) iPSCs lines. All cell lines were differentiated towards neural crest (NC) cells. No significant differences in proliferation capacity nor cellular ploidy were observed when compared to the parental *NF1*(-/-) cell line. These double KO cell lines were able to generate neurofibromaspheres using the protocol established by Mazuelas et al. (2022). When *SUZ12* was removed in these iPSC lines, colonies were formed but could not be expanded if p14ARF was still active. This result agrees with the finding that NF1-associated MPNSTs always have both genes encoded by the *CDKN2A* locus completely inactivated (Magallón-Lorenz et al, 2021) and somehow imposes a biological order of TSG step-wise inactivation: *NF1-CDKN2A*-PRC2. We indeed were able to generate triple KO cell lines, when both p14ARF and p16INK4A were inactivated. Our results indicate that the loss of PRC2 function is the cause that PNF-forming cells lose the identity of NC-SC axis of differentiation and acquires a NC-mesenchymal identity as it happens for MPNSTs in the PNF-ANNUBP-MPNST progression.

We are currently analyzing the impact of PRC2 loss on chromatin accessibility, methylation and gene expression, to better understand and characterize the mesenchymal phenotype exhibited by MPNSTs. Along passages, triple KO cells do not acquire genome instability generating the classic genome rearrangement and hyperploidy seen in MPNSTs, which makes us hypothesize the necessity of acquiring an additional alteration for these cells to gain such genomic characteristics.

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A Functional Genomic Ras GAP Atlas Reveals Distinct Neurofibromin Functions and Druggable Dependencies in the Peripheral Nervous System

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Purpose: Ras GTPase activating proteins (GAPs) such as *NF1* are important for human disease yet the specificity and functional overlap between different Ras GAPs remains incompletely understood. Here, we combine CRISPRi, RNA-sequencing, biochemistry, phosphoproteomic, and pharmacologic approaches to define the role of the Ras GAPs *NF1, RASA1, RASA2,* and *RASA3* in peripheral nervous system (PNS) cells.

Methods: The CRISPRi system was introduced into immortalized peripheral nerve (iPN) cells and stable cell lines were generated. Bulk RNA-sequencing and immunoblotting for Ras pathway components was performed on all cell lines. Pharmacologic inhibition was measured using both cell viability assays and immunoblotting for effector protein activation across a panel of iPN, neurofibroma, and malignant peripheral nerve sheath tumors (MPNST) cells. Phospho-proteomic mass spectrometry (MS) analysis was used to measure post-translational alterations.

Results: Following validation of CRISPRi iPN cells selectively repressing *NF1, RASA1, RASA2,* or *RASA3*, RNA-sequencing revealed *NF1* deficient iPNs harbor a distinct transcriptional program compared to other Ras GAPs enriched for cell proliferation, de-differentiation, and epithelial-mesenchymal transition (EMT). In addition, all Ras GAP deficient cells exhibited a conserved "pan-GAP" program enriched for mitotic spindle genes and growth factor signaling feedback regulators such as RNA-binding proteins. From a biochemical perspective, *NF1* deficient cells exhibited increased Ras GTP levels and downstream Raf/MEK/ERK induction. Pharmacologically, *NF1* deficient cells exhibited modestly increased resistance to MEK inhibition. Phospho-proteomic MS of control or *NF1* deficient iPNs with or without selumetinib treatment identified seven differentially regulated phosphopeptide clusters across all four conditions. Of these 7 clusters, Clusters 5 and 6 reflected differential selumetinib response in *NF1* deficient iPNs: Cluster 5 phosphopeptides enriched for a SOS1 network that includes regulators of Raf/MEK/ERK signaling while Cluster 6 phosphopeptides enriched for ERK1/2 targets were no longer selumetinib responsive. Consistent with *NF1* loss altering upstream regulation of Raf/MEK/ERK to increase signaling output, *NF1* deficient iPNs were more sensitive to SHP2 inhibition, a finding recapitulated in a panel of MPNST cells. Moreover, *PTPN11* (encoding SHP2) deficient iPNs were more sensitive to selumetinib and exhibited an inverse transcriptomic signature enriched for negative regulators of cell proliferation and positive regulators of differentiation.

Conclusions: *NF1* loss is distinct amongst Ras GAPs in the PNS and biochemically alters selumetinib responses due to dysregulated feedback. Pharmacologic and genetic SHP2 perturbation reveals a potential therapeutic window in *NF1* deficient cells.

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New Models to Define Cooperating Events That Drive PNF Transformation into MPNST

Ellen Voigt, *The University of Iowa*

Purpose of study: Malignant peripheral nerve sheath tumors (MPNSTs) are deadly sarcomas that arise either spontaneously or through transformation of benign tumors, called plexiform neurofibromas (PNFs), in patients with Neurofibromatosis Type 1 (NF1). Most MPNSTs have alterations that inactivate the *INK4a* and *ARF* tumor suppressor genes, and most overexpress the RABL6A oncoprotein. RABL6A is functionally linked to *INK4a*/*ARF* – it reduces ARF expression while promoting activation of cyclin-dependent kinases 4 and 6 (CDK4/6), the targets of $p16^{INK4a}$. We are developing models to determine the individual importance and cooperativity of *INK4a* loss, *ARF* loss, and/or RABL6A overexpression in driving PNF transformation into MPNSTs.

Methods: For *in vitro* studies, CRISPR/Cas9 editing of *INK4a* and/or *ARF* with or without RABL6A overexpression is conducted in primary patient-derived PNF cultures and normal human Schwann cells. Edited cells are evaluated for malignant-like phenotypes using cell transformation assays. For *in vivo* studies, a novel Cre-inducible LSL-CMV-HA*Rabl6a* transgenic (*Rabl6a* OE) mouse was generated. Rabl6a expression was measured by western blotting and immunostaining in mouse embryonic fibroblasts (MEFs) from multiple founder mice as well as tissues from *Rabl6a* OE mice crossed onto a RosaCreER background. Future work will assess *de novo* MPNST formation in *Rabl6a* OE;DhhCre mice following adenoviral CRISPR/Cas9 inactivation of *Ink4a, Arf,* or both *Ink4*/*Arf* in the sciatic nerve.

Results: Two primary PNF cultures were validated for the presence of intact *INK4a*/*ARF* and *RABL6A* genes by fluorescence-in-situ-hybridization (FISH) and protein expression by western blotting. Early passage PNFs were partially immortalized via stable overexpression of human telomerase reverse transcriptase (hTERT) prior to CRISPR editing. Guide RNAs that effectively and specifically target human *INK4a* or *ARF* were optimized, as were methods for successful CRISPR/Cas9 editing and delivery (e.g., ribonucleoparticle vs vector-based) in PNF and normal human Schwann cells. Early analyses suggest a more pronounced growth advantage for *INK4a* inactivated cells relative to those lacking *ARF.* One *Rabl6a* OE mouse founder line displayed Cre-inducible expression of Rabl6a protein, localized predominantly in the cytoplasm as expected, in isolated MEFs. Induction of Rabl6a provoked premature arrest in those MEFs, consistent with 'oncogene induced senescence,' a protective checkpoint in normal cells known to be controlled by $p16^{INK4a}$ and ARF.

Conclusions: We have developed new *in vitro* and *in vivo* models to evaluate putative drivers of PNF-to-MPNST transformation. Findings should guide better preventive and treatment strategies for this disease.

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Multiomic Analysis of Mouse and Human Malignant Peripheral Nerve Sheath Tumors (MPNSTs) Identifies G6PD Dysregulation as a Metabolic Vulnerability

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Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are highly aggressive and metastatic soft tissue sarcomas that arise from benign precursor lesions, neurofibromas. Homozygous loss of Neurofibromin-1 gives rise to neurofibromas. Additional mutations in either the TP53 gene or CKDN2a locus (encoding p16INK4a and ARF) are associated with progression to MPNSTs.

It is well established that cancer metabolism is altered over the course of tumor initiation and progression. Our lab is interested in investigating the altered metabolic pathways in MPNST progression. To investigate these effects, our lab utilized a CRISPR/Cas9 approach to induce primary MPNST formation in mice. We assessed tumor growth kinetics and performed mass spectrometry metabolic profiling on tumor derived cell lines. Our data shows that MPNSTs generated from NF1 and CDKN2a deletion have elevated utilization of the Pentose Phosphate Pathway (PPP) and upregulated activity of G6PD (the rate limiting enzyme of the PPP). Additional studies show that genetic and pharmacological inhibition of G6PD slows tumor growth *in vitro.* Furthermore, G6PD inhibition improves chemotherapy response in mouse and human MPNST cells. Genomic analysis of patient samples shows that PPP targets are upregulated in human MPNSTs. Taken together, these data identify key metabolic pathways in MPNSTs, suggesting that G6PD and redox metabolism are attractive targets to improve patient outcomes.

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Study of Nutraceutical Intervention with High Phenolic Extra Virgin Olive Oil and Curcumin for Neurofibromatosis Type 1

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The development of MEK inhibitors for the treatment of plexiform neurofibromas has been a major milestone in the NF1 field, and they may be useful for other manifestations of the disease. However, many patients are unable to benefit from this therapeutic approach, possibly lacking a qualifying indication. Moreover, MEK inhibitors can have complex side effect profiles to manage, do not show efficacy in all patients, and the response is not durable if therapy is discontinued. There has been much interest in the NF1 patient community in exploring nutraceutical interventions which have been shown to mimic pharmaceutical inhibition of Ras and other NF1 relevant pathways including inflammation. These interventions could conceivably treat or prevent benign tumors in NF1.

Two such compounds, oleocanthal and curcumin, have garnered particular attention. Oleocanthal is found in high phenolic extra virgin olive oil (HP-EVOO) and has been shown to have anti-inflammatory and anti-cancer properties. Curcumin is a bioactive compound found in turmeric and has been shown to have anti-inflammatory, antioxidant, and anti-cancer properties. A small clinical trial was conducted (Espositio *et al,* 2017), using dietary intervention with high phenolic olive oil and curcumin, which showed limited, yet promising, results in NF1 patients.

This study builds on the previous trial observations in the following ways: 1. We have an open and enrolling clinical trial to establish the safety and preliminary activity of a novel and more bioactive form of curcumin (SLCP Longvida®) and HP-EVOO supplementation in adult NF1 patients with cutaneous and plexiform neurofibromas (NCT05363267). 2. We have examined signaling pathways and neurofibroma-relevant cell phenotypes impacted by curcumin and/or oleocanthal using human plexiform-like neurofibroma cells *in vitro* and *in vivo.*

In our pre-clinical work, we investigated the effect of curcumin, oleocanthal and their combination on signaling pathways in NF1-related cell lines. Our results show that the combination of the compounds at doses achievable in humans, but not single agents, produced potent inhibition of mTORC1 as evidenced by dramatically reduced pS6K, in *NF1*-deficient, immortalized human Schwann cells, comparable to the FDA approved mTOR inhibitor, everolimus. The combination treatment also inhibited activating phosphorylation of STAT3 and ERK in *NF1*-deficient Schwann cells. Strong inhibition of signaling pathways known to be important for neurofibroma formation by this combination prompted *in vivo* follow-up investigation.

When used in an *in vivo* model of neurofibroma growth, promising results were again observed. Using *NF1*-deficeint immortalized human Schwann cells implanted into immunodeficient mice, control of tumor growth and an overall survival benefit was observed in animals receiving the oleocanthal/curcumin combination. The combination of oleocanthal and curcumin shows promise as a chemopreventive treatment for NF1 patients that could slow tumor growth and improve outcomes.

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Combining SOS1 and MEK Inhibitors in a Murine Model of Plexiform Neurofibroma Results in Tumor Shrinkage

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Individuals with neurofibromatosis type 1 develop RAS-MAPK-MEK driven nerve tumors called neurofibromas. While MEK inhibitors transiently reduce volumes of most plexiform neurofibromas in mouse models and in NF1 patients, therapies that increase the efficacy of MEK inhibitors are needed. BI-3406 is a small molecule that prevents SOS1 interaction with KRAS-GDP, interfering with the RAS-MAPK cascade upstream of MEK. Single agent SOS1 inhibition had no significant effect in the *DhhCre;Nf1$^{fl/fl}$* mouse model of plexiform neurofibroma, but PK-driven combination of selumetinib with BI-3406 significantly improved tumor parameters. Tumor volumes and neurofibroma cell proliferation, reduced by MEK inhibition, were further reduced by the combination. Neurofibroma are rich in Iba1+ macrophages; combination treatment resulted in small and round macrophages, with altered cytokine expression indicative of altered activation. The significant effects of MEKi plus SOS1 inhibition in this pre-clinical study suggest potential clinical benefit of dual targeting of the RAS-MAPK pathway in neurofibromas.

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Single-Cell Analysis Identifies Clinically Relevant Mesenchymal Stem-Like Cells in MPNST and Distinct Tumor Ecosystem in Benign Schwann Cell-Derived Tumors

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Neurofibroma and malignant peripheral-nerve-sheath-tumor (MPNST), tumors often seen in patients with neurofibromatosis type 1 (NF1), and vestibular schwannoma (VS), tumors frequently found in NF2, are heterogeneous peripheral nerve sheath tumors originating from Schwann cells (SC) or their embryonic neural crest-derived progenitors. These tumors are regulated by cell-intrinsic and cell-extrinsic factors that contribute to the establishment of a tumor niche. MPNST is classified as a highly aggressive SC-derived soft-tissue sarcoma. It arises from benign neurofibroma and is among the most challenging mesenchymal malignancies to treat. Changes in cancer behaviors during initiation, progression, metastasis, and relapse are critically dependent on the cellular interplay between tumor cells and the tumor microenvironment (TME). Moreover, cellular plasticity and heterogeneity that result from activation of the epithelial-to-mesenchymal transition (EMT) program endows benign tumor cells with malignant traits, such as migration, invasion, and chemoresistance. Thus, understanding the cellular diversity and plasticity during neurofibroma-to-MPNST progression is critical for identifying key regulators and vulnerabilities to target this deadly disease and predicting treatment outcomes. By applying single-cell multi-omics profiling to early/benign (plexiform neurofibroma) and advanced/malignant (MPNST) tumors, we defined the stage-specific cellular heterogeneity, tumor cell fate transitions, and TME landscape during malignant transformation in MPNST. Our analysis reveals a clinically-relevant nestin-negative stem-like mesenchymal neural-crest subpopulation underlying malignant transformation across mouse and human MPNSTs. These mesenchymal-like stem cells in MPNST no longer exhibit a SC lineage identity but show a strong EMT signature that is distinct from and more primitive than that in nestin^{+} progenitor cells. Integrative multi-omic profiling further identifies unique regulatory networks and druggable targets against the malignant subpopulations in MPNST. Targeting key EMT and stemness regulators, such as ZEB1 and ALDH1A1, impedes MPNST growth. To comprehensively decode tumor SC heterogeneity and the TME in benign SC tumors, we profiled the single-cell transcriptomes of NF2-associated and sporadic VS and compared them with plexiform neurofibroma and MPNST. Intriguingly, along with macrophages, T cells, and stromal cells, both NF2-associated and sporadic VS harbor repair-like SCs that resemble those in regenerating peripheral nerves post injury. Within the TME, macrophages display robust pro-tumorigenic M2 signatures in all three types of SC-derived tumors. However, we identified an increase in subtype heterogeneity of macrophages and lymphocytes in VS, compared with neurofibroma or MPNST. In particular, we identified multiple pro-inflammatory macrophages in VS, leading to an enhanced M1 signature score. In addition, all three SC-derived tumors display immune dysfunction within the TME, as reflected by low expression of immunostimulatory IL-2 but high levels of TNFalpha, TGFbeta, and interferon-gamma in the immune cell subsets. Several immune checkpoint receptors, including TIM3 in macrophage and T cell subsets and VISTA and LAG3 in T cells, are detected within the TME of all three SC-derived tumors. Together, in addition to highlighting a hitherto unrecognized mesenchymal stem-like subpopulation in disease progression of MPNST, our studies reveal the underlying principles of tumor cell-state evolution and heterogeneity, as well as tumor ecosystem diversity within SC-derived tumors.

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Drug Response Evaluation for NF1 Tumors Using High Throughput Screening Analysis and DREA Web Tool

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The study intends to identify potential drug candidates for Neurofibromatosis Type 1 (NF1) tumor treatment from an analysis of high throughput screening (HTS) of drugs in plexiform neurofibroma-derived cell lines. Drug candidates were investigated using an analysis with outcomes revealing drug resistance and sensitivity as found in non-tumor *(nf1 +/+, nf1 -/-)* and tumor cell lines. The analysis is based on an evaluation of delta log(Emax/EC50), commonly used in receptor pharmacology. Emax (effectiveness, also efficacy) is the maximum observed cytotoxic response. EC50 (potency) is the effective concentration at half maximal response. Delta log(Emax/EC50) incorporates and normalizes drug potency and effectiveness into a single value allowing comparison across drug classes and different cell lines. It was determined that ipnNF95.11C (nf1 -/-, -/+) was the best available control investigated to predict human clinical outcomes from available cell lines. Drug candidates were identified in various cell pools including cell surface receptors, mTOR pathway members, protein kinases, and targets related to the DNA/cell cycle. Another result was a ranked list of candidate drugs which may be useful in follow-on pre-clinical or clinical development. The method appears to be suitable for use in evaluating drug responses in either benign or malignant tumor variants of NF1.The methods used in the analysis were integrated into an interactive web platform [DREA – Drug Response Evaluation and Analysis] for the analysis of dose response curves, EC50, and delta log(Emax/EC50). It was concluded that delta log(Emax/EC50) and the web tool simplify HTS analysis across many cell lines, and may provide a springboard for further investigations of benign or malignant variants of NF1.

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Mechanisms of Immune Escape in NF1-Associated Peripheral Nerve Sheath Tumors

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Background: Neurofibromatosis type 1 (NF1) is a neurogenetic condition with stereotypic cutaneous findings and a predisposition for benign and malignant tumors. About half of patients will develop plexiform neurofibromas (pNF), non-malignant tumors that have a 10% lifetime risk of developing into aggressive soft tissue sarcomas called malignant peripheral nerve sheath tumors (MPNST). Some lesions, denoted atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP), exhibit histopathologic features and are precursors to MPNST. Despite many clinical trials, there has been little advancement in treatment outcomes and overall survival remains poor; therefore, new therapeutic approaches are needed. PNST are composed of transformed Schwann cell precursors that interact with infiltrating immune cells. An understanding of the relationship between the pre-existing immunity and tumor microenvironment (TME) will help unveil potential for immunotherapeutics.

Methods: We used complementary techniques to interrogate the TME in NF1-associated PNST. 15 pNF, 8 ANNUBP, and 18 MPNST specimens were analyzed to compare the quantitative and spatial resolution of the geography and nature of tumor infiltrating immune cells during progression to malignancy. We determined the interactions of immune cells and immunoregulatory molecules, using a combination of multiplex flow cytometry (MFC) and gene expression profiling studies. Lastly, we correlated these findings with clinical outcomes.

Results: Immunophenotyping confirmed the higher presence of infiltrating myeloid compared to lymphoid cells, with a predominance of CD163+ myeloid cells during malignant transformation. Similarly, transcriptomic data showed significant accumulation of immunosuppressive myeloid populations in MPNST. The MPNST TME was consistent with an immune excluded phenotype on spatial resolution. MFC further characterized CD163+ myeloid cells as immunosuppressive with a high PD-L1 expression. Higher expression of CD8+ T cells and lower PD-L1 in MPNST correlated with improved survival.

Conclusions: An immunosuppressive microenvironment characterizes PNST during the process to malignancy, generating an immune-excluded phenotype. This progression of pre-malignant tumors to malignancies can respond to the principles of immunoediting; thus, we believe that immunotherapies may be a therapeutic option in patients with MPST. Further studies on PD-L1 expression may reveal it as a useful biomarker. The mechanisms of immune modulation in PNST will inform interventions to stimulate anti-tumor immunity in this disease.

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Malignant Peripheral Nerve Sheath Tumors Demonstrate Distinct Patterns of Radiation Response Through Activation of Immunosuppressive Pathways

Iowis Zhu, *University of California, San Francisco*

Purpose: Peripheral nervous system (PNS) tumors such as plexiform neurofibromas (pNF) and malignant peripheral nerve sheath tumors (MPNSTs) are a significant source of morbidity and mortality in patients with neurofibromatosis type 1 (NF1). Despite multimodal therapy with surgery, systemic therapy, and radiation therapy (RT), no effective therapy exists for MPNSTs. Here, we investigate the molecular mechanisms underlying differential RT responses in NF1 associated pNFs and MPNSTs.

Methods: Patient derived *NF1* mutant pNF cells (NF95.11b), and *NF1*/*CDKN2A*/*SUZ12* mutant MPNST (ST88-14, JH02.2) cells were used to study RT responses *in vitro. In vitro* viability was measured by cell counts. CRISPRi was used to suppress *NF1* in iPNs. Transcriptomic signatures were measured by bulk RNA-sequencing and integrated with single-cell RNA sequencing (scRNA-seq) data from patient-derived pNF and MPNST resection specimens.

Results: To determine the radiation response of peripheral nerve tumor cells, we performed single fraction radiation dose response cell viability assessments in NF9511b pNF cells, and ST88-14 and JH02.2 MPNST cells. We observed pNF cells were significantly more radiosensitive than MPNST cells (IC50 pNF 0.61 Gy; 95% CI 0-1.737 versus IC50 ST88-14 MPNST 4.15 Gy; 95% CI 2.72-6.27 and IC50 JH02.2 MPNST 6.25 Gy; 95% CI 3.26-25.87). When treated with 2 Gy x 5 fractions, MPNST cells were significantly more viable than wildtype iPNs, *NF1* deficient iPNs, or pNF cells ($p=0.02$; t-test). Principal component analysis of bulk RNA sequencing at 5 or 14 days following 2 Gy x 5 fractions in iPNs, NF9511b pNF, or ST88-14 MPNST cells revealed cell line of origin accounted for the largest source of variation between samples (64.9% variance). Both iPNs and pNF cells upregulated genes enriched for pro-apoptotic pathways *(BAD, DAPK3)* at 5 days post-RT while MPNST cells instead induced genes enriched for pro-survival growth factor signaling *(FGF2, PDGFRB).* At 14 days post radiation, pNF cells expressed genes enriched for ER stress *(EIF4A1, EEF2, EIF4E)* consistent with their radiosensitivity, while MPNST cells uniquely upregulated a gene signature enriched for TGFβ signaling and interferon secretion. Integration of MPNST-specific RT signatures with patient derived radiation-naïve pNF and MPNST scRNA-seq data revealed enrichment of growth factor signaling and TGFβ signatures and significantly decreased immune populations in MPNST samples ($p=0.008$, t-test).

Conclusions: MPNST cells display increased radioresistance and altered transcriptional responses to RT compared to iPN or pNF cells. Integrated analysis of RT signatures with patient tumor scRNA-seq suggests that upregulated growth factor signaling and TGFβ associated immunosuppression are distinct features of MPNST.

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NF1: Clinical Science (The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Adeyemi	Ayo	2	Evaluating Risk of Progression with Selumetinib in Pediatric Patients with Neurofibromatosis Type 1 and Plexiform Neurofibroma: Propensity Score Method (PSM) Analysis of the SPRINT Trial vs a Natural History (NH) Control Arm
Allaway	Robert	4	Examining the Power of AI in Neurofibromatosis Care and Research with ChatGPT
Aschbacher-Smith	Lindsey	6	Comparative Analysis of Validated Measures of Cognitive, Behavioral, and Motor Impairments in a Pediatric Population of Neurofibromatosis Type 1
Azizi	Amedeo	8	Novel Austrian Care and Prevention Guide for Children and Adolescents with NF1
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NF1: Clinical Science

Evaluating Risk of Progression with Selumetinib in Pediatric Patients with Neurofibromatosis Type 1 and Plexiform Neurofibroma: Propensity Score Method (PSM) Analysis of the SPRINT Trial vs a Natural History (NH) Control Arm

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Purpose: Selumetinib is approved for children aged ≥2 (US) or ≥3 (EU) years with neurofibromatosis type 1 and symptomatic, inoperable plexiform neurofibromas (NF1-PN) based on data from the single-arm SPRINT trial (NCT01362803) and age-matched patients in the NH trial (NCT00924196). This PSM analysis estimates progression risk with selumetinib in SPRINT versus NH (external control arm), accounting for residual differences in baseline characteristics.

Methods: Balance in baseline characteristics between SPRINT and NH was assessed by standardized difference. Propensity scores (PS), accounting for age, sex, race, weight, height, and PN parameters (location, volume, progression status), were used in a 1:1 PS match without replacement (base case analysis), stabilized inverse probability of treatment weighting (sIPTW), and 1:2 PS match with replacement as sensitivity analyses. Selumetinib's effect (maximum duration 5.6 years) on progression risk was evaluated using univariate and multivariable Cox models (adjusted for aforementioned baseline characteristics) of progression-free survival (PFS) and reported as hazard ratios (HRs). Kaplan–Meier method was used for nonparametric PFS estimates.

Results: Fifty SPRINT and 75 NH patients were included. Before application of PSM, SPRINT versus NH baseline characteristics were generally balanced (standardized difference ≤20%), except for PN location (51%) and PN progression status (60%). Following a 1:1 PS match (n=37), balance was achieved across baseline characteristics, except for PN status (standardized difference 25%). Balance across baseline characteristics was achieved with sIPTW and 1:2 PS match (n=46:43).

PFS HRs (95% confidence interval) for the direct comparison, 1:1 PS match, sIPTW and 1:2 PS match were 0.11 (0.05, 0.25), 0.11 (0.04, 0.29), 0.12 (0.06, 0.25), and 0.11 (0.06, 0.24), respectively, with p-values consistently <0.001 across all methods.

Conclusions: Residual baseline differences in prognostic factors between SPRINT and NH were adequately accounted for. NF1-PN progression risk reduction with selumetinib was consistent with direct comparison and was statistically significant, robust, and comparable across methods.

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Examining the Power of AI in Neurofibromatosis Care and Research with ChatGPT

Robert Allaway, PhD, *Sage Bionetworks*

This presentation will introduce large language models (LLMs) such as ChatGPT, an artificial intelligence (AI)-powered technology that has the potential to revolutionize how researchers, patients, and healthcare providers approach their understanding of neurofibromatosis. ChatGPT is an easily-accessible LLM that generates human-like responses to user input, enabling people to ask complex questions and receive detailed answers. We will explore the transformative impact of this technology on medical research, and discuss the exciting possibilities and some of the risks that lie ahead as AI continues to advance.

For neurofibromatosis researchers, LLMs represent a new paradigm for summarizing and exploring large research corpora. When provided with context, its ability to parse and analyze large amounts of information could drastically reduce the time spent on literature reviews and data analysis, in turn expediting the research process. Additionally, LLMs might help researchers identify new patterns and insights that may otherwise be overlooked.

LLMs can also serve as an effective communication bridge between scientists and non-specialist audiences. By translating complex research findings into simpler, accessible language, LLMs can improve the understanding and dissemination of research outcomes. Notably, the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) Collaboration is exploring using ChatGPT to more efficiently communicate their research findings and response criteria to non-specialist audiences. This could lead to more effective patient education and more informed clinical trial design.

It is also important to consider potential limitations with LLMs like ChatGPT. We will discuss key concerns such as the generation of misinformation, data privacy issues, and the propagation of biases in the training data. We hypothesize that ChatGPT and other LLMs will be widely-adopted due to the ease of access. The NF community needs to be aware of and consider both the opportunities and the corresponding risks when using LLMs.

LLMs have the potential to transform the way neurofibromatosis patients, clinicians, and researchers explore medical information, providing accessible, on-demand knowledge and clinical decision support. But it's just the beginning—as AI continues to evolve, so too do the possibilities for transformative healthcare solutions. We encourage the neurofibromatosis community to embrace this exciting new frontier, and join us in exploring the potential of AI in healthcare while being vigilant of its limitations.

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Comparative Analysis of Validated Measures of Cognitive, Behavioral, and Motor Impairments in a Pediatric Population of Neurofibromatosis Type 1

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Purpose: To establish brain-based, objective measures that reflect 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 3,500 individuals. Manifestations can include skin pigmentation abnormalities, distinct osseous lesions, and/or benign or malignant tumors. Approximately 50% of children with NF1 exhibit problems with behavioral and emotional regulation that can include attention-deficit/hyperactivity disorder (ADHD) symptoms, learning disabilities, and delays in motor function. Currently, ordinal scales performed by subjective raters are used to evaluate these manifestations. This impedes accurate measurement of responses to potential therapeutics. We hypothesized that techniques such as Transcranial Magnetic Stimulation (TMS) and MRI-based measures would provide more objective data.

Methods: We recruited children ages 8- 16 years with NF1. Assessments of behavior and executive function included parent rating scales [ADHD-RS; Behavioral Rating Inventory of Executive Function Second Edition (BRIEF-2); Test of Variables of Attention (TOVA)], and motor development using the Physical and Neurological Examination for Subtle Signs (PANESS). We measured inhibition and excitation in motor cortex using single- and paired-pulse TMS. We also evaluated neurotransmitter concentrations using Magnetic Resonance Spectroscopy (MRS). Spearman Rank Correlations were performed to explore associations between clinical assessments and brain measures.

Results: Seventeen youth (age mean 12.4, S.D. 3.1; 8 female, 9 male; 16 Caucasian, 1 African American; 17 non-Hispanic) participated. Reduced left motor cortex excitability, reflected in low amplitude motor-evoked potential (MEP) and high resting motor threshold (RMT), was associated with more severe clinical ADHD (ADHD-RS and/or BRIEF composite scores). Impaired motor function, reflected in high PANESS scores, was associated with poor performance on the TOVA (high omission/commission error rates and high variability). High response variability (TOVA) was associated with lower glutamate/glutamine (GLX) levels.

Conclusion: Including TMS-associated MEP and RMT and TOVA-associated omission, commission, and variability error rates in future clinical trials might improve speed and accuracy in detecting clinically meaningful improvement. These measures show promise as brain-based biomarkers of impaired executive function and motor function in NF1.

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Novel Austrian Care and Prevention Guide for Children and Adolescents with NF1

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Background: Unified strategies for the care of children and adolescents affected by Neurofibromatosis type 1 (NF1) are lacking in Europe. While ERN GENTURIS developed guidelines for the tumour surveillance and management in NF1, these recommendations do not cover all other aspects of the disease. This holds not only true on an international level, but also on the national level in Austria. Despite being a small country with a uniform health and social security system, approaches to patient care may be comparable but not identical.

Aim: In order to standardise patient care for children with NF1 in Austria, a care and prevention sheet was developed.

Methods: In close cooperation with the Austrian patient organisation NF Kinder the *Austrian NF network* was established. This network is composed of dedicated health-care providers (medical as well as psychosocial) involved in the care of patients with NF1 in ten Austrian centres as well as patient representatives, some of whom were also involved in drafting the novel ERN GENTURIS recommendations. This network undertook the task to review existing guidelines and incorporate the national healthcare structure and best practice guidelines into a consensus document.

Results: The recommendations were condensed into a single form that is divided in four sections: clinical examination, lab investigations, imaging, additional consultations. For each parameter, the document advises on the age specific intervals for screening. Furthermore, it indicates the appropriate time points during follow-up (e.g. at initial presentation, at transition, if clinically indicated, …). The recommendations thereby focus not only on the inherent tumour risk but addresses the broad spectrum of possible symptoms (such as orthopaedic or surgical), specifically emphasising also psychosocial and neurocognitive issues.

Conclusion: The novel Austrian care and prevention guide for children and adolescents with NF1 will facilitate uniform best practice care for affected kids on a national level.

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Outpatient and Inpatient Health Care Utilization in Patients with Neurofibromatosis 1 in Ontario, Canada

Carolina Barnett-Tapia, MD, PhD, *Division of Neurology, Department of Medicine, University of Toronto*

Objectives: There are limited data regarding the health care utilization use of people with Neurofibromatosis type 1 (NF1). We aimed to assess the use of different health services in people with 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 of comorbidities, outpatient claims, hospitalizations, emergency department visits and same-day surgeries. We also compared mortality rates in NF1 patients and controls.

Results: We identified 1,240 individuals with NF1, and 1,205 met inclusion criteria. These were matched to 6,025 controls. At index date, the mean age was 7.4 ± 12.3 years (median 0, IQR: 0-11), and 50.8% were female. Mean follow up time was 19.6 ± 8.7 years in NF1, and 18.8 ± 8.5 years in controls, and at the end of the study window, mean age was 26.2 ± 16.9 years (median 23, IQR: 14-36). Individuals with NF1 had a higher prevalence of cancers than controls (11.7% vs. 2.3%, respectively, $p<0.001$). A higher proportion of NF1 patients had a family physician than controls (94.4% vs. 86.5%, $p<0.0001$). More adults with NF1 received disability benefits than controls (17.6% vs. 6.6%, $p<0.001$).

During the study window, NF1 patients had more visits to the emergency department (RR:1.35, 95% CI: 1.25-1.45), hospital admissions (RR: 3.41, 95%CI: 3.16-3.8), primary care visits (RR: 1.26, 95%CI: 1.2-1.3), specialist visits (RR:2.98, 95%CI: 2.8-3.2) and same-day surgeries (RR:1.93, 95%CI: 1.74-2.14). During the study window, there were 21 (1.7%) deaths in the NF1 group, compared to 111 (1.8%, p=ns) in the controls.

Conclusions: Individuals with NF1 in Ontario, Canada, have higher use of primary and specialty outpatient services, as well as higher rates of ED visits, admissions to hospital, surgeries and disability benefits. In this cohort there was no difference in death rates or age of death compared to matched population controls, but this is limited due to small numbers.

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Primary Healthcare Utilization in Individuals with NF1 in Ontario, Canada

Carolina Barnett-Tapia, MD, PhD, *Division of Neurology, Department of Medicine, University of Toronto*

Objectives: To assess the use of primary healthcare of individuals with Neurofibromatosis 1 (NF1) compared to controls.

Methods: We used UTOPIAN, which is a database of electronic medical records (EMRs) from primary care in Ontario, Canada. We used a validated free text search strategy to identify individuals with NF1; a clinician with NF1 experience validated NF1 records. NF1 patients were matched 1:5 by age, sex and EMR start date to controls. We compared the prevalence of the most common diagnostic codes used in individuals with NF1 compared to controls, during a 5-year window (2015 to 2019). We also compared the billing rates (number of billings per 100 individuals) for selected codes between patients and controls during the same period.

Results: In December 2020, there were 421,971 eligible patients in UTOPIAN, 127 were classified as NF1 (prevalence 1 in 1,323). These were matched to 635 controls; 53.5% were female and mean age at data extraction date was 36.4±22.6 years. Median time in the EMR was 8 years.

During the study window, NF1 patients had a higher rate of visits for anxiety/depression (rate difference= +32.6 /100 patients, 95%CI: 27.9-37.3); however, there was no significant difference in the prevalence of diagnostic codes for anxiety/depression between NF1 and controls (22.1% vs 18.0%, p=ns). More NF1 patients had a diagnostic code for skin/subcutaneous tissue disorders (10.2% vs 5.4%, p<0.0001) and for essential hypertension (11.0% vs. 9.7%, p<0.001) than controls.

NF1 patients had more periodic health visits for adults (18-64 years) than controls (rate difference: +13.1/100, 95%CI:9.2-16.9), but had fewer healthy child visits (rate difference: -15.3 /100, 95%CI: -20.5, -10.0). NF1 patients had fewer billings for contraception management than controls (5.5% vs. 8.4% p<0.001), with a rate difference of -11.3 billings/100 (95%CI:-14.5, -8.2). NF1 patients also had fewer billings for immunizations (rate difference: -9.9/100, 95%CI: -12.9, -6.9) and mammogram tracking (rate difference: -4.9/100, 95%CI: -8.0, -1.8) than controls.

Conclusion: In this cohort from primary health care records, individuals with NF1 had a similar prevalence of a diagnosis of anxiety/depression than controls, but needed more primary mental health services. NF1 patients had fewer healthy child and immunization visits than controls, which may reflect concomitant follow up by pediatricians. There were more primary health visits in adults than controls; however, there were fewer billings for mammogram tracking in NF1, despite increased risk of breast cancer in this population, and fewer contraceptive-related visits.

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Pre-Surgical Testing Considerations in Visually Impaired Patients with NF1: An Illustrative Case Study

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Neurofibromatosis type 1 (NF1) is a rare and complex, multi-symptom condition caused by variation in the NF1 tumor suppressor gene that results in neurofibromas . Optic pathway gliomas (OPG) are a common finding in NF1, affecting an estimate of between 5 to 20% of people diagnosed with NF1. Approximately 30-50% of NF1-patients with OPG demonstrate symptoms that require treatment, such as reduced visual acuity, endocrinological abnormalities, disturbances of color vision, visual field defects, and atrophies of the optic nerve. Approximately one-third of OPGs in NF1 require intervention, which is primarily reserved for individuals with worsening symptoms. Surgical resection of symptomatic or growing neurofibromas is necessary as these lesions can cause substantial pain, disfigurement, and neurological deficits. Owing to the elevated possibility of neurosurgical intervention, neuropsychological testing can be extremely beneficial to patients and their medical team as it can serve to establish a neurocognitive baseline, provide predictions of the neurocognitive risks associated with the proposed surgery, and contribute to the locational and lateralization conceptualization. Yet, there are often barriers to comprehensive assessment, including, but not limited to: developmental delays, attentional disturbance, and/or vision/visuospatial dysfunction. We present the case of a 21-year old patient with NF1 who was referred for a pre-operative neuropsychological assessment on a pre-operative basis. Additional medical history includes refractory focal epilepsy (onset at 18 months of age) and OPG, which resulted in profound visual impairment. Despite relatively intact intellectual functioning, the patient experienced reduced task vigilance and elevated levels of cognitive fatigue. Owing to his unique presentation, multiple testing accommodations and non-standard test administration practices were undertaken. The patient underwent complex neuropsychological assessment including a clinical interview and tests of general intellectual functioning, attention, working memory, executive functioning, learning and memory, language, motor, academic functioning, and mood. We aim to explore the diagnostic decision making and data interpretation necessary to potentially localize and lateralize underlying neurocognitive dysfunction, which was a core referral issue. The current presentation will also discuss the challenges inherent to more extensive neurosurgical work-up, such as functional MRI and/or intracarotid amobarbital procedure (i.e., Wada test).

Prognostic Factors Related to Pediatric NF1-Associated CNS Tumors

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Background: Neurofibromatosis type 1 (NF1) is a hereditary neurocutaneous tumor predisposition syndrome (TPS). As a TPS patients with NF1 have a high risk of developing central nervous system (CNS) tumors, predominantly optic pathway low-grade gliomas (OPGs). Clinical management is challenged by the variable natural history of these OPGs and gliomas outside the optic pathway (GOOPs), and the lack of well-established prognostic markers.

Purpose: to identify prognostic factors in disease course and outcome of pediatric NF1-CNS tumors patients.

Methods: this pediatric NF1-CNS-tumor-patient-cohort (pNF1-CNSt-C) study reports on NF1-related CNS tumors in a Dutch national-center cohort over a period of 8 years (2005-2023). Genetic and clinical data were obtained. Modified Dodge classification categorized the OPG location. Three final outcomes were evaluated: progression free survival, presence of visual and neurological symptoms.

Results: the pNF1-CNSt-C included 121 patients (63 males (52.1%) and 58 females), with median (min-max) age of 4.6 (0.8-17.8) years at diagnosis and 13.9 (1.8-26.0) at study end. Hundred-thirty-five (69.5%) optic pathway, 18 brainstem and 18 cerebellar (9.3%) tumors were identified. Nighty-seven (65.3%) patients were symptomatic at presentation; 29 (24.0%) with abnormalities in ophthalmological and/or neurological examination. Forty-nine (40.5%) tumors were indolent, 72 (59.5%) required treatment. Fifty-two (43.0%) patients had radiological tumor progression. Neurological symptoms (HR2.5, $p=0.007$), and one, two, and three or more lines of therapy (HR2.9 $p=0.008$, HR4.1 $p=0.005$, HR14.7 $p<0.005$, respectively) were statistically significant predictors of tumor progression, in multivariate analyses. (Post)chiasmatic tumor location showed highest correlation with visual symptoms and GOOPs with neurological symptoms in logistic regression (both $p>0.05$). Symptomatic patients at presentation had worse visual (OR10.0, $p=0.004$) and neurological (OR28.6, $p=0.003$) outcomes compared to asymptomatic patients.

Conclusions: in this pNF1-CNSt-Cohort poor prognostic factors for tumor progression are neurological symptoms and multiple lines of therapy. Tumor location and symptomatic presentation are associated with worse visual and neurological outcomes.

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The NF Data Portal: Accelerating Research of Neurofibromatosis

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Purpose: The Neurofibromatosis (NF) Data Portal provides a space for NF researchers to share, find, and re-use original research data and analysis tools. Sharing data can spark collaborative opportunities and accelerate scientific progress by improving the speed and accuracy of the research pipeline. Yet, following the F.A.I.R principles that data be Findable, Accessible, Interoperable and Re-usable presents several challenges including: developing data model standards for diverse data types, maintaining accurate and organized content, establishing data-sharing governance, protecting patient data privacy, harmonizing data from diverse sources, making data findable through an accessible user interface, and providing documentation for users with diverse expertise.

Methods: Expanded breadth of terms and data models for newer data types (https://github.com/nf-osi/nf-metadata-dictionary). Development of a more functional data sharing plan (DSP) interface and workflow at project start. In this workflow, governance and patient data privacy are captured in the DSP using data use ontology (DUO) terms. Efforts to optimize data harmonization and re-use via reprocessing of sequencing data and transmission of data to analytical platforms such as cBioPortal. Collected feedback from the NF community during a recent webinar about the types of analysis tools needed.

Results: Since 2016, the NF Data Portal has grown to host data for >20 initiatives supported across 5 funding agencies, ~200 studies, 33,000 files encompassing 87 distinct file types, and ~1000 research tools that altogether equate to over 150 terabytes of content. Overall, ~46 versions of the metadata dictionary have been released. The NF data portal has enabled research leading to 130 publications. The survey showed most users (15 of 21) see a need for more platforms that are highly specific, like iAtlas and cBioPortal which have pre-built analysis tools, as opposed to platforms which have flexible infrastructure but require larger programming knowledge.

Conclusions: The NF Data Portal is a platform that hosts data from studies focused on neurofibromatosis. As the tools for data annotation, harmonization, analysis, and re-use improve and requirements for data storage and sharing including the NIH data sharing policy are enacted, we anticipate additional demand for managing data upload, annotation, responsible access, and re-use. The improvement of the DSP interface has facilitated data ingress and implementation of robust fit-for-purpose data access governance. In conclusion, the NF Data Portal is a reliable option to share NF data responsibly and optimize the data utility for the benefit of NF patients.

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Amusia and its Electrophysiological Correlates in Neurofibromatosis Type 1: An In-Depth Analysis

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Background: Neurofibromatosis type 1 (NF1) is a rare genetic disease which often results in different cognitive problems. In one of our previous studies, we found that 70% of people with NF1 had a deficit in music perception, described in the literature as amusia. It was also observed that the worse this deficit was, the greater the latency for the occurrence of a long latency auditory evoked potential, known as Mismatch Negativity (MMN) (**Figure 1**), which is an objective marker of auditory sensory accuracy and assesses the pre-attentive cognitive operations of hearing. The objective of this study is to investigate in a larger sample if there really is a correlation between the occurrence and latency of MMN and the degree of musical perception in people with NF1.

Methods: 34 patients with NF1, aged between 14 and 35 years, were invited and agreed to participate. They were submitted to the assessment of musical perception through the Montreal Battery Evaluation of Amusia (MBEA) – short version. The integrity of the cortical primary auditory processing areas was assessed using the MMN (**Figure 1**)

Results: Although we did not find a statistically significant correlation between the absence of MMN and the degree of musical perception impairment among the evaluated subjects, there was a trend toward higher MMN latency values for lower MBEA scores ($p = 0.039$) (**Figure 2**).

Conclusions: The present study corroborates our previous findings about a possible correlation between MMN latency and music processing in people with NF1. Our next steps will be to evaluate possible correlations between this auditory evoked potential and other cognitive functions in NF1.

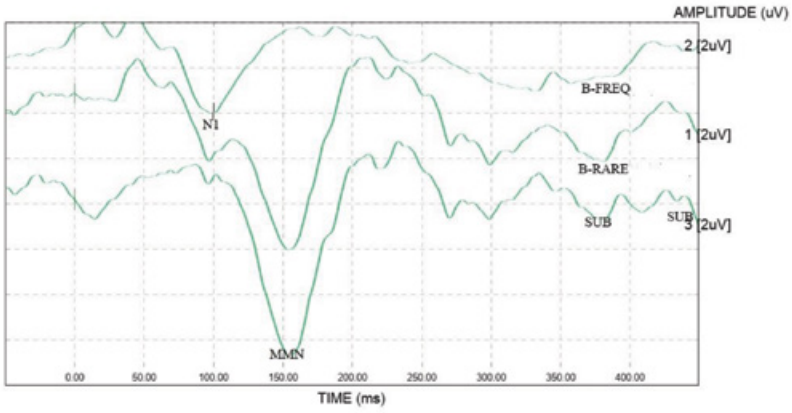

Figure 1. Mismatch negativity (MMN). Graphic record of long latency auditory evoked potentials of a patient with NF1. The MMN is the additional negative peak after N1, occurring here near 150 ms during a rare stimulus trace (B-RARE) and its absence during a frequent stimulus trace (B-FREQ). The MMN is more evident in the trace corresponding to the subtraction (SUB) of the standard stimulus from the deviant stimulus.

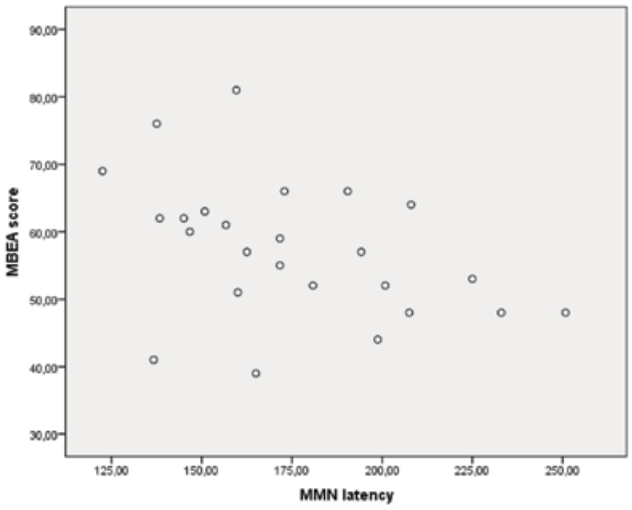

Figure 2. Scatter plot graphic: Correlation between Montreal Battery Evaluation of Amusia (MBEA) and mismatch negativity (MMN) latency in patients with NF1.

Full List of Authors: Cota BCL, Júlia Lemes JL, Araújo MA, Corgosinho MSC, Rezende NA, Rodrigues LOC; Resende LM.

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Amusia and its electrophysiological correlates in neurofibromatosis type 1. Arquivos de Neuro-Psiquiatria, 2018. 76(5), 287-295. doi:https://doi.org/10.1590/0004-282X20180031

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Effects of Music Training on Voice and Auditory Skills in Adolescents with Neurofibromatosis Type 1 – An Ongoing Case Series Study

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Background: A variety of non-neoplastic manifestations affect individuals with Neurofibromatosis type 1 (NF1), including some speech-language disorders, which result in an important impact on communication skills and quality of life. The auditory processing disorder stands out, which leads to difficulty understanding speech and language, reading and writing disorders; and voice disorder, characterized by hoarseness, reduced maximum phonation time, reduced vocal resistance and hypernasality. In people without NF1, it was demonstrated that musical training, addressing parameters such as height, duration, rhythm and melody, improves auditory skills, and that singing has positive effects on the voice, contributing to vocal health and plasticity. The aim of this study is to verify the effects of music training on voice quality in four adolescents with NF1, and the possible correlation between these findings and changes in central auditory processing.

Methods: Four teenagers were invited and agreed to participate in this study. They underwent vocal assessment and auditory processing tests (**Table 1**) before starting 20 musical training sessions aimed at developing rhythmic and melodic skills. At the time of submission of this abstract, the volunteers are in the sixteenth session of musical training, and after the last session they will be submitted again to the same tests performed previously. At the end, the effects of musical training on voice quality parameters and auditory processing will be evaluated.

Results: The data presented (**Tables 2 and 3**) correspond to the tests carried out before the beginning of music and singing lessons. Based on these data, we found mild to moderate dysphonia among the volunteers, in addition to impaired acoustic measures and temporal auditory processing, based on Musiek's criteria. The post-intervention analysis, including the effect of music training on voice quality and listening skills, will be performed in 6 weeks and will subsequently be available for presentation at the NF 2023 Conference.

Conclusions: Preliminary data from this study corroborate data from the literature that describe changes in voice and auditory processing in people with NF1. After completing the next steps, it will be possible to measure the effect of musical training on vocal quality and auditory skills in the volunteers of this study.

Function	Test	Description
Temporal Auditory Processing	Gaps In Noise (GIN)	Assesses temporal resolution ability, which refers to the accuracy of perceiving sounds in time.
	Pitch Pathern Sequence (PPS)	Evaluates the temporal ordering ability, which refers to the perception of two or more auditory stimuli in their order of occurrence in time.
Voice	Auditory-perceptual analysis	Evaluation based on the GRBASI (Hirano, 1981), which evaluates the characteristics of roughness, breathiness, asthenia, tension and voice instability from the recording of the sustained vowel /a/. It was performed by a speech therapist with more than 10 years of experience in the field of clinical voice.
	Short term acoustic measurements	Acoustic measures that evaluate, in general terms, the degree of aperiodicity of the sound wave, and the higher the values, the greater the degree of aperiodicity of the wave. The measures were extracted by the MDVP advanced software program from the recording of the sustained vowel /a/.

Table 1. Description of tests used in assessments.

Test	Parameter	Subject 1	Subject 2	Subject 3	Subject 4
Auditory-perceptual*	Grade	1	2	2	2
	Rough	0	0	0	0
	Breathy	1	2	2	2
	Astenic	0	2	2	2
	Strain	0	0	0	0
	Instability	0	0	1	1
Acoustic Parameters	F0 (Hz) 243 (norm)	240	230	224	206
	Jitter (%) < 0.366 (norm)	2.944	3.375	3.408	3.669
	PPQ (%) < 0.366 (norm)	1.817	1.993	2.062	2.233
	Shimmer (%) < 1.997 (norm)	9.057	7.447	6.382	6.669
	APQ (%) < 1.397 (norm)	6.367	4.792	4.342	4.515
	NHR < 0.112 (norm)	0.168	0.156	0.152	0.121

Table 2. Auditory-perceptual and acoustic voices evaluation before music classes.
*Rating Scale: 0: normal, 1: slight; 2: moderate; 3: severe. F0= fundamental frequency; PPQ= period perturbation quotient; APQ= amplitude perturbation quotient ; NHR= noise harmonic ratio. norm= normality value.

Auditory processing	Test	Subject 1	Subject 2	Subject 3	Subject 4
Temporal Resolution GIN (norm): > 54%	GIN RE	41,6%	20%	50%	60%
	GIN LE	48,3%	8,33%	35%	53,3%
Temporal Ordination PPS (norm): >75%	PPS RE	40%	40%	93,3%	100%
	PPS LE	33,3%	33,3%	96,6%	100%

Table 3. Temporal auditory processing evaluation before music classes.
GIN = Gaps in Noise; PPS = Pitch Pattern Sequence Test; RE = right ear; LE = left ear.

Full List of Authors: Ávila ALFS; Lopes BP; Cota BCL; Barbosa TAO; Rezende NA, Rodrigues LOC; Resende LM.

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High Cognitive and Musical Functioning in Neurofibromatosis Type 1 (NF1): A Case Report

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Background: People with NF1 often have difficulties in perceiving and performing music. The disease also results in various cognitive dysfunctions, including learning and communication disorders, impairment of attention, memory and motor skills, as well as visuospatial and executive dysfunctions. Some pathogenic variants have been described as possible determinants for a greater impairment of cognition, especially microdeletions, however there are no studies that correlate specific pathogenic variants with phenotypes of lower cognitive impairment. Therefore, the objective of our work was to evaluate possible factors that could be related to a possible high cognitive and musical performance in a young patient with NF1.

Methods: We invited a 27-year-old female patient, with no parental history of NF1, with 3 clinical criteria for the diagnosis of neurofibromatosis type 1, with no noteworthy complications arising from the disease, with no current or previous complaints of any cognitive impairment. Brazilian, master's student at a prestigious university in the USA, and with a history of great affection for music. She studied music from the age of 3, learned several musical instruments, and has been dancing ballet for 11 years. She agreed to participate in the study and underwent a battery of cognitive, musical and hearing electrophysiology tests (**Table 1**), in addition to the genetic analysis of the pathogenic variant that resulted in her disease.

Results: The patient performed above average in almost all cognitive skills assessed (**Table 2**). Her musical perception was far above the average described for people with and without NF1. Her MMN was identified, with a latency of 151 ms and 6.71 μV of voltage (**Figure 1**). Genetic analysis showed the pathogenic variant Chr17:31,334,927 C>T in the NF1 gene.

Conclusion: The evaluated patient has excellent cognitive and musical performance in several aspects, consistent with the absence of cognitive complaints and her history of great involvement with music. Its genetic variant is described in the literature as pathogenic for the NF gene, but there is no data on its phenotypic correlation. Her MMN had low latency and high amplitude, which are parameters often described as determinants of better cognitive and musical function. Could these findings be due to its pathogenic variant or to the influence of early external musical exposure? Are there other genetic or environmental factors to consider?

Test	Assessed Skill
WASI	Intelligence - Reduced version using the Vocabulary and Matrix Reasoning subtests.
Corsi Cubes	Working Memory, visuospatial processing.
Five digits test (FDT)	Processing speed, inhibitory control, attention and executive functions.
Alternating Verbal Fluency (AVF)	Semantic memory, language, cognitive flexibility, processing speed, working memory.
Five Points Test (FPT)	Visuoconstructive and visuospatial ability.
Attention network test	Attention, inhibitory control.
Montreal Battery of Evaluation of Amusia (MBEA)	Components of music processing: scale, contour, interval, rhythm, metric, and memory.
Mismatch Negativity (MMN) Long-latency auditory evoked potential	Auditory cognitive processing wich reflects a pre-attentive memory trace produced as response of a repetitive and identical auditory stimulus.

Table 1: Neuropsichologic, music processing and auditory electrophysiological tests.

Test	Score	Performance| Interpretation
WASI- IQ	128	p= 96 Upper average
Performance Corsi direct order- Hits	10	p=82 upper average
Corsi direct order-Span	6	p=60 Average
Direct order composite score	60	p=77 Upper average
Corsi- reverse order-Hits	11	p=82 Upper Average
Corsi- Reverse Order-Span	7	p=87 Upper Average
Composite score in reverse order	77	p=91 Higher
Reading	22	p=50 Average
read-errors	0	p>95 Higher
Score	23	p=63 Average
count- errors	0	p>95 Higher
choices	28	p=81 Upper Average
choice- mistakes	0	p>95 Higher
alternation	34	p=82 Upper Average
Toggle- errors	0	p>95 Higher
Inhibition	6	p=85 Upper average
Flexibility	12	p=86 Upper average
AVF-Animals	25	p= 81 Upper average
AVF-Fruits	16	p= 50 average
AVF-Animals And Fruits	17	p= 50 average
AVF-Animals And Fruits pairs	8	p= 50 average
FPT-Accesses	36	p= 81 Upper average
FPT-Errors	0	p= 95 Upper average
MBEA Total score	81	General population ~ 2P higher; NF people ~3DP higher
MBEA melodic score	40	General population ~ 2P higher; NF people ~4DP higher
MBEA rhythm score	28	General population ~ 1DP higher; NF people ~4DP higher
MBEA memory score	13	General population= average; NF people ~1DP higher

Table 2. Results of Neuropsychological Tests and evaluation of musical perception

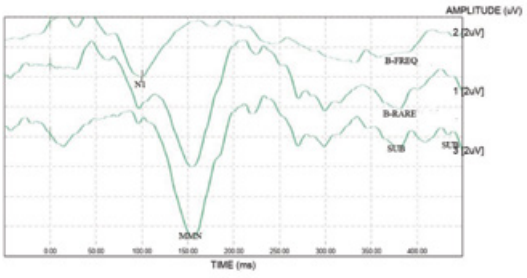

Figure 1. Mismatch negativity (MMN). Graphic record o long latency auditory evoked potentials of a patient with NF1. The MMN is the additional negative peak after N1, occuring here near 150 ms during a rare stimulus trace (B-RARE) and its absence during a frequent stimulus trace (B-FREQ). The MMN is more evident in the trace corresponding to the subtraction (SUB) of the standard stimulus from the deviant stimulus.

Full List of Authors: Cota BCL; Santiago VP; Andrade JN; Pereira GP; Santos BM; Rodrigues LOC; Resende LM, Rezende NA

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Musical Training for the Temporal Auditory Processing and Speech Coordination in Neurofibromatosis Type 1: Is There a Relation?

Bruno Cezar Lage Cota, MD, *Outpatient Neurofibromatosis Reference Center (CRNF), Federal University of Minas Gerais, Brazil*

Background: Neurofibromatosis type 1 (NF1) is a rare genetic disease that results in multisystem manifestations, including several speech-language disorders, which may be related to Auditory Processing Disorder (APD). In a previous study with 25 patients with NF1, everyone presented alterations in the temporal resolution test. Other speech-language disorders that may be present are related to stomatognathic system, speech fluency (non-stuttering disfluencies) and amusia. Considering that music training can lead to positive effects on auditory processing skills, and that auditory perception can be related to motor learning, the aim of this study is to verify whether music training can improve auditory processing, and consequently, coordination of speech in individuals with NF1.

Methods: This study is in progress and was approved by the ethics committee #4062315.7.0000.5149. Five individuals diagnosed with NF1 were invited and agreed to participate. They were submitted to an auditory processing evaluation, using the Gaps in Noise (GIN) test, and to an Orofacial Motricity (OM) assessment, using the oral diadochokinesia test. The evaluation of the oral diadochokinesis test was performed blindly by a trained evaluator. Twenty musicalization sessions were planned, with the purpose of rhythm and melody training. Of the five volunteers, three took singing lessons and two took keyboard lessons, beside the musicalization classes. At the end of the training, all volunteers will be submitted to the same tests described above.

Results: The results of the pre-intervention evaluations are shown in **Table 1**. The speech rhythm was evaluated in an auditory-perceptual way. At the time of this abstract submission, the music intervention is in its final phase (16th session). Post-intervention data will already be available during the NF Conference, where they can be presented.

Conclusions: Data from the assessment prior to musical training corroborate the literature findings regarding APD, except for one volunteer who presented normality in the GIN test. Our findings also showed changes in speed and rhythm in all volunteers. From the analysis of the results at the end of this study, it will be possible to state whether musical training has any effect on the evaluated aspects.

Functions/tests		Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
Temporal Auditory Processing	GIN RE	41,6%	60%	50%	71,6%	20%
	GIN LE	48,3%	53,3%	35%	75%	8,33%
Speech coordination	SS	/pa/ - 5,8 /ta/ - 4,9 /ka/- 5,0 /pataka/- 1,8	/pa/ - 4,4 /ta/ - 4,7 /ka/- 4,6 /pataka/- 1,6	/pa/- 4,7 /ta/ - 6,4 /ka/- 6,1 /pataka/- 1,9	/pa/ - 5,4 /ta/ - 7,1 /ka/- 6,5 /pataka/- 2,0	/pa/ - 3,0 /ta/- 5,1 /ka/- 3,5 /pataka/- 1,9
	SR	/pa/- inadequate /ta/- inadequate /ka/- inadequate /pataka/- inadequate	/pa/- inadequate /ta/- inadequate /ka/- inadequate /pataka/- inadequate	/pa/- adequate /ta/- inadequate /ka/- adequate /pataka/- inadequate	/pa/- adequate /ta/- adequate /ka/- inadequate /pataka/- inadequate	/pa/- adequate /ta/- inadequate /ka/- inadequate /pataka/- adequate
Music Learning Modality		Singing	Singing	Singing	Keyboard	Keyboard

Table 1: Results of the pre-intervention assessment of auditory processing, speech coordination and learning modality. AP= Auditory Processing; GIN = Gaps in Noise; RE = right ear; LE = left ear; SS = Speech Speed; SR = Speech Rhythm

Full List of Authors: Barbosa TAO, Cota BCL, Motta AR, Resende LM, Ana Letícia Ferreira Ávila ALFS, Cortes ABJC, Rezende NA, Rodrigues LOC.

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Expanding a Precision Medicine Platform for MPNSTs: New PDXs, Cell Lines and Tumor Entities

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Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas constituting the main Neurofibromatosis type 1 (NF1)-associated malignancy. Since MPNST's specific histological criteria have not been completely established, their diagnosis can be challenging, especially outside the NF1 context. In the pre-clinical context, there has been considerable heterogeneity in terms of treatment response. We reasoned that one possible cause of this response disparity could be the difficulty of getting a correct diagnosis in those tumors with a great histological and marker expression overlap with MPNSTs.

For several years, our group has been generating a large patient-derived orthotopic xenograft (PDOX) MPNST platform for testing new treatments. We describe here the expansion of this platform using six primary tumors clinically diagnosed as MPNSTs, from which we obtained six additional PDOX mouse models and three cell lines, generating three pairs of *in vitro/in vivo* models derived from the same tumors (**Figure 1**). We performed a comprehensive characterization of these tumors, including genomic, epigenomic, and histological analyses. Three of the tumors were clearly classified as MPNSTs. However, the initial MPNST diagnosis of the other three was questioned, one most probably being a melanoma (MPNST-SP-01), another bearing an *NTRK* fusion gene (MPNST-SP-05) and a third tumor (MPNST-SP-06) presenting a truncated variant in *SMARCA4,* inactivation of *NF2* and an activating mutation in *NRAS* (**Table 1**). We were able to generate cell lines from only 50% of the tumors, while the efficiency for PDOX generation was 100%. Newly derived cell lines and PDOX were also extensively characterized and compared to primary tumors, recapitulating main histological features, expression markers, and copy number profiles. Finally, we used the new cell lines for testing compounds against known altered pathways in MPNSTs. We found that the functional status of tumor suppressor genes *(NF1, CDKN2A, and PRC2)* and the identity of tumors both impacted treatment response (**Table 2**).

In summary, we generated a comprehensive precision medicine platform for MPNSTs, also extending it to tumor entities that might be misdiagnosed as MPNSTs, and thus being more representative of a real clinical scenario. Since genomic status and tumor identity impacts treatment response, our current platform may facilitate the identification of the best therapeutic strategy for all tumors diagnosed as MPNSTs.

Figure 1

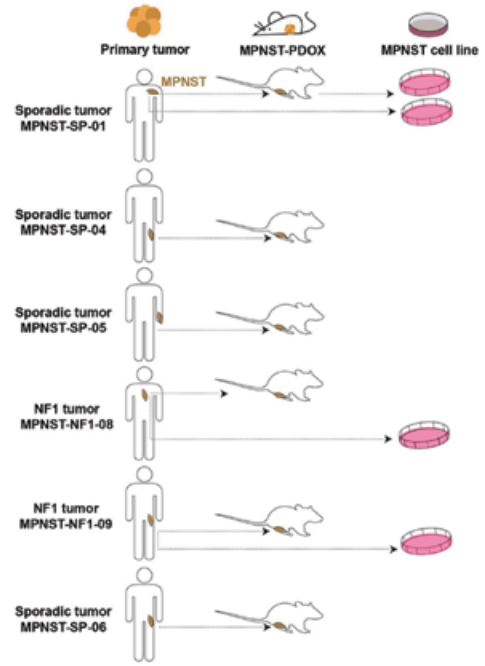

Table 1

	Genetics						Epigenetics	MPNST markers				
Tumor ID	*NF1*	*CDKN2A*	*SUZ12*	*EED*	*TP53*	Number of somatic exonic SNV	Cell lines methylome classification	Sox10	S100	H3K27	Additional alterations not related to MPNSTs	Final tumor classification
MPNST-SP-01	✗	✓	✓	✓	✗	1410	Melanoma	+	+	-	*ERBB4*:NM_005235:c.1834C>T: p.(Arg612Trp) COSMIC signature 7	Posible melanoma
MPNST-SP-04	✗	✗	✗	✓	✓	20	NA	-	(focal +) +	-	NA	MPNST
MPNST-SP-05	✓	✗	✓	✓	✓	23	NA	-	-	-	*LMNA-NTRK1* gene fusion	NTRK-associated sarcoma
MPNST-SP-06*	✓	✗	✓	✓	✓	23	NA	-	-	+	*NRAS*:NM_002524.5:c.35G>C: p.(Gly12Ala) *NF2*:NM_000268:c.1157del:c.p.(Lys387Argfs*39) + LOH	Posible other tumor entity
MPNST-NF1-08	✗	✗	✗	✓	✓	33	MPNST	-	-	-	NA	MPNST
MPNST-NF1-09	✗	✗	✓	✓	✗	NA	MPNST-like	-	-	+	*PIK3CA*:NM_006218.4: c.3035T>A p.(Met1012Lys)	MPNST

* Data obtained from Whole Exome Sequencing
LOH: Lost of Heterozygosi

Table 2

	IC50 values (μM)			
	S462 (Classic MPNST)	**MPNST-SP-01** (Melanoma)	**MPNST-NF1-08** (Classic MPNST)	**MPNST-NF1-09** (MPNST, PRC2 WT)
Alisertib (Aurora inh.)	3.872	105.7	12.59	>100
Mirdametinib (MEK inh.)	0.98	>100	9.267	>100
JQ1 (BET inh.)	1.1	22.85	1.606	7.276

Figures and Tables legend:
Figure 1: PDOX and cell lines generated from the six primary MPNSTs. **Table 1:** Summary of genetic and histological features of primary MPNSTs.
Table 2: Therapeutic response differences between genuine MPNST cell lines and the melanoma cell line.

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A Real-World Assessment of Pediatric Patients with Neurofibromatosis Type 1-Associated Symptomatic Inoperable Plexiform Neurofibroma in the United States: Evidence from a Retrospective Medical Record Review

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Background: Signs and symptoms of neurofibromatosis type 1 (NF1) often begin during early childhood. Plexiform neurofibromas (PNs) affect approximately 20% to 50% of patients with NF1 and can lead to pain, disfigurement, malignancy, and compression of vital structures (Nguyen et al., 2011; Tchernev et al., 2016; Miller et al., 2019). The aim of this study was to describe characteristics and diagnostic journey of pediatric patients in the US diagnosed with both NF1 and symptomatic, inoperable PNs.

Methods: This was a retrospective observational study of pediatric NF1 patients with at least one symptomatic inoperable PN from 3 NF treatment centers in the US. Patient records were abstracted if the patient (1) received NF1 diagnosis from 1 Jan 2000 to 31 Dec 2018, (2) was 2 to 18 years old at the time of diagnosis, (3) was diagnosed with ≥1 symptomatic inoperable PN, (4) received care on-site for ≥12 months, and (5) had ≥3 site visits prior to the end of the study period on 31 Dec 2019. Records were abstracted at the patient's first visit, last visit, and visit nearest the midpoint of observation. All study measures were summarized with standard descriptive statistics using SAS Studio statistical software (version 3.8) (Cary, NC; SAS Institute, Inc.; 2011).

Results: The sample included 102 patients with an average of 3.1 PNs treated over 8.25 years. Most patients were diagnosed with NF1 before the age 3 (57.8%) and with their first symptomatic, inoperable PN by the age 6 (51.0%). Nearly 85% of patients had their first symptomatic, inoperable PN diagnosed by age 10, which typically involved a geneticist (68.6%). Nearly all patients had at least 1 PN diagnosed using MRI (92.2%) while 41.2% reported at least 1 PN through clinical assessment without imaging. Patient PNs were most often managed by a geneticist (85.3%) and/or a pediatric oncologist (30.4%), pediatric neurologist (22.5%), or pediatric neuro-oncologist (22.5%). The most common symptoms at diagnosis of first PN were pain (55.9%) and disfigurement (42.2%). Throughout the review period, pain (76.5%) and disfigurement (45.1%) remained most common. Most patients experienced ≥1 instance of PN progression (73.5%) with a median of 2 episodes per patient.

Conclusion: The burden of PN among pediatric NF1 patients is substantial with most patients having PN symptoms, such as pain and disfigurement, by age 6 and experiencing progression of their PN during childhood, highlighting the importance of early diagnosis and access to care and efficacious treatment.

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Tchernev G, Chokoeva AA, Patterson JW, Bakardzhiev I, Wollina U, Tana C. Plexiform neurofibroma: a case report. Medicine (Baltimore). 2016 Feb;95(6):e2663.

This study was funded in full by AstraZeneca. TD, RR, MB are salaried employees of Alexion, AstraZeneca Rare Disease. XY is a salaried employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA.

Impact of Selumetinib Treatment for NF1 PNs in Pediatric Patients: Perspectives from Patient Caregivers

Theresa Dettling, BSN, JD, MPH, MS, *Alexion, AstraZeneca Rare Disease, Boston, MA*

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 pharmacological treatment licensed for the treatment of symptomatic, inoperable PNs in children with NF1 (approved by the US Food and Drug Administration in April 2020). 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 the patient's caregiver.

Methods: This was a qualitative study of caregivers of children aged 2-17 years who were prescribed selumetinib for the treatment of their PN(s). Study materials were IRB reviewed and approved. Caregivers had to be a parent or primary caregiver of a patient who had been taking selumetinib for ≥ 6 months. Telephone interviews were conducted with each caregiver and interview transcripts were developed from the audio recordings and thematic analysis of the data was performed to identify patterns. Demographic and clinical background information were summarized using descriptive statistics.

Results: The study included 12 caregivers who reported a mean patient age of 13.1 years (range 5 – 17 years) with an average duration of selumetinib treatment of 42.1 months (range 12 – 96 months.) Reported treatment goals mainly focused on tumor size reduction (n=5, 41.6%) and stabilization (n=4, 33.3%) of PN(s). Additional goals included a decrease in PN-associated pain (n=3, 25%). In terms of treatment response, a majority of caregivers (n=11, 91.6%) reported either stabilization or a reduction in size of PNs. While four caregivers (33.3%) reported no PN-associated pain pre-treatment, over half (n=7, 58.3%) reported that their child experienced a reduction in pain following selumetinib initiation. Of three caregivers who reported exhaustion as a pre-treatment symptom, all reported improvement in the child's energy level. More than half (n=7, 58.3%) attributed psycho-social benefits, including social, emotional and learning, to selumetinib therapy.

Conclusion: Caregivers of NF1 PN pediatric patients are concerned about the presence of PNs and associated morbidities in their children. Treatment goals primarily focus 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 in their children.

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This study was funded in full by Alexion, AstraZeneca Rare Disease. TD, RR, MB are salaried employees of Alexion, AstraZeneca Rare Disease. XY is a salaried employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA. AK is a salaried employee of Cerner Enviza, an Oracle Company.

The EU-PEARL Project: A Framework for a Basket-Platform Trial in Neurofibromatosis

B. A. E. Dhaenens, MD, *Department of General Pediatrics, Sophia Children's Hospital, Rotterdam, The Netherlands*

Purpose of the study: Variability in disease manifestations and severity, in combination with a low prevalence, complicates conduct of clinical trials in the Neurofibromatoses (NF). Innovative trial designs that efficiently and rapidly assess the efficacy of new agents are needed. As part of The European Patient-Centric Clinical Trial Platforms (EU-PEARL) project, we designed a framework for a basket-platform trial in NF.

Methods: The NF manifestations to be included in the trial were selected through a modified Delphi procedure. The trial was designed by NF experts, a dedicated statistician, and a trial designer. Collaboration with NF experts from the USA and pediatric oncologists was sought to ensure alignment with current and upcoming trials for NF. A stakeholder meeting was held at the 2022 European NF Conference to assess protocol feasibility. We sent surveys to European NF centers and oncology centers, inquiring about available research facilities and the expected number of eligible patient numbers for each manifestation.

Results: The NF basket-platform trial was designed for plexiform neurofibroma, cutaneous neurofibroma, optic pathway glioma (OPG) and low-grade glioma (LGG) in Neurofibromatosis Type 1, and tumor manifestations in *NF2*-related Schwannomatosis and non-NF2 related Schwannomatosis. The trial will consist of both an observational and a treatment phase. The observational phase will serve as longitudinal natural history study, providing comparator data for the treatment arms. Patients will be able to enter the trial for multiple manifestations sequentially, anticipating on varying treatment needs throughout time. Patients will be randomized to a sequence of available drugs, rather than one single drug: this allows the addition of newly identified drugs during the trial. If a drug concept fails or unacceptable toxicity arises, patients may re-enter the observational phase or be re-randomized to a different treatment arm if eligible. The stakeholder meeting revealed no major objections regarding feasibility as seen by the major stakeholders (patients, clinicians, researchers and pharmaceutical companies). The surveys identified potential research sites for the trial. Small expected patient numbers still remains a significant impediment, especially for OPG and LGG.

Conclusions: This innovative basket-platform trial for NF allows for optimal learning from a small number of patients. It will enhance the chances of finding beneficial treatments by optimizing patient inclusion and invigorating international collaborations. Although this trial design was considered feasible by the main stakeholders, significant challenges remain in terms of limited availability of investigational agents for NF and small patient numbers.

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NVD-003, an Osteogenic Implant Derived from Autologous Adipose Tissue Used for the Treatment of Congenital Pseudarthrosis of the Tibia: Does the Presence of a Neurofibromatosis Type 1 Mutation Influence the Efficacy and Safety of This Tissue Engineered Product?

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Introduction: Congenital pseudarthrosis of the tibia (CPT) is reported as related to neurofibromatosis-1 (NF1) in 55–84%. NF1 mutation results in a loss of neurofibromin activity, driving defective osteoblastic differentiation and increased osteoclastic activity, explaining the excessive bone resorption and increased (re)fracture rates in patients with CPT.

NVD003 is a scaffold-free, stem cell-based osteogenic implant supporting bone healing, even in severe pathophysiological pseudarthrotic conditions. The current standard of care bone graft, autologous bone harvested at the iliac crest, remains associated with significant comorbidities and is impeded by shortness of available bone graft material (**Figure 1**).

NVD-003 might become an alternative option for restoring bone healing capacity, by inducing osteogenicity, osteoconductivity and angiogenicity. In view of CPT treatment, potential influences of the NF1 gene mutation on NVD-003 safety and efficacy had to be investigated.

Methods and results: A series of preclinical studies were completed in mice and rats, implanted with NVD-003 derived from NF1 positive (NF1+) or negative (NF1-) patients. No signs of local or systemic toxicity nor tumorigenicity were reported, independent of the NF1 status of the graft.

The potential impact of the NF1 on NVD-003 efficacy was assessed in vivo by evaluation of the expression profile of osteogenic genes, by histology, histomorphometry and immunohistochemistry. Interference of the NF1 co-morbidity with the osteogenic gene expression profile of NVD-003 was rejected. Histology, histomorphometry and immunohistochemistry confirmed that an NF1 mutation does not impact the number of osteoblasts, new bone tissue formation, osteoblast activity confirmed by expression of RUNX2 and OCN proteins (**Figure 2**), increase in the number of blood vessels, sustained mineralization, and absence of resorption.

Within a compassionate use setting, two NF1+ pediatric CPT patients have been treated with NVD-003 and were followed for over 24 months. Besides the absence of any NVD-003 related side effects, patients showed early radiologically signs of bone formation (**Figure 3**) and had confirmed bone union within 12 months post-implant surgery, confirmed by progressive clinical improvement.

Conclusion: The pre-clinical and clinical results, support the osteogenic potential of NVD-003, irrespective of the NF1 status. From a short- and long-term safety perspective, no signs of toxicity or tumorgenicity were observed. A phase I trial in pediatric patients with CPT with or without NF1, *for which a detailed study outline can be retrieved from Clinical trials.gov, has been approved and is currently open for recruitment (identifier: NCT05693558 – Study Sponsor: Novadip Biosciences SA/NV).*

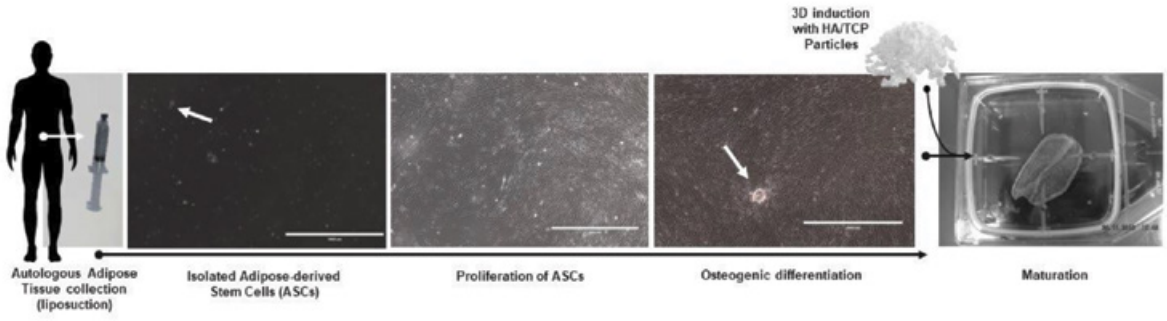

Figure 1: GMP controlled production process: Using stem cells isolated from adipose tissue, proliferated and differentiated to osteogenic cells, complemented with HA/TCP particles and maturated to an autologous 3D scaffold-free osteogenic bone graft.

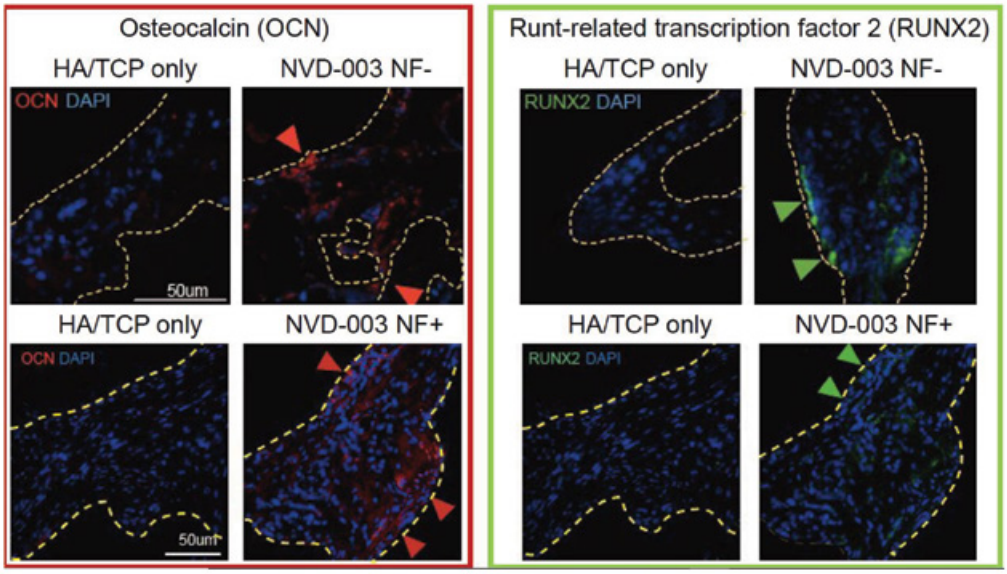

Figure 2: Osteogenicity, expressed by OCN and RUNX2, 12 weeks post- implantation confirmed both for NF1+ and NF1- NVD-003 bone grafts. Osteocalcin (OCN) and Runt-related transcription factor 2 (RUNX2) immunohistochemical staining within NF1-/NF1+ NVD-003 and control HA/TCP-treated implant sites, respectively presented in the red and green panel. OCN immunostaining appears red, RUNX2 immunostaining appears green, while nuclear counterstain appears blue. RUNX2 immunostaining present within bone- lining cells was more conspicuous among NVD003-treated implant sites (green arrowheads). OCN immunostaining present within bone-lining cells was more conspicuous among NVD003-NF1+-treated implant sites (red arrowheads). Dashed yellow lines indicate margins of implant material.

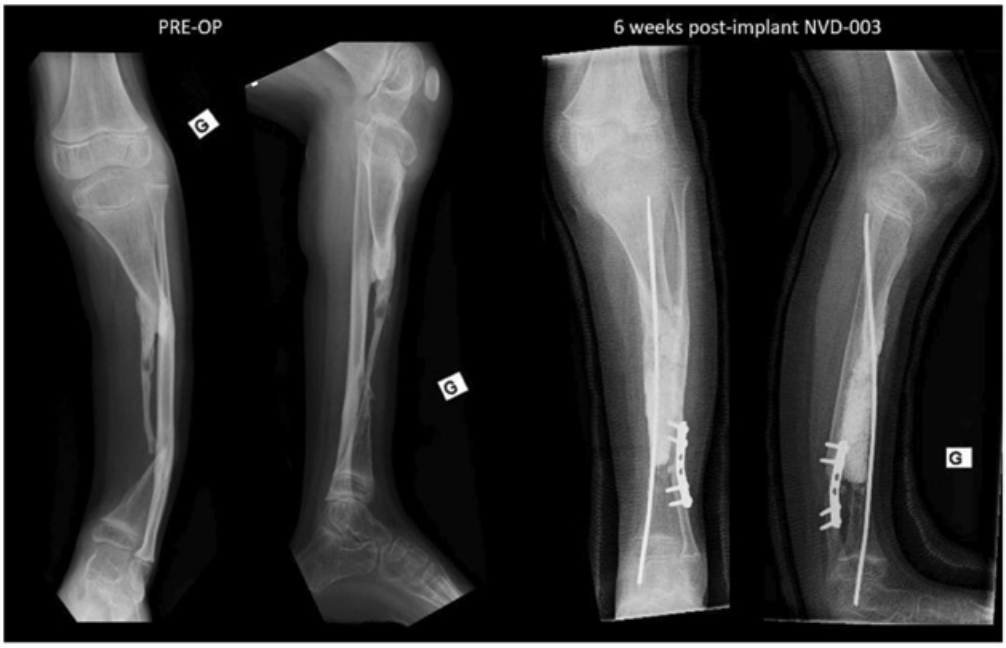

Figure 3: NVD-003 implanted in a 9-year-old NF1+ CPT patients: Baseline versus 6 weeks post-implant surgery.

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Evaluation of Plexiform Neurofibromas Tumors Through 3D Volumetric Segmentation Modeling

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Introduction: Plexiform neurofibromas (pNF) are present within up to 60% of Neurofibromatosis Type 1 (NF1) cases. Typically, pNFs are benign nerve sheath tumors that exhibit diffuse growth sporadically, are present at birth, and are a cause of morbidity with limited medical treatment solutions[1,2]. Current guidelines for NF1 diagnosis are centered around high specificity and sensitivity, where screening is optimized to target pNFs tumors while limiting misdiagnoses and is accomplished through surveillance imaging methodologies[3,4]. However, uncertainty exists in how patients with pNFs should be imaged, as some doctors prefer to perform a baseline MRI of observable tumors while others solely image symptomatic cases[5]. The aim is to establish a retrospective chart review and a unified protocol to advance tumor segmentation processes under a single methodology and obtain the 3D volumetric magnitude of NF1 tumors.

Methodology: A total of 12 case-study NF1 patient MRI scans were extracted from the Yale New Haven Health (YNHH) system YNHH EPIC server in conjunction with radiology treatment. Eight MRI scans were selected due to their adequate visibility and identification. Segmentation and 3D volumetric analysis were carried out using image processing software (Simpleware ScanIP, Synopsys, Mountain View, CA), which allowed for visualization of each tumor. Size of the tumor was also assessed by measuring its longest linear dimension on an appropriate plane.

Results: pNF tumors were located in the left wrist, neck, pelvis, thorax and abdomen (**Figure 1, 2**). Indications involved tumors ranging between 3D volumetric sizes of 1.39 to 1.45 mm^3. These 3D models were then examined by neuroradiologists at Yale School of Medicine (YSM), who verified the boundaries of the included voxels. The linear measurements ranged from 35 to 159 mm. A comparison of linear measurement to the 3D volumetric analysis was demonstrated to distinguish the difference between the growth patterns of tumors.

Discussion: The current protocol shows promising results enabling the accurate segmentation of pNFs. With a shortage of 3D modeling protocols for NF1, the analysis of tumor size variation is intended to add credence to our methodology and understand the growth of NF1 pNFs. Presentations of pNFs tumors are inherently difficult to measure linearly, given the complexity of tumor formation and the location of the tumor.

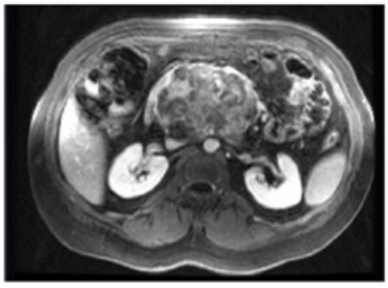

Figure 1: Axial post-contrast scan representing pNF tumor in the abdomen

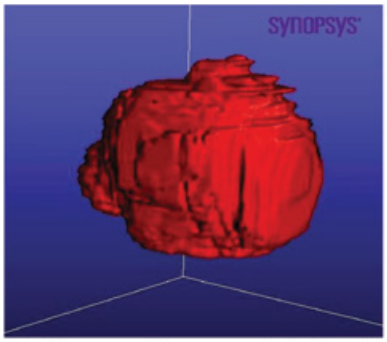

Figure 2: 3D model representing segmented abdominal pNF tumor

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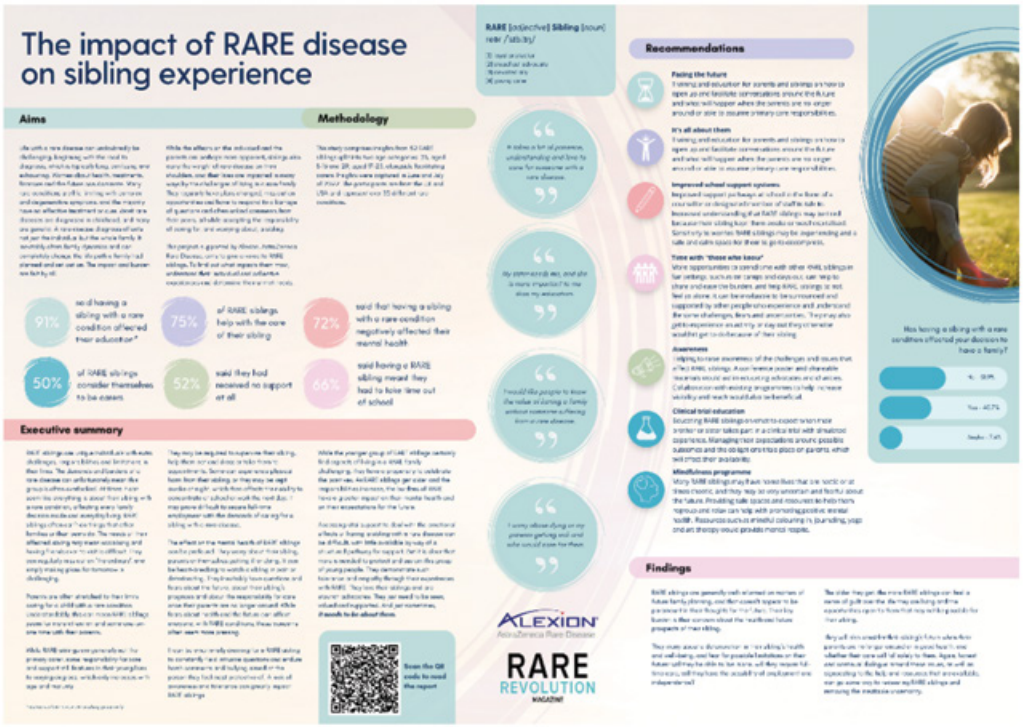
The Impact of RARE Diseases on Sibling Experience

Wendy Erler, VP, Head of Patient Experience & Insights, *Alexion, AstraZeneca Rare Disease*

The impact of RARE disease on sibling experience impact report, undertaken by RARE Revolution Magazine and supported by Alexion, AstraZeneca Rare Disease, aims to give a voice to RARE siblings. The report shares the personal insights of young people living with RARE embedded into the fabric of their family life, who want—and deserve—to be recognised, supported and heard. The study comprises insights from 52 RARE siblings across two age categories: 23 participants aged 8–16 and 29 participants aged 17–25, alongside facilitating carers, captured in June and July of 2022 from both surveys and interactive focus groups held over zoom. Participants from the UK and USA represent over 35 different rare conditions. Through the invaluable insights provided by these RARE siblings, the report highlights the impact siblings experience in their education, family life and relationships and makes recommendations to support and nurture this unique group of young people. Insights and recommendations can serve as a useful guide to all organisations operating in a support capacity within rare disease—aiding their short and long-term strategic aims for whole family support. While the effects of rare disease on the individual and their parents are perhaps more acknowledged, siblings also carry the weight of rare disease on their shoulders, and their lives are impacted in many ways by the challenges of living in a rare family.

Fig.1: Poster summarizing report.



Full report: https://rare-revolution-wp-images.s3.eu-west-1.amazonaws.com/wp-content/uploads/2023/01/20092626/The-impact-on-RARE-disease-on-siblings-1.pdf?

Full List of Authors: N. Miller, E. Bishop, R. Pender: RARE Revolution Magazine, W. Erler: Alexion Astra Zeneca

Research sponsorship provided by Alexion Astra Zeneca.

Prevalence of Obesity in Adults with Neurofibromatosis 1: A Pilot Study

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The role of metabolism in the pathophysiology of neurofibromatosis 1 (NF1) is still misunderstood. Reviews and textbook classically describe patients with a distinct anthropometric and metabolic phenotype including a short stature and a low body mass index (BMI).

Therefore, we conducted an observational retrospective pilot study to confirm the anthropometric phenotype of persons living with NF1.

Form the database of the French rare skin diseases network (BAMARA), 1999 in- and out-patients followed in our referral centre (CERENEF, Henri Mondor Hospital, Créteil, France) were identified. Among them, 1301 were included: 743 women (57%), mean age 39 years (from 18 to 81 years). The NF1 population was compared to the general population evaluated in the French Obepi study including 9598 adults (Fontbonne et al., J. Clin. Med, 2023).

The mean BMI was significantly lower in the NF1 population (24.1 vs 25.5kg/m2, IC95[23.9-24.4], $p<0.001$). The prevalences of overweight (BMI between 25 and 30kg/m2) and obesity (BMI ≥30kg/m2) were significantly lower in the overall NF1 population: 24.6% vs 30.3%, IC95[23.9-27.0.], $p<0.001$, and 10.2% vs 17.0%, IC95[8,7-12,0.], $p<0.001$, respectively. These differences were significant regardless of gender ($p<0.001$). In individuals with overweight or obesity, the distribution of the different socio-professional categories was similar, except for the "employees" category (public and private sectors) whose proportion was increased in the general population in comparison with the NF1 population ($p=0.008$) (**Table 1**). Of note the degree of diploma was lower in the overall NF1 population (<0,001). The mean size was significantly lower in the NF1 population: 164.4 cm, SD 9.7 vs 169.9cm, SD 8.9, $p<0.001$.

We confirmed the anthropometric phenotype of persons living with NF1, featuring a short stature and a low BMI. Multiple regression analyses testing the role of several variables, including sex, age, and socio-professional category, will be presented. In addition, the study of a potential genotype/phenotype correlation is pending.

Table 1: Comparison of the demographic and clinical characteristics of the NF1 population with the general population.
Data for the general population were extracted from the French Obepi study (Fontbonne et al., J. Clin. Med, 2023) including 9598 adults. For continuous variable, the Wilcoxon test was used, and for categorical variables, the one-proportion z-test was used. Significance was based on a two-sided p-value 0.05 throughout.

	General population (N=9598)	NF1 population (N=1301)	95% CI	P value
Mean BMI (Kg/m²)	25.5	24.1	23.9-24.4	<0.001
Overweight (%)	30.3	24.6	22.3-27.0	<0.001
Overweight +Obesity (%)	47.3	34.8	32.3-37.4	<0.001
Obesity (%)	17.0	10.2	8.7-12.0	<0.001
Morbid obesity (%)	2.0	0.7	0.4-1.2	<0.001
Sex				
Male				
Overweight +Obesity (%)	53.5	36.4	32.5-40.5	<0.001
Obesity (%)	16.7	9.3	7.2-12.0	<0.001
Female				
Overweight +Obesity (%)	41.3	33.6	30.4-37.1	<0.001
Obesity (%)	17.4	10.9	8.9-13.3	<0.001
Age (Overweight +Obesity)				
18-24 years	23.2	22.3	17.3-27.6	0.667
25-34 years	35.2	32.6	27.9-37.6	0.301
35-44 years	44.0	36.6	31.0-42.6	0.015
45-54 years	50.7	44.8	38.2-51.5	0.085
55-64 years	57.2	41.1	33.5-49.0	<0.001
65 years and more	57.3	40.8	30.2-52.5	0.005
Socio-professional category (Overweight +Obesity)				
Workers Formal and informal sectors	51.1	39.6	30.6-49.4	0.021
Employees Public and private sectors	45.3	36.8	31.0-43.0	0.008
Intermediate profession	43.0	37.0	30.0-44.7	0.125
Manager	35.0	26.2	18.7-35.5	0.062

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Missense or Splicing? Assessing Exonic Variants Adjacent to Canonical Splice Sites in the *NF1* Gene

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Classifying variants near the canonical AG/GT splice site can be challenging. For example, single nucleotide substitutions at the first or last two nucleotides of an exon, have the potential to be splicing variants instead of a missense variant. Data obtained through RNA-based genetic testing of *NF1* has identified 109 single nucleotide substitutions located at these four positions, among which 96 were missense/synonymous variants, and 11 were nonsense variants. Whether there was an effect on splicing or not, all nonsense variants were classified as pathogenic. Of the 85 missense variants, 69 (81%) were proven with functional data to affect splicing and therefore were all classified as likely pathogenic or pathogenic. Of the 13 synonymous variants, 11 (85%) were proven to lead to mis-splicing and were classified as pathogenic. There are 13 missense variants (15% of total missense) that were proven to not affect splicing, all of which were initially classified as variants of uncertain significance. These results suggest that single nucleotide substitutions at the first or last two nucleotides of an *NF1* exon have a high likelihood (83%) to be pathogenic. This data is currently being used to optimize the settings of SpliceAI to enable it to predict the effect of all possible single nucleotide substitutions at these four nucleotides in the *NF1* gene more accurately. Ideally, when SpliceAI predicts a variant at these four positions to affect splicing, very strong pathogenic evidence (PVS1) should be applied.

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The Long and Short of it—Expanding the Phenotype of NF1 Microdeletion Syndrome

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Background: *NF1* microdeletion accounts for 5 to 11% of patients with neurofibromatosis type 1 (NF1). Individuals with microdeletions are reported to have a more severe clinical phenotype with larger tumor burden, malignancy risk, and learning difficulties.

Purpose of the Study: To characterize a large, diverse cohort of individuals with *NF1* microdeletion for additional associations and outcomes.

Methods: We performed a retrospective chart review across 30 years (1994-2023) of individuals with confirmed *NF1* microdeletion syndrome seen in the Neurofibromatosis Clinics at the Ann & Robert H. Lurie Children's Hospital of Chicago and Cincinnati Children's Hospital Medical Center.

Results: A total of 44 patients (26 children and 18 adults) were identified with *NF1* microdeletion (22 type 1, 4 type 2, 2 type 3, and 11 atypical deletion). Mosaicism was identified in 2 individuals with type 2 deletion. Three families (a father-son, a mother-twins, and a mother-son) have first degree relatives with *NF1* microdeletion. The median age at diagnosis was 1 year old (range 3 months to 67 years), and 24 individuals were assigned female sex at birth. Characteristic dysmorphic features were identified in 30 individuals (68%). The most common ocular manifestations included Lisch nodules (17/44), strabismus (12/44), and symptomatic optic pathway gliomas (OPG, 7/44) with 2 requiring chemotherapy. Developmental delays were observed in 32 patients, and 21 received early intervention services. Expressive and/or receptive speech delays were diagnosed in 18 individuals. Learning difficulties were observed in 28 individuals, and 23 required individualized education programs. In this cohort, FSIQ ranged from 51-87 (15/31). Attention-deficit/hyperactivity disorder was noted in 17 individuals. Among adults (18/44), 11 graduated from high school, one was a college graduate, and two were attending college at time of this study.

Conclusions: In this *NF1* microdeletion cohort, there was a more favorable neurocognitive outcome in adults than typically reported in the literature. There was no increased occurrence of OPG compared to other known NF1-related tumors in this population.

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Acute Symptomatic Hemorrhage Associated with Peripheral Nerve Tumors in NF Patients

Kimberly Harbaugh, MD, *PennStateHealth*

Acute symptomatic hemorrhage associated with a peripheral nerve tumor is a rare event. Most reports in the literature discuss single cases, many with fatal outcomes. We present the Penn State Hershey Medical Center experience with 6 patients who had symptomatic large hemorrhages associated with peripheral nerve tumors of the trunk and lower extremity over approximately 6 years.

A retrospective review of patient records and imaging was carried out.

Median age at time of presenting hemorrhage was 41 years and 4 of the 6 patients were men. One patient with NF2 suffered a spontaneous hemorrhage and rupture of a sciatic nerve tumor at the buttock requiring urgent surgical intervention for intractable pain. The remaining 5 patients carried a diagnosis of NF1 and suffered symptomatic hemorrhages in association with plexiform neurofibromas located in the trunk, gluteal region, and thigh. One hemorrhage occurred 2 months after resection of a malignant peripheral nerve sheath tumor. The remaining bleeds occurred after minor or no trauma. Two or more hemorrhages occurred in 3 patients, all of whom had at least one endovascular treatment. Evidence of prominent vasculature was seen in most and in some cases arteriovenous shunting was noted. All patients survived their hemorrhages. Misdiagnosis was common leading to delayed or inappropriate treatment in some cases.

Given the emergent nature of these hemorrhages, initial treating physicians may not be aware of the association of vascular anomalies in NF patients and the potential for spontaneous massive hemorrhage. For this reason, it is important to educate NF patients and families about this uncommon but potentially devastating complication.

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Comparison of Medication Adherence Tracking Methods in Adults with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PN) on a Clinical Trial of Selumetinib: Results of a Pilot Feasibility Study

Cynthia Harrison, *Pediatric Oncology Branch, National Cancer Institute*

Background: Given the recent FDA approval of selumetinib for children with NF1, attention is turning to the challenges of prolonged treatment, including long-term medication adherence. Along with pill counts (PCs) and patient diaries, technology offers more objective measures of adherence such as the medication event monitoring system (MEMS™) pill caps, which record the opening of pill bottles. It is important to examine the feasibility and reliability of these options in NF1 research and care. The primary objective of this small pilot study was to examine the feasibility of using MEMS™ caps to monitor adherence in a NF1 clinical trial. Secondary objectives were to compare adherence rates between PCs, diaries, and MEMS, and to examine demographic and medical correlates of adherence.

Methods: This longitudinal study recruited adults with NF1 and plexiform neurofibromas (PNs) enrolled in a trial of MEK1/2 inhibitor selumetinib, targeting PN volume reduction (NCT02407405). Patients were given a MEMS™ cap at baseline and followed for 18 cycles (cycle=28 days). At restaging visits, diaries and PCs were collected/completed, and MEMS™ data was uploaded. MEMS™ feasibility was determined a priori to be met if two or more cycles could be monitored for ≥75% of patients. Adherence was calculated as correct doses/total doses prescribed. Using SPSS, analyses of variance were used to examine differences between adherence methods and repeated measures examined changes in adherence over time (cycles 1-6 vs 7-12 vs 13-18).

Results: Twelve adults were enrolled (*M* age=34.4, range 20-46 years); nine completed 18 cycles of treatment to date. PC and diary data were compared over cycles 1-18, while cycles 1-6 for all methods were compared (MEMS™ cycles 7-18 could not be compared due to missing data resulting from patient error and technical problems). The mean adherence rates for diaries and PC from cycles 1-18 were not significantly different from each other (ps>0.05). Mean MEMS™ adherence over cycles 1-6 was significantly lower than both diaries (p=0.03) and PCs (p=0.03) over cycles 1-6. Mean MEMS™ adherence cycles 1-6 was unrelated to age, education, disease severity, or baseline pain levels (ps>0.05).

Conclusions: These results support the use of MEMS™ cap as a feasible method of tracking adherence, with the caveat that technical problems can occur. MEMS™ appears more sensitive to nonadherence, especially when considering dosing intervals, as demonstrated by the significantly lower adherence via MEMS™ vs PCs and diaries. We support the use of MEMS™ in adults with NF1 for both clinical and research purposes, ideally in combination with another method to account for any technical issue/user error. Improving adherence tracking betters our understanding and clinical management of NF1 to benefit patients, clinicians, and researchers.

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Neurofibromatosis Therapeutics Program: Program Development, Tumor Treatment, and Side Effect Management

Molly Hemenway, DNP, MS, RN, AC/PC-CPNP, *Children's Hospital Colorado, University of Colorado School of Medicine, Aurora, CO*

Purpose: To outline how to develop and grow a multidisciplinary neurofibromatosis therapeutics program (NTP) at a tertiary care center. Discuss the need for a team approach to treatment and side effect management of Neurofibromatosis Type 1 (NF1) and *NF2*-related Schwannomatosis (NF2) related tumors.

Summary: The advanced practice provider (APP) is instrumental in designing, developing, and growing a program to treat tumors associated with NF1 and NF2. Both NF1 and NF2 are autosomal dominant genetic disorders associated with the growth of both benign and malignant tumors of the peripheral and central nervous system. In the last three years selumetinib, a mitogen-activated protein kinase enzyme (MEK 1/2) inhibitor (MEKi), gained Federal Drug Administration approval for treatment of plexiform neurofibromas in the children with NF1. With new treatment available the patient care load increased significantly. As with all MEKi, the side effect management is extensive and labor intense. A tertiary care hospital formed a formal program, the Neurofibromatosis Therapeutics Program, within the Center for Cancer and Blood Disorders. The aim of the program is to work in a multidisciplinary setting to care for patients with plexiform neurofibromas, meningiomas, low grade gliomas, high grade gliomas, and malignant peripheral nerve sheath tumors (MPNST). While treatments may vary, the most common treatment in the large volume of patients with NF1 is a MEKi. Significant teaching by providers, nurses, and pharmacists is necessary regarding administration of the medications as well as management of the skin and gastrointestinal side effects. Our centers standardized side effect management protocols will be shared as well as the program development pearls that have been gleaned. In addition to the clinical program, a brain tumor, MPNST, and plexiform neurofibroma tissue back allows scientific research to be conducted on the same campus.

Results: The development and growth of the NTP has increased patient care visits, consults from primary care and other subspecialty providers, enrollment on therapeutic clinical trials, and improved collaboration with intradisciplinary teams. The program has improved patient and family education, side effect management, obtained faster insurance authorization of medications, and streamlined referral and second opinion processes.

Conclusions: The development and growth of the NTP is an ongoing process to ensure the program is strong and vibrant with the aim to provide high quality of care for the whole child as well as conduct lab and clinical research.

The Development of a Schwann-Cell Targeted Adeno-Associated Virus (AAV)-Mediated Gene Therapy for Neurofibromatosis Type 1

Caleb Holaway, *Center for Gene Therapy, The Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH*

This study aims to inform the selection of vector regulatory elements and viral administration route to develop a Schwann-cell targeted adeno-associated virus (AAV)-mediated gene replacement therapy for neurofibromatosis type 1 (NF1). NF1 often presents with debilitating peripheral nerve tumors that arise from Schwann cells. Due to the monogenic nature, NF1 is a promising candidate for AAV gene therapy, but the large gene size and difficult to target cell population have remained a challenge in developing a translatable therapy. First, we designed AAV reporter constructs expressing enhanced green fluorescent protein (eGFP) to compare the transcriptional activity of a promoter with high selectivity for Schwann cells, myelin protein zero (P0), and a ubiquitously expressing promoter, chicken beta actin with a CMV enhancer (CAG). Expression of the AAV constructs, *pAAV-P0.eGFP* and *pAAV-CAG.eGFP,* was verified in cultured Schwann cells and they were packaged into AAV9, a serotype with known tropism for central and peripheral nervous systems (CNS, PNS). To assess viral particle biodistribution and expression using clinically feasible administration routes, *AAV9-P0.eGFP* or *AAV9-CAG.eGFP* were injected into wildtype mice pups via intracerebroventricular (ICV) injection and wildtype mice weanlings via intrathecal lumbar (IT-L) injection (**Figure 1**). By expression data from qPCR and Western blot, we show both *pAAV-P0.eGFP* and *pAAV-CAG.eGFP* drive robust eGFP expression in Schwann cells *in vitro. In vivo,* vector biodistribution and expression data by qPCR and immunohistochemistry demonstrate expression is driven in the peripheral nervous system (**Figure 2**). These findings are currently being validated by IT-L or subpial injections of both AAV9 vectors into wildtype minipigs (**Figure 1**). This proof-of-concept work demonstrates the capability of an AAV to drive expression to the Schwann cells of the peripheral nervous system, therein establishing a strong foundation for the development of an AAV-mediated gene replacement therapy for neurofibromatosis type 1. Next, we will examine the therapeutic efficacy of a truncated version of the *Nf1* gene, *miniNF1,* generated by the Sena-Esteves lab and which fits in the confines of AAV. This *miniNF1* transgene has been cloned into our P0- and CAG-driven AAV gene replacement constructs, packaged into AAV9, and will be delivered to a murine model of NF1 to assess therapeutic efficacy (**Figure 3**). The lead candidate AAV vector from this study will be evaluated in a porcine model of NF1 (**Figure 3**).

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These research efforts are funded by the Gilbert Family Foundation.

Figure 1.

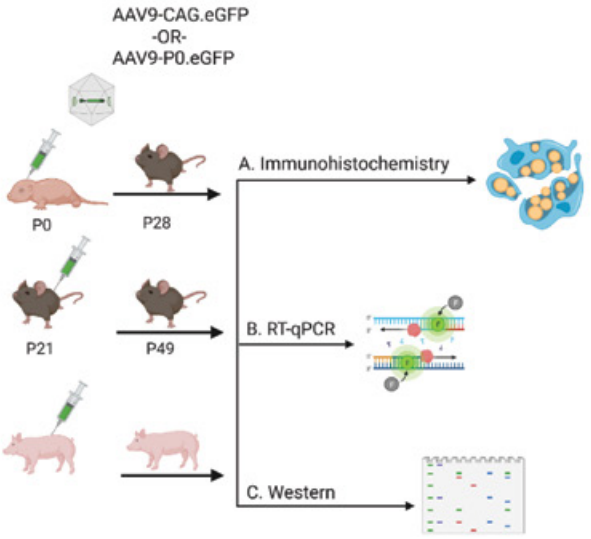


Figure 2.

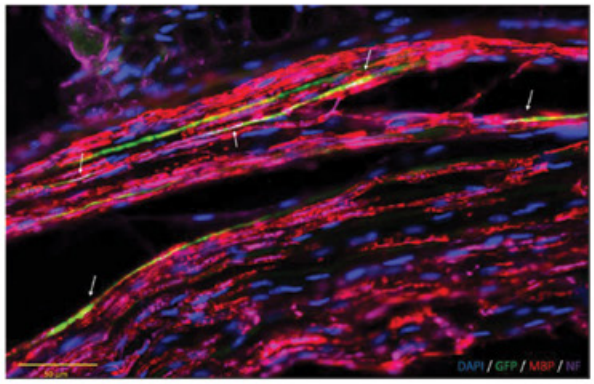
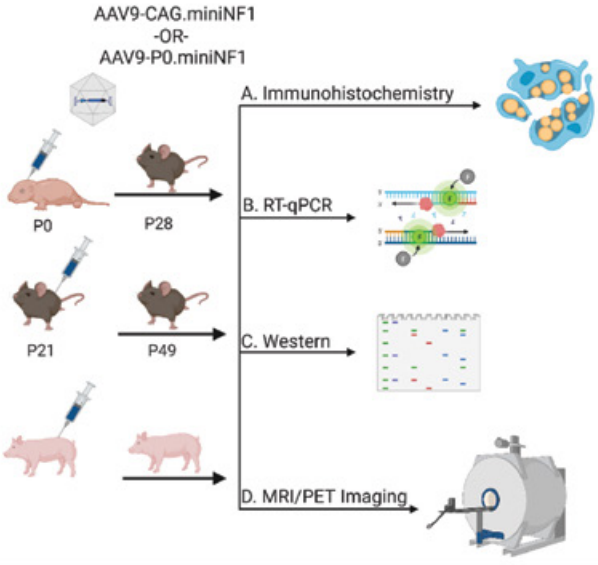


Figure 3.



A Meta-Analytic Study of ADHD Symptoms in Individuals with Neurofibromatosis Type 1

Yang Hou, PhD, *Florida State University*

Background: Children with (versus without) Neurofibromatosis type 1 (NF1) have higher risk for attention-deficit/hyperactivity disorder (ADHD). However, prior studies showed inconsistent findings on group differences between children with versus without NF1 in the extent of ADHD symptoms. The inconsistencies are likely due to different study and sample characteristics and the heterogeneity of ADHD symptoms in the NF1 population. This study aims to provide a robust estimate of the extent of ADHD symptoms in individuals with NF1 relative to the control or normative group and examine how study and sample characteristics are associated with the group differences in ADHD symptoms through meta-analyses.

Methods: This study used data from a larger meta-analysis project on the neurobehavioral functions of individuals with NF1. Systematic literature searches were conducted in Scopus, PsycINFO, Web of Science, PubMed, and ProQuest. Search terms included a combination of NF1 terms (e.g., neurofibromatosis type 1, NF1) and neurobehavioral functioning terms (e.g., attention, hyperact*, impuls*, ADHD). **Figure 1** shows the study selection procedure. About 159 papers met the inclusion criteria of this ADHD study, and 69 papers with effect sizes were used in analyses. Hedges' g was calculated for differences in ADHD symptoms (total symptoms, inattention, hyperactivity) between the NF1 group and the controls (healthy community control, unaffected siblings, and normative data). A robust standard error estimation technique was used to handle dependence between study effect sizes. Meta-regression was used to analyze potential moderators of group differences in separate models.

Results: Results indicated that individuals with NF1 had significantly more ADHD symptoms than the control or normative group, with medium effect sizes (g = 0.62 to 0.67, **Table 1**). There are large variability of effect size across studies (I^2 = 96% to 98%). The variability was partly explained by study and sample characteristics (**Table 2**). Specifically, higher percent of participants with familial NF1 is associated with greater group differences in ADHD total symptoms and attention problems, but not hyperactivity problems. Also, group differences in attention problems were higher in informant-reported (vs. performance-based) measures. The nonsignificant effects of some moderators explored in the current study may be due to small number of studies analyzed. Thus, we will request more data from authors and conduct additional literature search and coding to include more studies. Moreover, publication bias test and sensitivity analysis will be conducted.

Conclusions: The higher levels of ADHD symptoms indicate the need for more support and interventions for children with NF1 to help improve their behavioral health. The heterogeneity in effect sizes suggests the need to identify predictors of ADHD within the NF1 group. Individuals with familial NF1 may be particularly at risk for ADHD symptoms.

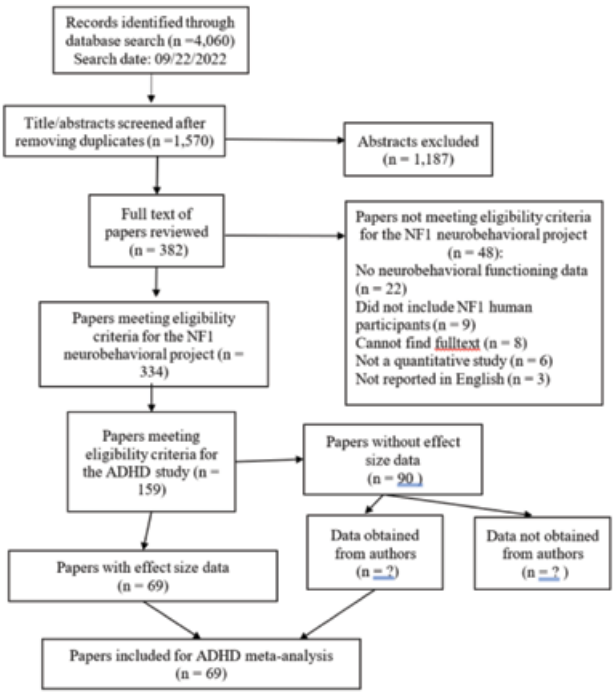


Figure 1. Flow diagram for inclusion and exclusion in meta-analysis.

Table 1. Summary of Mean Effect Size across Studies

	Hedge's g	*LL*	*UL*	*SE*	*df*	p-value	*n*	*k*	Tau^2	I^2 (%)
ADHD total	0.620	0.272	0.969	0.166	18.989	.001	20	55	1.586	97.588
Attention	0.666	0.473	0.859	0.096	59.928	<.001	61	219	1.086	96.393
Hyperactivity	0.627	0.144	1.110	0.236	29.989	<.013	31	110	2.236	98.158

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.

Table 2. Tests of Moderators

Moderator	*n*	*k*	estimate	*SE*	*LL*	*UL*	*df*	*p*-value
ADHD total								
Mean age	19	52	0.017	0.039	-0.087	0.121	4.350	0.683
% Girl	19	53	-0.004	0.013	-0.035	0.026	7.573	0.740
% White	5	11	0.014	0.007	-0.013	0.042	2.052	0.159
% familial NF1	**8**	**21**	**0.030**	**0.006**	**0.004**	**0.057**	**1.900**	**0.040**
Mean IQ	13	38	-0.004	0.019	-0.049	0.041	7.043	0.846
% ADHD diagnosis	12	32	0.004	0.014	-0.030	0.038	5.890	0.758
Excluded brain tumors (yes vs. no)	20	55	0.171	0.333	-0.530	0.873	17.151	0.614
Siblings (vs. community)	11	32	-0.540	0.288	-2.262	1.181	1.506	0.241
Normative data (vs. community)	18	48	-0.506	0.356	-1.262	0.250	15.987	0.175
Reported vs. performance-based	17	50	0.627	0.926	-6.398	7.653	1.291	0.599
Attention								
Mean age	57	171	-0.010	0.009	-0.035	0.015	3.977	0.343
% Girl	56	208	-0.006	0.007	-0.024	0.011	7.366	0.411
% White	9	30	0.010	0.015	-0.040	0.060	2.740	0.544
% familial NF1	**27**	**90**	**0.035**	**0.007**	**0.010**	**0.059**	**2.728**	**0.022**
Mean IQ	45	128	-0.017	0.004	-0.039	0.005	1.597	0.074
% ADHD diagnosis	33	112	-0.011	0.009	-0.035	0.013	5.187	0.285
Excluded brain tumors (yes vs. no)	61	219	0.171	0.199	-0.227	0.569	56.796	0.393
Siblings (vs. community)	31	115	-0.331	0.256	-0.964	0.302	5.716	0.245
Normative data (vs. community)	54	175	-0.148	0.214	-0.578	0.282	51.587	0.493
Reported vs. performance-based	61	213	**0.424**	**0.179**	**0.065**	**0.782**	**50.325**	**0.022**
Hyperactivity								
Mean age	28	95	0.037	0.072	-0.129	0.202	8.387	0.625
% Girl	31	110	-0.015	0.011	-0.042	0.013	5.483	0.230
% White	5	18	0.118	0.113	-0.736	0.972	1.295	0.453
% familial NF1	14	49	0.074	0.044	-0.121	0.269	1.954	0.240
Mean IQ	17	46	-0.033	0.032	-0.110	0.043	6.850	0.335
% ADHD diagnosis	17	60	-0.012	0.016	-0.060	0.036	3.346	0.493
Excluded brain tumors (yes vs. no)	31	110	0.902	0.459	-0.038	1.841	28.879	0.059
Siblings (vs. community)	17	62	-0.757	0.499	-4.550	3.035	1.291	0.329
Normative data (vs. community)	28	93	-0.603	0.506	-1.642	0.436	26.879	0.244
Reported vs. performance-based	29	105	0.497	1.134	-3.576	4.571	2.484	0.697

Note: n = number of studies; *k* = number of effect size; *SE* = standard error; *LL* = Lower limit of 95% confidence interval; *UL* = Upper limit of 95% confidence interval; *df* = degrees of freedom. Significant moderators are bolded.

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Funding Sources: This research was supported by (a) federal funds from Neurofibromatosis Research Program, Congressionally Directed Medical Research Programs, Department of Defense (grant number W81XWH2110504) (b) Florida State University Faculty Startup Funding, and c) the University of Kentucky Faculty Startup Funding.

A Systematic Review and Meta-Analytic Study of Internalizing and Externalizing Problems in Children with Neurofibromatosis Type 1

Yang Hou, PhD, *Florida State University*

Background: Children with Neurofibromatosis type 1 (NF1) are at higher risks for internalizing and externalizing problems compared to those without NF1. However, the differences are inconsistent across studies, likely due to variations of study and sample characteristics. This study aims to conduct the first systematic review and meta-analysis of internalizing and externalizing problems in children with NF1. It will determine the extent to which children with NF1 experience internalizing and externalizing problems compared to the control or normative group and explore the moderators of group differences.

Methods: Literature searches were conducted with NF1 terms (e.g., neurofibromatosis type 1, NF1) and neurobehavioral function terms (e.g., internaliz*, externaliz*). Approximately 220 papers met the inclusion criteria, and 63 papers with effect sizes were used in analyses (**Figure 1**). Hedges' g was calculated for differences in internalizing (total symptoms, depression, anxiety, and somatization) and externalizing problems (total symptoms, aggression, and delinquency) between the NF1 group and the controls (healthy community control, unaffected siblings, and normative data). Dependence between study effect sizes was accounted for using a robust standard error estimation technique. Meta-regression was used to analyze potential moderators of group differences.

Results: Individuals with NF1 had significantly more internalizing (total symptoms) and externalizing problems (total symptoms and delinquency) than the control or normative group (**Table 1**). The observed between-study heterogeneity in effect sizes was partly accounted for by study and sample characteristics (**Table 2**). Specifically, 1) Group differences in total internalizing were larger in studies with older participants; 2) group differences in depression were larger in studies with unaffected siblings as the controls; 3) group differences in aggression and delinquency were larger in studies with more participants diagnosed of ADHD; 4) group differences in delinquency were also larger using parent report (vs. self-report). The next steps include requesting more data from authors, additional literature search and coding, publication bias test and sensitivity analysis, and a qualitative review of prior findings on the topic and discussion of gaps identified in exitant literature.

Conclusions: The higher levels of internalizing and externalizing problems found in children with NF1 indicate the need for more support and interventions for these children to help improve their mental and behavioral health. The findings that certain study and sample characteristics predicted discrepancies between children with and without NF1 also suggest the need to explore predictors of the problems within the NF1 group and the importance for research to provide clear study and sample information (e.g., sample mean age, measure type).

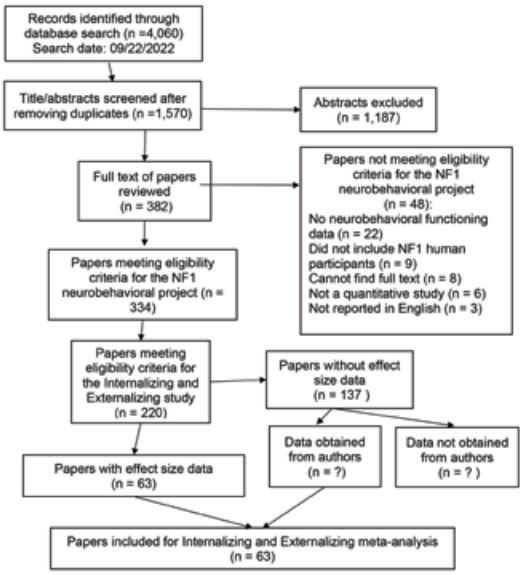

Figure 1. Flow diagram for inclusion and exclusion in meta-analysis.

Table 1. Summary of Mean Effect Size across Studies

	Hedge's g	*LL*	*UL*	*SE*	*df*	p-value	*n*	*k*	Tau^2	I^2 (%)
Total Internalizing	0.412	0.205	0.619	0.101	24.063	<.001	26	220	0.175	80.649
Depression	0.163	-0.158	0.484	0.135	6.790	.268	8	58	0.093	74.320
Anxiety	0.119	-0.451	0.690	0.241	6.955	.635	8	27	0.457	89.706
Somatization	0.315	-0.054	0.684	0.172	13.794	.089	15	141	0.334	88.965
Total Externalizing	0.192	0.059	0.032	0.064	21.812	.007	25	153	0.069	63.171
Aggression	0.131	-0.108	0.369	0.111	14.236	.260	16	151	0.137	76.432
Delinquency	0.275	0.058	0.493	0.102	14.459	.017	16	144	0.154	78.726

Notes: g = Hedge's g, *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.

Table 2. Tests of Moderators

	Total Internalizing			Depression			Aggression			Delinquency		
Moderator	*n*	*k*	*b*	*n*	*k*	*b*	*n*	*k*	*b*	*n*	*k*	*b*
Mean age	**23**	**197**	**0.082***	7	57	0.038	14	137	0.086	15	133	-0.161
% Girl	24	203	-0.004	8	58	-0.031	16	145	-0.005	15	134	-0.009
% White	6	25	0.005	-	-	-	2	14	0.021	2	14	-0.004
% familial NF1	14	132	-0.003	5	36	-0.049	10	93	-0.005	8	83	-0.004
Mean IQ	17	173	-0.004	4	39	-0.003	9	107	0.006	10	105	0.003
% ADHD diagnosis	13	141	0.006	6	55	0.007	**10**	**112**	**0.013****	**11**	**110**	**0.012***
Excluded brain tumor[1]	26	220	-0.448	8	58	0.075	16	151	0.172	16	144	-0.021
Unaffected siblings[2]	26	220	0.1	**8**	**58**	**0.489***	16	151	0.363	16	144	-0.044
Normative data[3]	26	220	0.135	8	58	0.035	16	151	0.174	16	144	0.023
Self report[4]	26	220	-0.002	8	58	-0.329	16	145	-0.436	**16**	**144**	**-0.666*****
Teacher report[5]	26	220	0.016	8	58	-0.084	16	145	-0.011	16	144	-0.315

Note: n = number of studies; *k* = number of effect size; *b* = meta-regression estimate. Significant moderators are bold-faced. [1]yes = 1; no = 0. [2]unaffected sibling control = 1; healthy community control = 0. [3]normative data = 1; healthy community control = 0. [4]self-report = 1; parent report = 0. [5]teacher report = 1; parent report = 0.
*p < .05. **p < .01. ***p < .001.

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Funding Sources: This research was supported by (a) federal funds from Neurofibromatosis Research Program, Congressionally Directed Medical Research Programs, Department of Defense (grant number W81XWH2110504) (b) Florida State University Faculty Startup Funding, and c) the University of Kentucky Faculty Startup Funding.

Identifying Lesions of the Corpus Callosum in Patients with Neurofibromatosis Type I

Nora Jandhyala, BS, *NYU Grossman School of Medicine*

Purpose: To investigate the rate of lesions of the corpus callosum, including both unidentified bright objects (UBOs) and gliomas, in a large cohort of Neurofibromatosis Type 1 (NF1) patients.

Methods: We reviewed the medical records of 1373 patients (aged 3 months to 86 years) with NF1 followed at our institution over 2012-2021. Patients with UBOs or gliomas found on MRI in the corpus callosum were identified. MRI reports and imaging were reviewed to determine location of lesions and change over time.

Results: Of the 1373 patients analyzed, 34 were reported to have UBOs in the corpus callosum. This represents 6.3% of the 538 patients with any UBO. The majority of corpus callosum UBOs were in the splenium (41%), followed by the body (18%), and genu (12%). Nine patients were reported with corpus callosum gliomas, representing 5.6% of the 161 patients with any glioma. Of these, 7 (78%) were in the splenium. Over the course of follow-up imaging, 5/7 remained stable, 2/7 decreased in size and then stabilized, and 1/7 increased in size.

Conclusions: Our study indicates a 2.5% and 0.7% prevalence of corpus callosum UBOs and gliomas, respectively. The majority of lesions are present in the splenium, and frequently remain stable over time. This adds to growing data regarding imaging findings in NF1 patients to better inform appropriate follow-up.

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Noninvasive Optical Detection of Invisible, Developing Cutaneous Neurofibromas

Wangcun Jia, PhD, *Beckman Laser Institute, University of California, Irvine*

Purpose of the study: The goal of our research is to detect developing cutaneous neurofibromas (cNF) using a translational optical instrument before they become clinical visible, and subsequently remove developing cNF with minimal side effects to maintain patients' quality of life.

Methods: We imaged face, chest, back and arms on twelve subjects diagnosed with neurofibromatosis type 1 using a spatial frequency domain imaging (SFDI) device. SFDI projects a patterned illumination over a large field-of-view (15 cm x 18 cm) at eight wavelengths and five spatial frequencies while collecting reflectance images. In combination with a mathematical model of light propagation, spatially resolved optical properties (scattering and absorption) can be deduced over the entire region of interest. SFDI optical properties maps were used to identify developing cNF, which were imaged with high-frequency ultrasound (HFUS) and some were biopsied for histological analysis.

Results: Suspected developing cNFs, previously not apparent to the unaided eye, were identified in patients as young as ten years old. Up to seven developing cNFs were found in a 12x17 cm^2 skin area where eleven visible cNFs can be seen on an adult patient. Combinations of wavelength and spatial frequency were identified to give enhanced contrast between invisible cNF and the surrounding uninvolved skin. As shown in Fig.2, developing cNF with low scattering (dark) regions (yellow arrows) can be clearly seen in the SFDI scattering map, but not apparently visible in color image of the same area (**Fig. 1**). HFUS imaging and histological analysis confirmed the presence of cNF in areas identified with SFDI. We also found that developing cNF have higher absorption at some wavelengths which can guide non-invasive laser treatment.

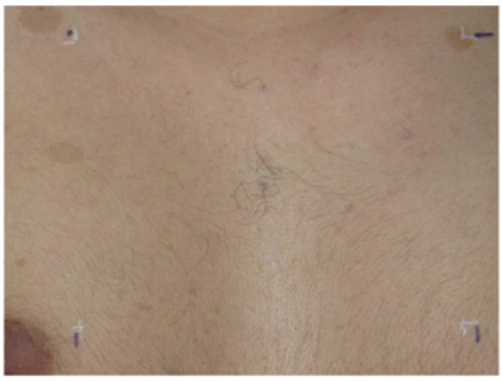

Fig. 1: Image of the chest of a NF1 patient

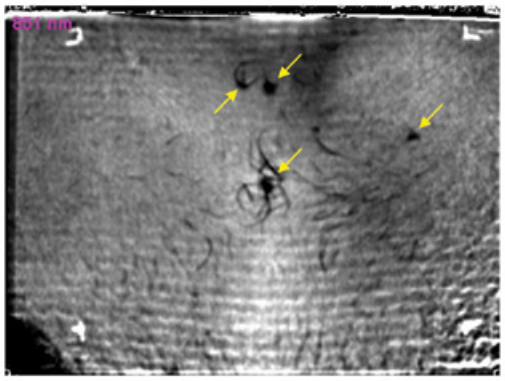

Fig. 2: Optical scattering map of the same area.

Conclusions: Our results suggest that optical scattering can be used as a cNF biomarker for non-invasive detection and monitoring. The ability of SFDI was demonstrated to identify invisible cNF.

Full List of Authors: Wangcun Jia, Gordon T. Kennedy, Nitesh Katta, Junsoo Lee, Rachel Elsanadi, Emily Nguyen, Anat Stemmer-Rachamimov, J. Stuart Nelson, Rafael Sierra, Kristen M. Kelly, Anthony J. Durkin, and Thomas E. Milner

Acknowledgement: The research is supported financially by the DHART SPORE Development Research Program and the Neurofibromatosis Therapeutic Acceleration Program (NTAP). Patient recruitment support is provided by the Neurofibromatosis Network and the Children's Tumor Foundation.

Knowledge-Driven Active Learning for NF1-OPG Volumetric Segmentation

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

Purpose: Limited labeled data could compromise the robustness of machine learning segmentation models trained on medical images. Active learning (AL) can reduce the amount of labeled data required to train a model by iteratively selecting only the subset of data to be labeled that is most beneficial for the model training. We develop a novel AL strategy that incorporates knowledge related to a segmentation model's performance to optimally and efficiently select the subset of data worth labeling at each AL iteration.

Methods: This study utilizes 479 pediatric magnetic resonance images (MRI) acquired from three institutions: Children's National Hospital, Children's Hospital of Philadelphia, and Children's Hospital of Colorado. Each MRI contains a high-resolution T1-weighted volumetric sequence generated by different imaging protocols and scanners. The data included 194 children with optic pathway gliomas associated with neurofibromatosis type 1 (NF1-OPG). To prepare the data, the anterior visual pathway (AVP) ground truth was manually segmented by medical experts and all volumes were resampled to a median resolution of $0.9\times0.82\times0.79$ mm^3. We used a 3D convolutional neural network (SegResNet) as the AVP segmentation model. An initial training set of 20 volumes was used. At each AL iteration, we first identified 5 failed validation cases with lowest Dice similarity coefficient (DSC), then selected 20 unlabeled volumes similar to the failed ones to be segmented for the next iteration. The localized similarity was determined by the structural similarity index measure within the region of AVP. The average DSC was used to evaluate the performance of the proposed AL strategy.

Results: Using only a small portion of the available data (24.2%) was sufficient to achieve comparable performance ($DSC=0.793\pm0.066$) to using the full NF1-OPG dataset of 331 volumes ($DSC=0.823\pm0.06$). Compared to a baseline method of AL that estimates uncertainty for each unlabeled case using variance, our method is computationally more efficient. Specifically, the baseline required 1,244 runtime inferences at each AL iteration, while our method required 98, and each inference required 2-3 seconds.

Conclusion: Knowledge-driven AL offers a solution to optimally rank unlabeled cases that is model-independent, flexible, and optimized for iterative AL. This approach performed well on AVP segmentation using MRIs from multiple sites and different imaging protocols to enable volumetric analysis of NF1-OPG.

Full List of Authors: Zhifan Jiang, Vishwesh Nath, Holger R. Roth, Abhijeet Parida, Nicholas Foreman, Michael J. Fisher, Roger J. Packer, Syed Muhammad Anwar, Robert A. Avery, Marius George Linguraru.

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Addition of Trametinib to Radiation and Temozolomide in NF1-Associated Glioblastoma: A Case Report

Justin T. Jordan, MD, MPH, FAAN, *Massachusetts General Hospital*

Purpose: The biology of high-grade gliomas in neurofibromatosis 1 (NF1) differs from sporadic gliomas, and physicians are employing innovative treatment strategies into routine clinical practice to account for those differences. Here we sought to report a case of NF1-associated glioblastoma (GBM) with a unique therapeutic approach and a durable therapeutic response.

Methods: A review of medical records including clinic notes, pathology results, and imaging was conducted. This was performed as a part of a protocol confirmed as exempt by the Partners' IRB.

Results: A 23-year-old left-handed female with sporadic NF1 underwent routine surveillance MRI of the brain and was noted to have increased size of a chronic focus of FLAIR hyperintensity in the right thalamus without enhancement. Prior to planned follow up imaging, she presented to the emergency room for fatigue and left hemiparesis and a new MRI revealed significant increase in size of the right thalamic lesion with new heterogeneous enhancement. A stereotactic biopsy revealed GBM, IDH-wild type, p53 mutant, MGMT promoter unmethylated. The patient received radiation therapy with concurrent temozolomide (TMZ) 75mg/m^2/day, followed by six cycles of adjuvant temozolomide 200mg/m^2 with addition of trametinib 2mg daily. Since completing 6 cycles of TMZ, trametinib 2mg daily has continued as monotherapy for 11 months to date. Brain MRI reveals reduction in size of enhancement by 83% between the post-radiation baseline scan and the most recent imaging 15 months post-radiation, with marked reduction in FLAIR hyperintensity, consistent with ongoing partial response.

Conclusion: A recent series reported a median overall survival of 14 months for 9 patients with adult-onset NF1-associated GBM at a median age of 38.[1] Moreover, in that study only 2 partial responses were seen (both in the setting of bevacizumab)[1] and no patients were treated with MEK inhibitors. Our case is unique in the therapeutic approach taken by inclusion of MEK inhibition, and the durable and ongoing response despite negative prognostic factors of IDH-wildtype, no resection performed, and MGMT promoter unmethylated status. This case highlights the potential for improved outcomes by adding MEK inhibition to standard therapy for adult-onset NF1-associated GBM.

Additional Authors: Christina Orr, NP (MGH); Christine Lu-Emerson, MD (Maine Medical Center); Maya Alvarez, BS (MGH).

References: 1. Romo CG, Piotrowski AF, Campian JL, et al. *Neuro-Oncol* 2023 Feb 25.

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Effect Modification on the Association Between Neurofibromatosis-1 and Clinical Outcomes in Patients with Malignant Peripheral Nerve Sheath Tumors

Eun Key Kim, MD, PhD, *Asan Medical Center, University of Ulsan College of Medicine*

Purpose: Sporadic and neurofibromatosis-1 (NF1)-associated malignant peripheral nerve sheath tumors (MPNSTs) have different presentations and prognoses. We compared the presentations, outcomes, and prognostic factors of sporadic and NF1-associated MPNSTs and examined the role of NF1 as a prognostic factor in relation to other risk factors.

Methods: A retrospective cohort study of 97 MPNST patients was conducted between 2004 and 2020. Compared to sporadic MPNST, hazard ratios of NF1-associated clinical outcomes were estimated, and effect modification (heterogeneity) on the NF1 association was assessed in subgroup analyses.

Results: Sporadic (n = 56) and NF1-associated MPNST (n = 41) groups differed in age at diagnosis (54.54 years vs. 38.12 years), tumor size (5.3 cm vs 9.0 cm), and stage distribution. Disease-specific death (DSD) risk was higher in the NF1 (41.5%) than that in the sporadic group (26.8%), with a marginal difference ($P = 0.065$), while any tumor-related event was significantly higher in the NF1 (56.1%) than that in the sporadic group (32.1%) ($P = 0.014$). Intracorporeal, deep, metastatic tumors, and an incomplete resection margin were significant risk factors for DSD in univariate analyses, and stage 4 disease and NF1 in multivariate analyses. NF1 acted as a significantly poor indicator of DSD or disease-free survival in only those subgroups without known strong risk factors.

Conclusion: The current study showed that NF1 was a significant risk factor for any tumor-related events and DSD, but only in subgroups where a good prognosis was expected. Patients in these subgroups might benefit from vigilant surveillance and active intervention.

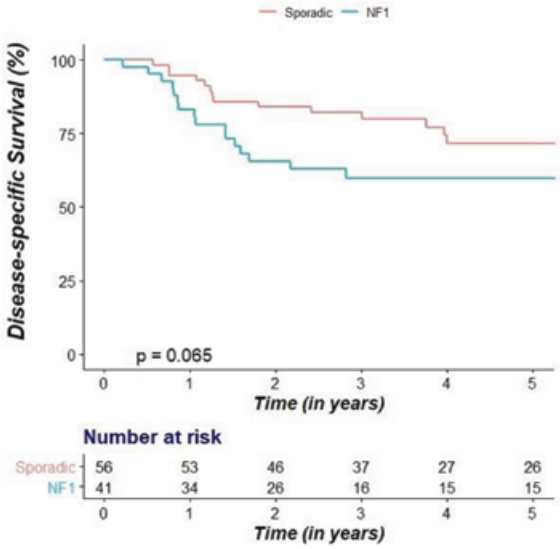

Figure 1. Kaplan–Meier for disease-specific survival (DSS) of sporadic versus NF1-associated MPNST

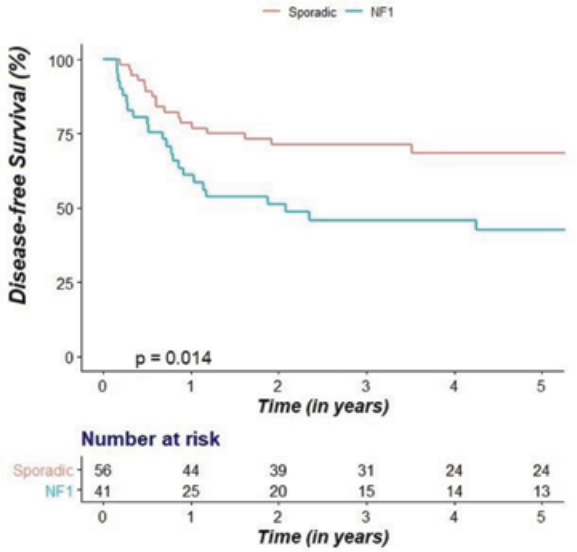

Figure 2. Kaplan–Meier for disease-free survival (DSS) of sporadic versus NF1-associated MPNST

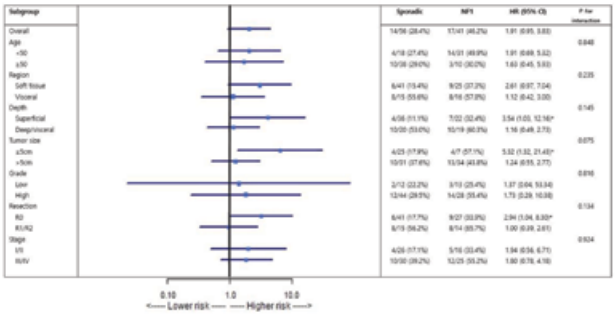

Figure 3. Forest plot for disease-specific survival (DSS) showing effect modification on the NF1 association in subgroup analyses

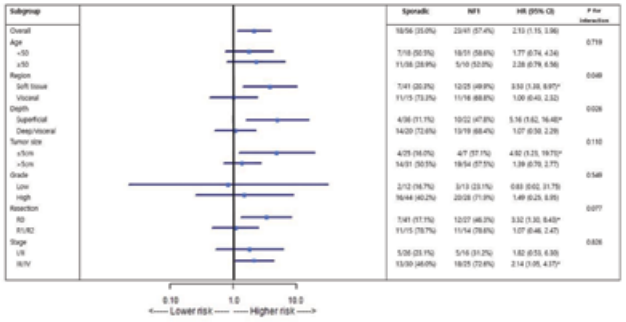

Figure 4. Forest plot for any tumor-related event showing effect modification on the NF1 association in subgroup analyses

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Diverse Clinical Effects of Selumetinib in Korean Children and Adults with Neurofibromatosis Type I

Hyery Kim, MD, PhD and **Hee Mang Yoon, MD, PhD**, *Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Republic of Korea*

Oral selective MEK inhibitor, selumetinib decreases the volume of plexiform neurofibroma (PN), resulting in quality of life (QOL).

The current study is an open-label, phase 2 trial, which enrolled 90 Korean patients with NF1 (≥3 yrs) with inoperable, symptomatic or potentially morbid, measurable PN (≥3 cm). Oral selumetinib was given 20 or 25 mg/m^2 or 50 mg q 12hrs as 28-day cycles for 2 years. Findings of pharmacokinetics, including the maximum concentration (Cmax) and the area under the curve from 0 to 12 hr ($AUC_{0\text{-}12hr}$), volumetric magnetic resonance imaging, growth, neuropsychiatric function, clinical photography Café-au-lait spots (CALS), and quality-of-life (QOL) questionnaires were evaluated.

A total of 89 patients (59 children, 30 adults) were received 27±9 (range, 9∼45) cycles of selumetinib. Partial responses (≥ 20% volume reduction) were confirmed in 91% of all patients and in 100% in patients after 2 years of treatment. Scores of verbal comprehension, perceptual reasoning, processing speed, and full-scale IQ were improved after 2 years of treatment. Prepubertal patients showed increases in height score and growth velocity. CALS notably decreased in 86.1% of patients after 2 years. The QoL scores improved in most of children, parents and adult patients. Following a 25 mg/m^2/dose, the mean C_{max} and $AUC_{0\text{-}12hr}$ of our subjects were higher than those in Caucasian children in prior trials. All adverse events were CTCAE grade 1 or 2 and managed without discontinuation. In conclusion, selumetinib may have a broad therapeutic role in NF1, including tumor shrinkage and improvements in neurocognitive function, overall growth, and cutaneous manifestations.

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Chronic Pain in Neurofibromatosis 1: The Relevance of Self-Determination Theory in Understanding Self-Care Behaviors

Erica Leif, MA, *Alliant International University, San Francisco Bay Area*

Self-Determination Theory (SDT) is a theoretical framework for understanding people's intrinsic motivation based on their perception of autonomy, competence, and relatedness. SDT has been used in health psychology research to predict health behavior (e.g., exercise participation among individuals with musculoskeletal pain). The current study is part of a larger research program to measure self-care among adults with neurofibromatosis type 1 (NF1), and to evaluate the applicability of SDT to understanding motivation to engage in self-care. Self-care in our research is defined broadly, including acquiring and engaging in medical and complementary care, use of services and devices to manage symptoms, mental and physical health strategies, utilizing social support, and engagement in personal and public advocacy. Self-care is particularly relevant to NF1 because as a progressive chronic condition involving chronic pain and affecting nearly all aspects of daily living, self-care among a wide variety of domains is required. The current study represents the first step in creation of a disease-specific patient reported outcome measure of self-care in NF1. This study involved individual interviews with 22 adults with a confirmed diagnosis of NF1 (4 male, 15 female, and 3 non-binary/genderfluid, between 18 and 56 years of age). They responded to questions regarding aspects of self-care, experienced barriers to care, SDT-related factors (sense of autonomy in health care decisions, perceived competence to manage symptoms, and experience of relatedness to the health care team), and their chronic illness experience. The interviews yielded specific themes related to self-care in the areas of symptom management via medications, non-medication substances, and devices, services, primary and specialty physicians, complementary care, activities, and personal strategies for emotional and symptom regulation. Primary barriers described included financial challenges and insurance coverage, the difficulty of acquiring providers with knowledge and competence, and experiences of stigma and discrimination. Interviews yielded specific themes related to autonomy in health care decisions, sense of competence to manage NF1, and experience of relatedness to the health care team. Emergent and unexpected themes included impact of COVID-19, concerns about lack of public understanding of NF-1, pushing through the pain, and impact of puberty and hormonal treatments on NF-1 symptoms. Major and minor themes and illustrative quotes will be presented. The results provide themes that inform the generation of items and scales to support our creation of a patient related outcome measure that can be used to evaluate self-care in NF 1 and identify areas for support and intervention.

Additional Authors: Diane Zelman, PhD, Alliant International University, San Francisco Bay Area

Granting agency: This study has been funded by the Children's Tumor Foundation Contract Award (ID: 2023-04-001).

Children and Adolescents with Neurofibromatosis Type I Have Lower Height and Weight Growth Percentiles that are Inversely Associated with Plexiform Neurofibroma Volume

Kathryn M. Lemberg, MD, PhD, *Johns Hopkins University School of Medicine*

Background: In the pediatric NF1 population, relationships between anthropometric measurements (e.g., height, weight), plexiform neurofibroma (pNF) tumor volume, and treatment history are unknown. The goal of this study was to describe anthropometric parameters for pediatric and adolescent/young adult (AYA) patients and to investigate their relationship to pNF volume. We hypothesized that people with NF1 would have lower height and weight percentiles compared to reference populations, and that height and weight percentiles would be negatively associated with whole body pNF burden. We also hypothesized that treatment of pNF with a MEK inhibitor (MEKi) would impact anthropometric measurements in this population.

Methods: We retrospectively investigated anthropometrics in pediatric and AYA patients on the NCI NF1 Natural History Study (NCT00924196) who had baseline volumetric MRI measurements of total body pNF burden. In the entire population (n = 106; ages 2.74-33.4 years) we identified heights and weights measured within one week of whole-body MRI (WB-MRI) and determined CDC height and weight percentiles. The association between whole body pNF volume and growth percentiles was assessed by Spearman correlation. In the subset of pediatric patients < 19.99 years old (n = 93, ages 2.74-19.99 years) we modeled height measurements over time. For the patients with >2 repeated height measurements (n=83 patients and 1062 measurements) we applied a non-linear modeling approach to estimate Preece-Baines (PB) growth curve parameters. PB estimated final adult heights for all treatment histories and in the absence of MEKi were compared to each other and to CDC reference growth data.

Results: Evaluation of anthropometric measurements taken at the time of WB-MRI revealed that for male NF1 patients (n = 60), 23% of patients (95% CI 13% – 36%) had height < 5th percentile and 13% of patients (n = 61; 95% CI 5.8 – 24%) had weight <5th percentile. For female NF1 patients (n = 45), 20% of patients (95% CI 9.6%-34%) had height < 5th percentile. The associations between height percentile and weight percentile and pNF tumor volume were both weakly negative (Spearman's r = -0.277; 95% CI -0.448, -0.088 for height and -0.216; 95% CI -0.395, -0.022 for weight). Using longitudinal data obtained across the full NF1 patient cohort, median estimated final adult height for males was at the CDC 23rd percentile (171.562 cm) and for females was at the CDC 14th percentile (156.186 cm). Trivial changes in the estimated median final adult heights were observed when measurements collected during MEKi treatment were excluded from the analysis.

Conclusions: In the NCI NF1 pediatric population, >5% of males and females have heights < the CDC 5th percentile, and > 5% of males have weights < 5th percentile. Whole body pNF burden shows a weak negative association with height and weight percentiles. NF1 patients achieve median final adult heights < CDC 25th percentile for each sex and this estimate was not affected by exclusion of patients treated with MEKi. These findings are consistent with previous studies of patient growth in NF1 but are the first to correlate anthropometrics with whole body pNF burden and treatment history. Additional analyses are ongoing.

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Familial Co-Segregation of Neurofibromatosis Type 1 and Legius Syndrome: Phenotype and Management

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Purpose of the study: Neurofibromatosis type 1 (NF1), caused by *NF1* pathogenic variants, is a tumor predisposition syndrome. Typical manifestations include neurofibromas and café-au-lait macules (CALMs)[1]. Legius syndrome, caused by *SPRED1* pathogenic variants is a rarer condition with a skin hyper-pigmentary phenotype similar to NF1, but without the increased tumor load[2]. Both NF1 and Legius may associate with some degrees of developmental delay and learning difficulties, but cognitive disability is overall rare. However, it is more frequent, often in the context of characteristic dysmorphic features, among NF1 individuals harboring a whole-gene deletion[3,4]. We present a family segregating both NF1 and Legius. To our knowledge, this is the first documented occurrence of such an event.

Patients and Methods: The proband, a 10-year-old boy, initially presented with severe developmental, attention and learning difficulties, relative macrocephaly, prominent forehead, bitemporal narrowing, hypertelorism, low set ears, about 6 CALMs, skinfold freckling, pectus excavatum and short stature. Later, plexiform neurofibromas, and leg-length discrepancy became obvious. His 38-year-old mother also had seizures, severe cognitive and global developmental delay, similar craniofacial features and short stature. She had had leukemia in childhood and was more recently diagnosed with multiple sclerosis and intestinal polyposis. On skin exam she had ≤ 5 CALMs, and 2 small cutaneous neurofibromas. Her skin was velvety and hyper-elastic. Mother and son displayed a shy, passive personality, with tendency to social isolation and required frequent supervision. The proband's 75-year-old maternal grandmother also had ≤ 5 CALMs and hyper-elastic skin but was otherwise healthy. She was the main caregiver of her daughter and grand-son. *NF1* and *SPRED1* were analyzed by next generation-sequencing. Written informed consent was obtained from the patients prior to testing.

Results: The proband, his mother and maternal grand-mother harbored the pathogenic heterozygous variant NM_152594.3*(SPRED1)*:c.796_797del, p.(Met266Valfs*4). In addition, the proband and his mother also harbored the pathogenic heterozygous variant NM_000267.3*(NF1)*:c.2970_2971delAA, p.(Met991Aspfs*29). Mosaicism for the same *NF1* variant was ruled out in the maternal grand-mother.

Conclusion:

- The co-segregation of *NF1* and *SPRED1* pathogenic variants likely represents an exceedingly rare occurrence.
- We suggest that patients with NF1, high burden of cognitive, developmental and behavioural difficulties, dysmorphic features classically associated with RAS pathway dysfunction, and relatively mild skin manifestations, should be screened for the co-segregation of *NF1* and *SPRED1* pathogenic variants.
- Regular screening for leukemia should be added to the management of these patients.

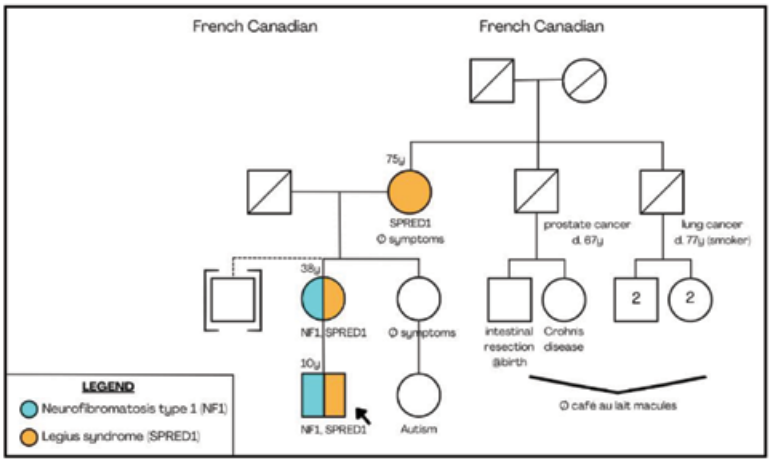

Figure 1: Pedigree of the family segregating both NF1 and SPRED1 mutations.

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Adherence to Selumetinib in Children on SPRINT over Two Years: A Comparison of Diary and Pill Count Data and Relationship to Pain, Quality of Life, and Tumor Burden

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Purpose: Medication adherence in children with neurofibromatosis type 1 (NF1) is an understudied topic. Since nonadherence leads to poorer outcomes in other medical populations, understanding medication adherence in NF1 is a crucial step towards developing and implementing interventions. There is currently no gold standard measure of adherence; methods range from pill counts (PCs) to patient-reported diaries. Multiple methods are often employed to increase reliability, using more resources and increasing burden for patients and staff. Some adherence data from the phase 2 trial of selumetinib for inoperable plexiform neurofibromas (PNs) (SPRINT, NCT01362803) was presented previously; this abstract extends that work through analysis of adherence diaries, comparison of adherence measures, and exploration of relationships between adherence, pain, quality of life (QOL), and tumor burden. We hypothesized that patients with more pain and poorer QOL at baseline would have better adherence over the first 6 cycles of selumetinib (1 cycle = 28 days).

Methods: This analysis includes children enrolled on SPRINT at the National Cancer Institute (diagnosed with NF1 with ≥1 symptomatic, inoperable PN). Adherence was measured by patient daily diaries and by PCs at restaging visits over the first two years. Patients completed Patient Reported Outcome (PRO) measures of pain (Numeric Rating Scale 11; NRS-11, Pain Interference Index; PII) and QOL (Pediatric Quality of Life Inventory; PedsQL [higher scores = worse functioning]). Tumor burden was measured through volumetric analysis. Nonparametric statistics were used to compare adherence on diaries vs PCs; adherence was compared with tumor burden and PROs.

Results: Of 35 children enrolled, 33 (mean[*M*] age=10.9 years, range=5-17) had adequate adherence data (>50% of diaries and PCs), and 22 of those had analyzable PRO data. Mean adherence rates remained high over the first 24 cycles for both adherence methods ($M_{diaries}$ year 1: 97.2% ± 5.0, year 2: 97.6% ± 4.1; M_{PC} year 1: 98.0% ± 2.6, year 2: 95.3% ± 9.4). There was no significant difference between diaries and PCs during year 1 (t=-.90, p=.38) or year 2 (t=1.4, p=0.17), and both measures were highly correlated over both years (ps<0.05). Neither mean PC nor diary data over cycles 1-6 were correlated with baseline pain measures (ps>0.05). Mean adherence for diary cycles 1-6 was negatively correlated with the PedsQL Physical Functioning scores (p<0.05), but became insignificant when controlling for baseline pain intensity. PCs over cycles 1-6 were not correlated with any PedsQL domain scores. Neither diaries nor PCs were significantly related to any parent-reported PedsQL scales. Adherence by diaries or PCs was unrelated to change in tumor burden over the first year.

Conclusions: Adherence to selumetinib was very high according to both adherence methods. Pill count adherence did not differ significantly from patient-reported diaries; this suggests a lack of necessity for using both measures to determine patient adherence on these trials, thus potentially lowering patient and staff burden. Contrary to hypotheses, baseline pain and poorer QOL were unrelated to adherence, which should inform future interventions targeting nonadherence.

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Case Series Illustrating Challenges in Defining Atypical Neurofibromas (aNF) Using Clinical, Histopathologic, and Genomic Features: A Need to Redefine aNF

Sana Zahra Mahmood, BS, *Pediatric Oncology Branch, National Cancer Institute*

Background: Atypical neurofibromas (aNF) are peripheral nerve sheath tumors (PNST) histologically defined by cytologic atypia, hypercellularity, loss of neurofibroma architecture, and/or increased mitotic activity. aNF often have heterozygous loss of *CDKN2A/B* in addition to *NF1* double inactivation. Clinically, aNF frequently appear as distinct nodular lesions (DNL) on MRI, grow faster than plexiform neurofibromas (pNF), and have increased avidity on fluorodeoxyglucose (FDG)-positron emission tomography (PET). However, some PNST demonstrate a discrepancy between clinical, histologic, and genomic criteria, where a PNST without significant atypical histologic features may have *CDKN2A/B* loss and clinical features concerning for aNF. The purpose of this case series is to raise awareness to this discrepancy by highlighting examples of PNST with clinical and genomic changes consistent with aNF, without significant atypical histologic features.

Methods: This is a case series of patients with Neurofibromatosis type 1 (NF1) enrolled on a biospecimen and tissue acquisition protocol at the National Cancer Institute (NCI), NCT01109394, who had PNST resected as part of their clinical care. Histopathology was reviewed by experts in the Department of Pathology at the National Institutes of Health. Molecular pathology was examined using the TruSight Oncology (TSO) 500 Gene Panel (DNA) v3.0. Imaging and clinical findings were collected retrospectively from electronic medical records.

Results: Four patients with resected DNL are included in this case series (3 male, 1 female). Median age at time of initial detection of the resected DNL was 10.25 years (range 9.8-13.9). Median DNL volume at time of resection was 32.2 mL (range 24-115). In all four patients, increased rate of DNL growth compared to typical pNF was the primary indication for resection, with one patient also reporting pain in the DNL. In three patients, DNL growth occurred while on treatment with Selumetinib for a pNF. The annualized growth rates of DNLs far exceeded the growth of target pNF preceding the resection: 31% vs. 5%, 79% vs. 9%, and 147% vs. 33%. One patient was not on treatment at time of resection, but clinically evident rapid tumor growth and pain were concerning for possible malignant transformation. FDG-PET scans were obtained for two patients, showing max SUVs of 4.2 and 6.0. Histopathology for all 4 tumors was consistent with neurofibroma; 3 of the 4 had "scattered" or "benign" nuclear atypia but no samples exhibited hypercellularity, loss of neurofibroma architecture, increased mitotic rate, or necrosis. *CDKN2A/B* loss was found in all 4 tumors (*CDKN2A* only in 1, *CDKN2B* only in 1, and *CDKN2A/B* loss in 2).

Discussion: In our case series, the discrepancy between the clinical features consistent with aNF and histologic findings without significant atypia suggests that histology alone is not sufficient to define aNF. Presence of *CDKN2A/B* loss in these cases supports inclusion of genetic sequencing to define aNF, to determine the biologic potential for future malignant transformation, and to select appropriate clinical management. We are expanding our study by including additional cases to more comprehensively assess the relationship between clinical, histologic, and genomic features.

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Neurofibromatosis Type 1 in Identical Twins: Case Study

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Introduction: The mutated NF1 gene presents extreme variability of expression, even within members of the same Family. It is difficult to predict which patients are at risk of specific complications and, therefore, to properly manage those at higher risk.

Objectives and method: The objective of this report is to present the case of two twin sisters with very different phenotypic presentation.

Case report: The patients are the result of the fourth pregnancy of a young, healthy, non-consanguineous couple, which evolved uneventfully. they evolved without significant delay in neuropsychomotor development and have satisfactory school performance. The diagnosis of neurofibromatosis type 1 occurred in childhood. Both present cafe au lait spots, freckles and cutaneous neurofibromas, but several different complications throughout life. Twin 1 has short stature, unilateral tibial dysplasia ovarian rhabdomyosarcoma at 3 years of age, plexiform neurofibroma in the clitoris and left labia majora, in addition to severe progressive scoliosis. Twin 2 had a plexiform neurofibroma in the breast and in the region under the right mandible, symptomatic and inoperable due to the involvement of important vascular structures in the cervical region. He has been using Koselugo for 5 months with a 10% decrease in the lesion and symptomatic improvement so far. Molecular study was not performed on the patients or their families. Parents do not show clinical signs of neurofibromatosis.

Discussion and conclusion: The distribution of involvement in patients reflects the difficulty in following a family member with the same mutation, but without predicting the evolution, despite similar genetic materials. Monozygotic twins with phenotypic discordances are a useful tool to assess which traits are influenced by non-inherited changes such as postzygotic mutations, environmental agents, epigenetic modification or somatic second occurrence events.

Sharing High-Quality Samples and Data to the NF1 Research Community: The Role of the Johns Hopkins NF1 Biospecimen Repository

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Background: Neurofibromatosis type 1 is a common neuro-genetic condition caused by mutations in the *NF1* gene. It constitutes an autosomal dominant condition that has a predisposition to benign and malignant tumors, including cutaneous neurofibromas (cNF), plexiform neurofibromas (pNF), atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP) and malignant peripheral nerve sheath tumors (MPNST). Progress in the development of nonsurgical therapy for pNF and MPNST has in the past been limited by several factors, including the limited access to primary tissue from patients with NF1 and the limited number of cell culture-based and animal models to adequately support research advances.

Methods: We have established and expanded the Johns Hopkins NF1 Biospecimen Repository, which includes quality-controlled, clinically, and genomically-annotated samples from patients with NF1. Samples included in the biorepository are blood fractions, frozen tumor tissues, slides from paraffin embedded tissue, single cell suspensions, and cell line and xenograft models to propagate primary human tissue and cells. Banked specimens also undergo comprehensive genomic characterization using whole exome sequencing (WES) and RNAseq and these data are available through the NF Data Portal. A comprehensive, clinically annotated database includes NF1-associated clinical symptoms, tumor characteristics, and outcomes data. An internal scientific review process allows for researchers outside our institution to request access to specimens, cell line and patient-derived xenografts models, genomic data, and accompanying de-identified clinical information for their research.

Results: Since its inception in January 2016, over 300 unique samples have been banked (from 157 unique patients), including pNF (n=78), MPNST (n=47), cNF (n=76), blood fractions, and xenograft (n=5) specimens. 54 researchers from outside institutions have requested access to our specimens. RNAseq (n=67) and WES data (n=102) are available through the NF Data Portal.

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.

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Accurate Diagnosis of MPNST Requires Both Consensus Histological Review and Interrogation of Genomic Data

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Purpose: The purpose of this study was to evaluate the correlation between the original pathology diagnosis of malignant peripheral nerve sheath tumors (MPNSTs) based only on clinical NF1 status and histology versus diagnosis in the context of histology plus germline *NF1* variant status and trimethylation of histone H3 at lysine 27 (H3K27me3) status.

Design: Ninety-nine frozen tumors were collected from participating institutions, and analyzed by whole-genome sequencing (WGS), RNA-sequencing and Infinium DNA methylation arrays. Hematoxylin-eosin (H&E) staining and H3K27me3 immunohistochemistry (IHC) were performed from formalin-fixed paraffin embedded (FFPE) material. Histopathologic analysis was performed on a whole slide digital platform. Clinical data were abstracted from the consortium REDCap database.

Results: Among tumors designated MPNST on submission (n=99); ten tumors were excluded because H3K27me3 expression data were unavailable. Among 89 tumors remaining, nine were not considered MPNST because there was no *NF1* germline variant or in sporadic cases in which the histological features were equivocal at best for MPNST. The remaining 80 tumors (54 NF1-related; 26 sporadic) from 69 individuals were divided into four categories based on the presence/absence of germline *NF1* pathogenic variants, conventional/non-conventional histopathological features (WHO), and presence/absence of H3K27me3 immunoreactivity (**Table 1**). Conventional tumors showed monomorphic spindle cells in intersecting fascicles with alternating densely cellular and hypocellular areas, some with a herringbone architecture. Non-conventional tumors were composed predominantly of large epithelioid cells with marked pleomorphism and occasional round cell morphology. Heterologous elements occurred only in MPNSTs (n=15) with conventional morphology and more commonly in the sporadic setting. H3K27me3 loss was seen in 53% of conventional tumors and in the context of NF1 (50%). Upon consensus review of these 80 tumors: two tumors were re-classified as neurofibroma (NF), and 3 as atypical neurofibromatous neoplasm of uncertain biological behavior (ANNUBP). The remaining cases were MPNST (low grade 5; high grade 70). Tumor grade was modified (6) or added (6) from original submission in 12 cases. Overall, 14 tumors were re-classified based on knowledge of *NF1* germline status and/or H3K27me3 status for accurate classification.

Table 1: MPNST tumor category compared to H3K27me3 status and presence of heterologous elements

Category	N	H3K27me3 loss (n)*	H3K27me3 retained (n)*	H3K27me3 partial loss (n)*	Heterologous elements #
GL – C	50	25 (50%)	24 (48%)	1 (2%)	5
GL – NC	4	1 (25%)	3 (75%)	0	0
S – C	23	16 (65%)	8 (35%)	0	10
S – NC	3	0	3 (100%)	0	0

*H3K27me3 performed on 80 tumors. GL-C: germline conventional, GL-NC: germline nonconventional, S-C: sporadic conventional, S-NC: sporadic nonconventional. #: rhabdomyosarcoma (10), angiosarcoma (2), osteosarcoma (1), rhabdomyosarcoma-chondrosarcoma (1), osteosarcoma-liposarcoma (1).

Conclusion: Among 89 tumors submitted as MPNST with H3K27me3 expression data available, 14 (15.7%) were reclassified as not being MPNST or a neurofibroma (9) or reclassified as a lower grade MPNST-related neurofibroma (5) after review that incorporated *NF1* germline variant and H3K27me3 status. Diagnosis was more challenging in sporadic cases and those with non-conventional morphology. We highlight the importance of both consensus histological review and knowledge of both *NF1* germline variant and H3K27me3 status for reaching an accurate diagnosis of MPNST.

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Perceived Quality of Life Differences Between Teens with NF1 and Their Parents

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Introduction: Neurofibromatosis type 1 (NF1) is a multisystemic genetic condition characterized by medical, behavioral, and cognitive manifestations, which are variable, even within families. Previous studies have shown that individuals with NF1 view their diagnosis as something that negatively impacts their quality of life (QoL). This is of particular interest for healthcare providers who may follow individuals with NF1 over their lifetime. Throughout the lifespan, adolescence can be particularly challenging because autonomy is gradually transitioned from parents to patients. This study aims to identify areas related to QoL in which parents and their children with NF1 may differ on views of the impact of NF1 on QoL.

Methods: Investigator-developed complementary surveys aimed to assess aspects of perceived QoL for individuals between the ages of 14-22 years with a diagnosis of NF1 and parents of this population. Surveys were distributed via the NF Registry's worldwide database with responses from 38 adolescents and 87 parents.

Results: Parents were more likely to perceive their child's QoL as fair (p .038), while adolescents tended to report good or better QoL (p .045). Perceived psychosocial effects of skin findings, cafe-au-lait macules and neurofibromas, did not differ between adolescent and parental cohorts ($p > .05$). Adolescents and parents who did not report public visibility of the skin findings were more likely to report a quality of life of "fair" or better (adolescents p .015; parents p .003). Parents were more likely to report higher scores for QoL when indicating that the skin findings did not have a perceived impact on clothing choices ($p < .001$) and worry about worsening in the future ($p < .001$), which was not seen in the adolescents (p .251; p .086). For some of the extracutaneous findings associated with NF1, adolescents' QoL scores did not appear to demonstrate any significant trends for any of the domains ($p > .05$). Based on parental reports, QoL scores were shown to have a relationship with pain (p .015), speech concerns ($p < .001$), and getting in trouble ($p < .001$). While the relationship did not suggest that those who reported concerns is correlated with lower QoL scores, it was found that for the parents who do not report these concerns in their children, they were more likely to report lower QoL scores.

Conclusion: Given these findings, adolescent and parent cohorts demonstrated some level of disagreement, especially regarding perceived QoL, which was overall lower in the parent cohort. Gaining understanding of the ways in which parents and adolescents view NF1 can be helpful in guiding healthcare professionals' approach when working with families.

Additional data pending.

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An Evaluation of Paediatric Pain Assessment Tools in a National Paediatric NF1 Out-Patient Clinic Setting

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Introduction: The purpose of this evaluation is to look at the best way to assess pain in a National Paediatric NF1 outpatient clinic using three general, validated pain assessment tools. Pain in NF1 can be complex due to variability in pain phenotypes as well as differing cognitive and psychosocial profiles between children and within families. Reporting of pain may be more challenging due to learning difficulties and the effect on pain measurement by non-physical factors[1]. Pain is a common reason for parents to contact clinical nurse specialists and other health professionals and accurate assessment is important if it is to be managed correctly and holistically[2].

Methods: 30 parents/carers and children aged 0-18 attending the National Paediatric NF1 out-patient clinic at Guy's and St Thomas' National Foundation Trust, London, who identified pain as an issue were assessed by the paediatric clinical nurse specialists. Both parent/carer and child were asked to score the pain using 3 different pain assessment tools; the verbal numerical rating scale which is the current method of pain assessment[3], the faces scale (revised)[4] and the Face, Legs, Activity, Cry, Consolability scale (FLACC revised) scale[5] for non-verbal children or children with significant learning difficulties.

The Michigan Body map[6] was used to indicate location of the pain. The nature of the pain, impact on function, observation of the site of the pain and things that may have exacerbated the pain were recorded. A record was made of demographics and current medication.

Evaluation of the pain assessment tools was obtained via a brief, structured, verbal questionnaire.

Results: Results will be presented on demographics, physical or mental disability or impairment. The results will present any differences in reporting of pain by children and their carers and evaluation of the pain assessment tools, including ease of use and how accurately pain is assessed. Location of pain, onset, pain descriptors, current medication and the impact of pain on lifestyle will be highlighted.

Conclusion: This evaluation aims to ensure that pain assessment tools are understandable and easy to use by our patients. The goal is to use these tools on a regular basis to assess and evaluate pain in a National Paediatric NF1 out-patient setting.

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Selumetinib for Symptomatic, Inoperable Plexiform Neurofibromas in Children with Neurofibromatosis Type 1: Experience In Japan

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Purpose: Selumetinib was allowed to use for patients with symptomatic, inoperable plexiform neurofibroma (PN) in children of neurofibromatosis type 1 (NF1) from November 2022 in Japan. In pre-approval clinical trials in Japan, selumetinib was used in only 12 patients. Therefore, post-approval clinical use experience is important for evaluating safety and efficacy of selumetinib, particularly for Asian patients. The purpose of this study is to report the short-term use experience after selumetinib was approved in Japan in November 2022.

Methods: Subjects were patients for whom prescription of selumetinib was started for symptomatic, inoperable plexiform neurofibromas in children with neurofibromatosis type 1 at our institution for 2.5 months from November 16, 2022 to January 31, 2023. Clinical factors of patients, site of symptomatic PN, maximum diameter, dose of selumetinib, administration period, adverse events (AE), presence or absence of dose reduction were investigated.

Results: Selumetinib was prescribed to a total of 9 patients, 5 males, mean age 13 years (8-18). The sites of symptomatic PN were the head in 4 patients, the pelvis to the lower extremities in 3 patients, and the paraspinal lesions in 2 patients. The morbidities of the patients were disfigurement in 5 patients, pain and motor dysfunction in 4 patients, and impending spinal cord injury in 1 patient. Six patients had other PN lesions. The median maximum diameter of PN was 91 mm (39-814). The dose of selumetinib was 50 mg/day in 4 patients, 60 mg/day in 4 patients, and 70 mg/day in 1 patient. The mean treatment duration was 2.8 months (median 2 months, range: 1.5-4.5 months).Regarding AE, Grade 2 was observed in only 1 patient with diarrhea. The others were Grade 1, including 6 patients of dermatitis, 2 patients of stomatitis, 1 patient of acne, 1 patient of paronychia, and 1 patient of elevated CK. AE tended to improve gradually. There were no patients of dose reduction or discontinuation of selumetinib due to AE.

Conclusions: Prescription of selumetinib has just started in Japan for symptomatic, inoperable plexiform neurofibromas in children with neurofibromatosis type 1. Despite the short-term results, the prescription was continued without major adverse events. It is necessary to create data on efficacy and safety through patient accumulation.

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Disclosure: Yoshihiro Nishida: Lecture fee, Consultant fee from AstraZeneca, Alexion Pharma

A Precision Therapy Strategy Guided by Tumor Suppressor Gene Inactivation Status Significantly Reduces MPNST Volume *In Vivo*

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Malignant peripheral nerve sheath tumor (MPNST) is a highly aggressive soft-tissue sarcoma that develops sporadically or in patients with neurofibromatosis type 1 (NF1). MPNST initiation is marked by the inactivation of tumor suppressor genes (TSGs), particularly *NF1, CDK2NA/B,* and *SUZ12* or *EED* (Polycomb repressive complex 2, PRC2). While the implication of these TSGs in MPNST pathogenesis has been studied, the use of their loss as potential vulnerabilities for therapeutic strategies has not been systematically tested. The main objective of this project is to provide a conclusive view of the therapeutic potential of the combined use of drugs against these three recurrent TSG-losses in MPNSTs, *in vitro* and *in vivo.*

For the loss of *NF1, CDK2NA/B,* and PRC2 function, we evaluated the effect of MEKi, CDK4/6i and BETi, respectively. To analyze the potential differences within the same class of inhibitors, we first used a quantitative high-throughput screening performed at NIH-NCATS using 14 different MEKi, 12 different CDK4/6i, and 3 different BETi, all contained in the NCATS Mechanism Interrogation PlatE (MIPE) library (**Figure 1**). We selected all drugs active as single agents and analyzed them in a pairwise combination matrix screen to explore synergistic activity. We then validated the best 26 drug combinations in a panel of 3 MPNST cell lines, using fibroblasts as a toxicity control.
The validation of the 6 most synergistic combinations were extended to 6 additional genomically characterized MPNST cell lines (**Table 1**). We observed significant redundancy for drugs of the same inhibitor class, as well as different vulnerabilities to these co-treatments among the distinct MPNST cell lines used. We selected the best non-toxic pairwise combinations using the three inhibitor classes and tested them *in vivo.* We used two patient-derived orthotopic xenograft mouse models (PDOX), one sporadic and one NF1-related bearing the inactivation of the three TSGs, in which a piece of primary MPNST was engrafted near the sciatic nerve of athymic mice. *In vivo* results in the sporadic MPNST showed a reduction of tumor volume of about 65% for one of the combinations (MEKi-2 + BETi-1) (**Figure 2 and Figure 3**). Currently, we are analyzing drug action and tumor regrowth after treatment, and performing the same co-treatments in the NF1 MPNST xenograft model.

In summary, we performed a systematic co-treatment analysis in genomically characterized MPNST cell lines using 26 MEKi, CDK4/6i and BETi combinations. One of them significantly reduces tumor volume *in vivo.*

Figure 1

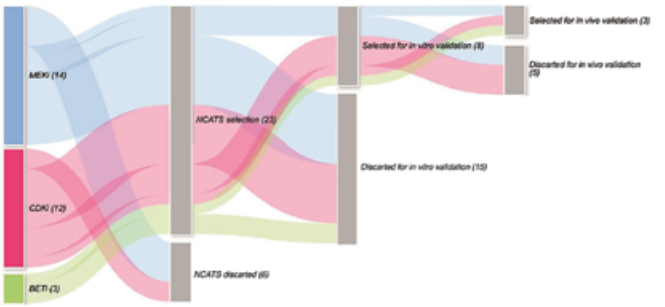

Table 1

	NF1-related MPNST cell lines								Sporadic MPNST cell	Toxicity
	S462	sNF96.2	STSB-14	NMS-2	90-8TL	MPNST-NF1-08	MPNST-NF1-09	MPNST-NF1-18D	MPNST-SP-10	HFF
Combination 1 (MEKi-2 + CDKi-1)	✓	✓	=	✓	Not effective	✓	Not effective	✓	Not effective	NO
Combination 2 (MEKi-3 + CDKi-1)	=	✓	Not effective	✓	Not effective	Not effective	=	✓	Not effective	NO
Combination 3 (BETi-1 + CDKi-1)	✓	✓	✓	✓	✓	✓	✗	✓	✓	NO
Combination 4 (BETi-1 + CDKi-2)	✓	✓	✗	✓	✓	=	✓	=	Not effective	NO
Combination 5 (BETi-1 + MEKi-2)	✓	✓	✓	✓	✓	✓	=	✓	=	NO
Combination 6 (BETi-2 + MEKi-1)	✓	✓	✓	✓	✓	✓	=	✓	✓	NO

✓ Synergistic effect = Additive effect ✗ Antagonistic effect NO No toxicity observed

Figure 2

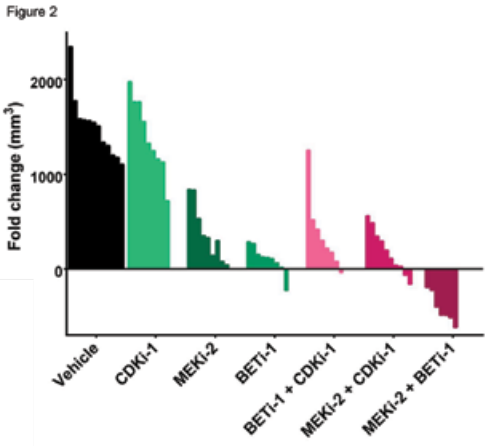

Figure 3

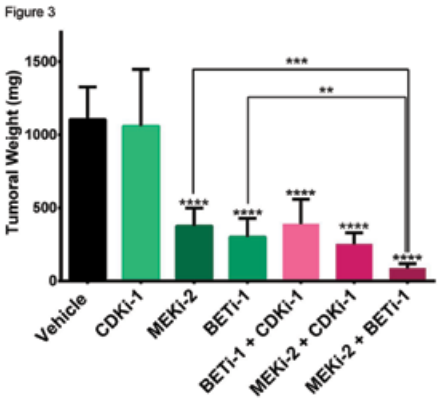

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Figure Legend:
Figure 1: Sankey plot as a summary to represent the flow from the first selection of compounds (29 drugs) to the final three selected drugs for *in vivo.*
Table 1: *In vitro* validation of the six most synergistic combinations in a broad panel of sporadic and NF1-related MPNST cell lines. Toxicity assays were also performed in a non-tumoral cell line (HFF, Human Foreskin Fibroblasts).
Figure 2: Waterfall plot of tumor volume at the end of the *in vivo* assay of sporadic PDOX-MPNST mice treated with the three selected combinations, individual drugs and vehicle. Each bar is a different replicate.
Figure 3: Plot of the tumor weight at the end of the experiment of sporadic PDOX-MPNST mice treated with the three selected combinations, individual drugs and vehicle.

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Longitudinal Modeling of Parent Ratings of Early Executive Function in Children with NF1 and Their Unaffected Siblings

Sara K. Pardej, MS, *University of Wisconsin – Milwaukee*

Purpose: The purpose of the present study is to identify whether there are differences between children with NF1 and their unaffected siblings in parent ratings on a measure of early executive functioning across the early childhood period using a longitudinal design.

Methods: Children with NF1 (n=62, 37 males) and their unaffected siblings (n=36, 23 males) were seen at least once between the ages of 3-8 years old. Parents completed the Behavior Rating Inventory of Executive Function- Preschool or Second Edition (appropriate editions was administered according to the child's age). Linear mixed model growth curve analyses were used to examine whether there were differences between children with NF1 and their unaffected siblings in the trajectories of their development on the following indices, which are available in both editions: Working Memory, Inhibit, Plan/Organize, Shift, Emotional Control, and the Global Executive Composite. T-scores (M=50, SD=10) were used, with higher scores indicating more challenges.

Results: There were significant interaction effects of age by NF1 status on Shift ($p=.035$), Plan/Organize ($p=.022$), and Global Executive Composite ($p=.036$) scores. There were significant main effects of NF1 Status on Inhibit ($p=.027$), Working Memory ($p<.001$), Plan Organize ($p=.006$), and Global Executive Composite ($p=.002$) scores. There were also significant main effects of age on Plan/Organize ($p=.040$) and Global Executive Composite ($p=.009$) scores.

Conclusions: Children with NF1 and their unaffected siblings show different developmental trajectories across many parent-reported executive functions, with children with NF1 showing more difficulties than their siblings across time on their ability to shift between tasks/activities and plan and organize, in addition to their global executive function. Furthermore, children with NF1 have poorer inhibition and working memory as compared to their unaffected siblings, which is consistent with prior literature. The present findings suggest that interventions to scaffold early executive function skills may be needed as early as the 3–8-year period for children with NF1.

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Harmonization Across Imaging Locations (HAIL): Implications of Image Harmonization for NF1-OPG Clinical Trials

Abhijeet Parida, MSc, *Children's National Hospital, Washington DC*

Purpose: To achieve high accuracy and reproducibility using deep learning algorithms, very large MRI datasets are required. For rare conditions like pediatric low-grade gliomas of the brain associated with NF1, clinical trials include numerous sites that acquire MRI data using different scanners and protocols. The ability to compare different MRI acquisitions from different sites, while leveraging the power of deep learning algorithms, the images must undergo processing to ensure accurate comparison—termed "multi-site harmonization." We propose a deep-learning algorithm to harmonize MRI data and enable accurate and reproducible data analysis across multiple clinical sites.

Methods: One-hundred eighty MRI scans from children with optic pathway gliomas secondary NF1 were included. T1-weighed MRIs were acquired from three sites, Children's National Hospital (site A, N=60), Children's Hospital Colorado (site B, N=60) and Children's Hospital of Philadelphia (site C, N=60) using different device manufacturers (GE, Phillips, and Siemens, respectively). Images from sites A and B were used to train a branched neural network that simultaneously identifies the differences in imaging protocols and harmonizes them to match a chosen protocol, e.g., the protocol of site A. Images from site C were used for independent testing of the method. The network was trained in an unsupervised fashion with loss functions to preserve the patient anatomy and harmonize the image intensities. For the evaluation of harmonization, we used a normalized Wasserstein distance (nWD with range 0-100%) to measure the distance between the intensity histogram of the input and target domains between the site pairs A↔B, A↔C, and B↔C. The preservation of anatomy was measured by the relative absolute volume difference (rAVD with range 0-100%), between the gray matter regions prior to and following the harmonization. The segmentation of gray matter regions was performed using FreeSurfer.

Results: For the harmonization between sites A↔B, we obtained nWD of 94.22±2.01% and rAVD of 6.88±4.71%. The image harmonization between sites A↔C and B↔C resulted in nWD of 94.59±2.25% and 91.92±3.92%, respectively with a rAVD of 13.08±4.67% and 5.32±3.56%, respectively.

Conclusion: Deep learning analysis enables harmonization of images from different MRI manufacturer platforms acquired at different institutions. The algorithm is scalable to include new sites and support clinical trials for rare pediatric diseases.

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Understanding Autism Spectrum Disorder In Children With Neurofibromatosis Type 1: Recent Findings from the PANDA Study

Jonathan M Payne, DPsych, *Murdoch Children's Research Institute*

Purpose: To characterize autistic behaviors in children with NF1 and investigate their association with other common co-occurring neurodevelopmental symptoms.

Methods: This is an international, multisite, prospective, cross-sectional cohort study of children with NF1 and typically developing controls (3-15 years of age). Participants completed a detailed assessment of their cognitive and language abilities, behavior, and adaptive functioning. Children screening *high* for autism spectrum disorder (ASD; Social Responsiveness Scale-2; SRS-2 *T*-score $\geq$60) completed a comprehensive autism assessment consisting of a play-based observational assessment (Autism Diagnostic Observational Schedule-2; ADOS-2) and a semi-structured, standardized parent interview (Autism Diagnostic Interview-Revised; ADI-R). Data from each child, including videos of their ADOS-2 assessment, were presented to an expert multidisciplinary assessment panel of clinicians to determine whether they met criteria for a DSM-5 diagnosis of ASD.

Results: To date, data have been collected and analyzed in 180 children with NF1 (95 males, 85 females; mean full scale IQ=88.62; SD=12.93) and 105 typically developing controls (50 male, 55 female; mean full scale IQ=106.90, SD=14.33). NF1 SRS-2 total scores were elevated by Cohen's d=1.3 relative to the control group (mean NF1 SRS total T-score, 62.39; SD, 14.5). 52% of children with NF1 scored at or above the *high* range for SRS-2 total scores, with 21% scoring in the *severe* range (*T*-score $\geq$75). SRS-2 Social Communication and SRS-2 Restricted Interests and Repetitive Behaviors scales were highly correlated (r=0.86, p<.001) in children with NF1, and SRS-2 total T-scores were also highly related to attention deficit hyperactivity disorder (ADHD) symptoms (r=0.76, p<0.01). Examination of autistic characteristics using the ADOS-2 and ADI-R revealed that social communication difficulties were common and wide-ranging in children with NF1. However, restricted interests and repetitive behaviors were mostly consistent with an insistence on sameness, such as circumscribed interests and difficulties with minor changes in routine. The prevalence rate of ASD in NF1 was 29%, with comparable rates between males and females. Diagnosis of ASD was generally associated with a broader range and increased severity of neurodevelopmental symptoms, including ADHD, anxiety, and lower adaptive skills. Links between intellectual functioning and ASD, however, were not strong. Further analyses are underway.

Conclusions: Results from this study provide strong support for elevated autistic behaviors in children with NF1 and help our understanding developmental strengths and weaknesses in children and adolescents with NF1. These findings have important implications for the identification of ASD and related behaviors in NF1, and clinical management.

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A Phase I Trial of Diphencyprone for Cutaneous Neurofibromas in Adult Patients with Neurofibromatosis Type 1: Preliminary Results

Dina Poplausky, BA, *Icahn School of Medicine at Mount Sinai*

Purpose: Cutaneous neurofibromas (cNFs) are one of the most burdensome features of Neurofibromatosis Type 1 (NF1), and to date, there are no Food and Drug Administration-approved pharmacotherapies for these benign tumors. We are investigating the use of diphencyprone (DPCP) for the treatment of cNFs in adults with NF1 in an ongoing single-center, phase I, open label trial (NCT05438290). DPCP is a topical hapten that causes a delayed-type hypersensitivity reaction in the skin and has been used successfully for the treatment of cutaneous melanoma metastases.

Methods: The primary aim of this clinical trial is to determine the safety and tolerability of DPCP ointment in the treatment of cNFs. DPCP 0.04% ointment was administered topically once weekly to up to 20 cNFs in a localized area for 10 weeks. Adverse events (AEs) are self-reported at each visit and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. To assess subjective tolerability, participants score their symptoms, including pain, stinging, burning, and pruritus from 0 (none) to 3 (severe). Skin reactions from DPCP were scored at each visit according to the International Allergic Contact Dermatitis Research Group (IACDRG) guidelines (**Figures 1-3**). Enrollment is ongoing with a target of 20 participants.

Results: Since September 2022, 12 adults (six male and six female) ages 26-60 with NF1 were enrolled. Fitzpatrick Skin Types range from I to V. This is a classification scheme for skin phototyping, ranging from I-VI. Seven participants completed the study including the 30-day follow-up visit. There were nine AEs in six subjects, five were Grade 1 and four were Grade 2. Eight of the nine adverse events were not attributed to DPCP and included COVID-19 infection, sinus infection, flu-like symptoms, nausea, diarrhea, back pain, and tooth infection. There were no serious adverse events. Two patients required transient drug interruptions due to excessive inflammation, deemed as an extreme positive (3+) skin reaction, which was an expected possible effect of the treatment. **Table 1** depicts median symptom scores and skin reaction scores on Day 21 (post-first treatment), Day 49, and Day 77 (last treatment).

Conclusion: These data support the safety and tolerability of DPCP ointment in adults with NF1. Inflammation was successfully induced by DPCP, but the investigation is ongoing to determine the efficacy of decreasing the cNF tumor burden in this study population.

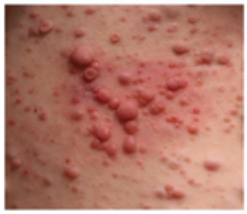

Figure 1: Weak positive (1+) skin reaction

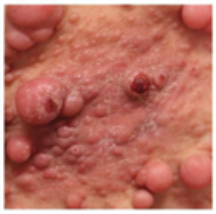

Figure 2: Strong positive (2+) skin reaction

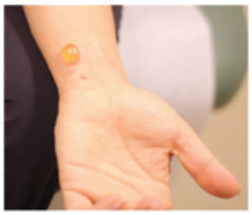

Figure 3: Extreme positive (3+) skin reaction

Table 1: Median Symptom Scores and Skin Reaction Scores

		Day 21 (n=12)	Day 49 (n=12)	Day 77 (n=10)[a]
Median Symptom Scores	**Pain**	0	0	0
	Stinging	0	0	0
	Burning	0	0	0
	Pruritus	2	2	1
Skin Reaction Score[b]	**(-) Negative Reaction**	--	--	--
	(?+) Doubtful Reaction	1 (8%)	--	--
	(1+) Weak Positive Reaction	8 (67%)	7 (59%)	8 (80%)
	(2+) Strong Positive Reaction	1 (8%)	4 (33%)	1 (10%)
	(3+) Extreme Positive Reaction	2 (17%)	1 (8%)	1 (10%)
	(IR) Irritant Reaction	--	--	--

[a]Ten participants have completed the study through Day 77 as of 03/2023. Seven of these participants completed the trial through Day 107, however, skin reaction scores were all negative 4 weeks after the final treatment.

[b]According to the International Allergic Contact Dermatitis Research Group guidelines

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Disclosures: G.S. has stock in Regeneron, Amgen, Johnson&Johnson, and Pfizer.

Characterizing Sleep Disturbance in Children and Adolescents with NF1: Preliminary Findings from the NF1 Sleep Study

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Purpose: To characterize sleep disturbance in children and adolescents with NF1 and investigate their association with other neurodevelopmental outcomes.

Methods: This is a multisite, cross-sectional cohort study of children with NF1 and typically developing (TD) controls (6-15 years). Participants completed an assessment of their cognitive skills, academic skills, behavior, and quality of life. A comprehensive sleep history and parent/guardian and self-report measures of sleep were collected including the Sleep Disturbance Scale for Children (SDSC).

Results: To date, SDSC data have been collected and analyzed in 112 children with NF1 and 24 TD controls. Preliminary results suggests that compared to controls, children with NF1 experience more severe sleep problems including initiating and maintain sleep (Welch's $F_{(1,73.69)} = 13.18$, $p<0.001$), sleep disordered breathing (Welch's $F_{(1,57.48)} = 9.3$, $p=0.003$) disorders of arousal (Welch's $F_{(1,135.90)} = 20.30$, $p<0.001$, and total sleep problems (Welch's $F_{(1,67.15)} = 10.63$, $p = 0.002$). Analyses are underway to investigate the association between sleep disturbance and patient characteristics and neurodevelopmental outcomes. Preliminary data involving a subset of NF1 participants suggests more severe sleep problems are associated with higher levels of fatigue, inattentive ADHD symptoms, autistic behaviors, pain interference and reduced literacy.

Conclusion: This study will further define the sleep phenotype, potential risk factors and the functional impact of poor sleep in children and adolescents with NF1.

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Funding: US Army Medical Research and Materiel Command, Department of Defense Neurofibromatosis Research Program, award number W81XWH1910254 New Investigator Award, Awarded to N. Pride

Use of Denosumab to Treat a Central Giant Cell Granuloma in a Pediatric Patient with Neurofibromatosis Type 1

Nicholas Pytel, DO, *University of Wisconsin School of Medicine and Public Health*

Introduction: Here we report a case of a pediatric patient with a clinical diagnosis of neurofibromatosis type 1 (NF1) who was incidentally found to have a central giant cell granuloma (CGCG) along with numerous non-ossifying fibromas. He started on denosumab therapy with marked clinical improvement. Using denosumab for this indication, and others, within the NF1 population is uncommon but may be beneficial.

Case Report: A 12-year-old male with a clinical diagnosis of NF1 presents to the dental office for a routine examination. His past medical history is significant for global hypotonia, macrocephaly, and prior plexiform neurofibroma within the right forearm, for which he recently underwent partial resection. He noted jaw discomfort, and an orthopantomogram film was obtained, noteworthy for a mass within the left mandibular area. The biopsied mass pathology was significant for a CGCG. He initially started on intratumoral corticosteroid injections with minimal improvement. His treatment then transitioned to subcutaneous denosumab therapy. Around this time, he also suffered a fall with lower extremity plain films obtained and significant for numerous non-ossifying fibromas.

Denosumab is a monoclonal antibody that binds to the cytokine RANKL (receptor activator of NFκB ligand), blocks osteoclast maturation, and reduces bone resorption utilized for treating CGCG and, in rare instances, symptomatic non-ossifying fibromas. Our patient responded well to this therapy with marked improvement in his initial jaw discomfort, and it could be possible that this may help with the non-ossifying fibromas, as well.

In conclusion, the use of denosumab within the pediatric NF1 population has rarely been described. As illustrated here, denosumab may benefit numerous indications, including associated osteoporosis, CGCG, and non-ossifying fibromas. Our patient remains on denosumab currently and continues to tolerate this well.

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Current Characteristics of the Children's Tumor Foundation US NF Clinic Network

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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. To date, the CTF has awarded over $2.5 million in stipends to NFCN sites to support NF-related activities. Sites are added through an application process with reviews performed by Clinical Care Advisory Board (CCAB) members. To promote education for providers, member sites have access to an NF list serve and monthly case conferences led by leaders in the field. Since it was established, the network has more than doubled in size, with 69 sites in the US and Canada awarded Affiliate Clinic status. In the last year, 21,145 NF patients (15,787 NF1, 1667 NF2, 521 SWN, 3170 other) were evaluated in NFCN clinics. Seventy-four percent of clinics see pediatric patients as a majority of their patient volume, and eight clinics provide almost exclusively adult care. The majority of care provision is based in Medical Genetics (32%), Neuro-Oncology (25%); and Pediatric Neurology (19%). The average wait time at an NFCN site is 10.7 weeks for a routine visit and 1.3 weeks for an urgent visit. Telehealth is offered at 87% of centers. Seventy-eight advanced practice practitioners and 83 genetic counselors support patient care within the NFCN. All clinics except one are involved with training and teaching activities. On-site NF clinical trials or other NF-related research studies are available at 68% of sites, with 53% of clinics informing patients of available research studies outside of their institution. NF-related publications were generated at 56% of clinics. Eight virtual and two hybrid educational NF symposia were held in 2022, with 70% of sites reporting that they had held a patient event in the past. Ninety-one percent of sites reported promoting the NF Registry, and 75% of clinics had clinic staff who attended the CTF NF Conference within the last 3 years.

Conclusions:

- The formation of an NFCN over 15 years ago has been highly beneficial in providing a large NF population with access to expert medical care, NF education, and research opportunities.
- There is still a need for additional NF centers in underserved areas of the US and Canada as well as an opportunity to expand the network to other countries.
- 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 research activities and clinical trials.
- The majority of NFCN clinics promote NF educational events for families and CTF initiatives such as the NF registry.

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Durability of Binimetinib Response and Retreatment in Pediatric and Adult Patients with Neurofibromatosis Type 1 Associated Plexiform Neurofibromas: A Report from the NFCTC and PNOC

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Background: Patients with NF1-associated plexiform neurofibromas (PN) often suffer debilitating complications including disfigurement. MEK inhibitors have shown promise in treating PN. We conducted a phase II clinical trial with the MEK inhibitor binimetinib, allowing treatment for up to 24 cycles for responders. Patients who completed all planned treatment but had subsequent PN progression after stopping binimetinib were eligible for a retreatment stratum. This is the first trial to systematically evaluate progression after stopping MEK-inhibitor and response to retreatment.

Methods: Patients ≥ 1 year of age (pediatric stratum < 18, adult stratum ≥ 18) with progressive and/or symptomatic PN causing significant morbidity were eligible. Patients with >15% reduction in PN MRI volume by 8 cycles and >20% by 12 cycles (partial response, PR) continued treatment for up to 24 four-week cycles. For those with progressive disease (PD) of their target PN (≥ 20% volume increase) within one year of therapy completion, enrollment on retreatment was offered. Patients could receive binimetinib for up to 24 additional cycles.

Results: Forty-five patients (20 pediatric, 25 adult) were enrolled on primary trial. Of 19 evaluable pediatric patients, 14 (74%; 95% CI, 48.8% to 90.9%) had PR; 13 of 20 evaluable adult patients (65%; 95% CI, 40.8% to 84.6%) had PR. Nineteen patients (9 pediatric, 10 adult) completed all planned treatment. Of these, 7 of 9 pediatric and 4 of 10 adult patients had PD after stopping binimetinib at a median of 4 months. Five pediatric and two adult patients enrolled on retreatment. Six patients remain on retreatment: 4 in second PR and 2 with stable disease. One patient came off therapy after 6 months due to grade 2 cardiac toxicity. Three patients with PD resumed treatment with a MEK inhibitor outside of the trial.

Conclusion: Binimetinib led to PR in NF1-associated PN for most patients. In this series, PD after stopping Binimetinib was common (78%) for pediatric patients and was less frequent (40%) but also seen in adult patients. Although the optimal duration of MEK-inhibitor therapy has not been determined, these data suggest most pediatric patients require prolonged treatment to maintain PR. Stopping after 24 months helps identify patients who may benefit from sustained treatment and does not negatively impact response to retreatment. Continued monitoring for toxicity is warranted.

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Treatment of Cutaneous Neurofibromas (cNF) in Neurofibromatosis Type 1 (NF1) with the MEK Inhibitor Selumetinib

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Background: cNF tumors can cause itching, disfigurement, pain and emotional difficulties. The results of an incompletely enrolled and terminated clinical trial of selumetinib for cNF, undertaken based on the efficacy of selumetinib in the treatment of NF1-related plexiform neurofibromas (PN), is reported here.

Methods: Adults with NF1 and ≥9 measurable cNF (≥4 mm longest diameter) were enrolled at 2 sites (University of Alabama at Birmingham & National Cancer Institute). Participants received continuous dosing of selumetinib (50 mg twice daily, optional increase to 75 mg if tolerated after cycle 1), for a maximum of 24 cycles (1 cycle=28 days), with restaging visits every 4 cycles. A 10x10 cm paper frame was used to photograph large (>4 mm) and small (≤4 mm) cNFs in 3 body regions. Calipers were used to calculate the cNF volume (length x width x height). Percent volume change from baseline was calculated at each restaging and averaged for all cNF. Participants also completed the Skindex-29, a patient-reported outcome (PRO) measure to assess quality of life (QOL) in adults with skin conditions, after cycle 1 and at each restaging to evaluate clinical benefit. Higher scores indicate worse QOL. Adverse events (AEs) were graded using CTCAE v5.0.

Results: 11 participants enrolled (median age=54 years; range=28-75; male=5) out of a total target of 16. The study was terminated early due to poor enrollment, primarily due to COVID-19. 209 total cNF tumors were evaluated (small=140, large=69) at 3 different body sites (back=39.7%, abdomen=33%, extremity=27.2%). The median duration of treatment was 9 cycles (range=1-24). Five participants (45%) had a drug-related AE requiring a drug hold (2 taken off study), with one requiring a dose reduction from 75 to 50 mg after cycle 20. All participants experienced ≥1 AE, with a total of 650 AEs reported, including AEs which resolved but recurred (80.7% grade 1, 18.6% grade 2, 0.6% grade 3 and 4). Most AEs were grade 1/2 (99.3%) and the most common AEs were acneiform rash or dry skin (37.5%), gastrointestinal events (14%), increased CPK (8.1%), and fatigue (6.5%). The grade 3 and 4 AEs were drug reaction with eosinophilia and systemic symptoms and hypertension, respectively. For those with a post-cycle 4 evaluation (n=7 participants;126 cNF), average volume reduction was -17.9% (range= –1.9% to –43.1%). For those who completed 12 cycles (n=6 participants; 103 cNF), average volume reduction was -36.7% (range= –8.3% to -81.4%). Skindex-29 median Emotion domain scores improved ten points from baseline after cycle 1 (p=0.0254) but no other statistically significant differences were observed.

Conclusion: The participants treated with selumetinib had some cNF volume reduction, similar to percent volume reduction in PN trials. However, all experienced at least one drug-related AE and it is not clear if the degree of cNF volume reduction led to clinical benefit. This study highlights the challenges of treating cNF with a medication that often causes cutaneous and other AEs. In future studies, enrolling a larger and diverse cohort would help to improve the generalizability of these results to the broader NF1 population.

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Non-Invasive Treatment of Cutaneous Neurofibromas (cNF): Results of a Prospective, Direct Comparison of Four Methods

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Purpose: We assessed the safety, tolerability, and tumor response of four different treatment modalities commonly used in dermatology applied to cNF.

Methods: 19 adults with an adequate number of small (2-4mm) cNFs (3-10 cNFs treated per modality) were recruited. The modalities tested are: (1) electrocautery with an insulated radiofrequency needle, (2) 755nm alexandrite laser with negative pressure (8mm spot size, 100J/cm2 fluence, 3ms pulse duration), (3) 980nm diode laser (delivered via 8mm sapphire skin-contact window) and (4) intratumoral injection of 10mg/mL deoxycholic acid (Kybella®) at a volume approximately equal to that of the tumor. Topical anesthetic (5% lidocaine/prilocaine) was applied for 40 minutes before treatment. Tumors were randomized to treatment versus control. At baseline, 3 and 6 months after treatment, tumor height and volume (via 3D Cherry Imaging®), clinician assessment of tumor clearance, adverse events (AEs) including inflammation, pigmentation, and scarring were assessed. Biopsies were obtained from 10 subjects 3 months after treatment.

Results: All 19 participants treated (24-70 years old, average age of 49; 4M/15F) have now completed the 6-month assessment. A total of 307 cNFs were treated. No adverse events (AE) >grade 2 occurred in any group (**Table 1-2**). Mild (grade 1 or 2) AEs in the form of pain, erythema, bruising and swelling were noted for all modalities. Pain during treatment was reported in 21-47% participants across all modalities and resolved rapidly with no pain reported 1 month post-treatment. Erythema was observed in 73-100% participants across all modalities on the day of treatment, improved at 1 month post treatment and resolved by 6 months post treatment. At 6 months, mild hypopigmentation was still present in 26% of the subjects treated with the alexandrite laser. All modalities reduced some cNFs by 6 months post-treatment, with large variation between tumors and between participants (**Table 3-4**). When residual cNF was present histologically, its appearance was similar to that of control cNFs. Mild fibrosis was present at the sites of prior tumor. Moderate to severe pain limited the 980nm laser treatment dose, and this treatment produced the least amount of tumor reduction in most participants. The alexandrite laser was only mildly painful and caused immediate gray-blue purpura localized to the tumor. In some participants, it led to complete tumor clearance without scarring or pigment abnormality. Deoxycholate injection pain was minimal, and the resultant cNF reduction ranged from none to nearly complete. Pain during RF coagulation was mild, though more than deoxycholate or alexandrite laser. Tumor reduction associated with this modality appeared to correspond to the coagulation zone. No cNF growth stimulation or recurrence has been noted with any modality.

Conclusions: Effective, well-tolerated treatment of small cNF without surgery is feasible. All four modalities assessed were safe and demonstrated at least some degree of efficacy. Based on this data, follow-up pre-clinical and clinical studies to optimize the dosing and dose frequency of alexandrite laser and deoxycholic acid are underway.

Table 1. Adverse events across modalities

	Deoxycholate injection (% immediately post-treatment/% 6 months post-treatment)	RF Coagulation (% immediately post-treatment/% 6 months post-treatment)	Alexandrite laser (% immediately post-treatment/% 6 months post-treatment)	980nm laser (% immediately post-treatment/% 6 months post-treatment)
Erythema				
None	0/100	26.3/100	15.8/100	22.2/100
Mild	84.2/0	68.4/0	10.5/0	72.2/0
Moderate	15.8/0	5.3/0	73.7/0	5.6/0
Edema				
None	10.5/100	63.2/100	52.6/100	72.2/100
Mild	89.5/0	36.8/0	42.1/0	27.8/0
Moderate	0/0	63.2/0	5.3/0	0/0
Purpura				
None	84.2/100	94.7/100	52.6/100	77.8/100
Mild	10.5/0	5.3/0	26.3/0	22.2/0
Moderate	5.3/0	0/0	21.1/0	0/0
Hyperpigmentation				
None	94.7/94.7	100/94.7	100/94.7	100/94.4
Mild	5.3/5.3	0/5.3	0/5.3	0/5.6
Hypopigmentation				
None	94.7/100	100/94.7	100/73.7	100/100
Mild	5.3/0	0/5.3	0/26.3	0/0
Blistering				
None	94.7/100	100/100	100/100	100/100
Mild	5.3/0	0/0	0/0	0/0
Ulceration				
None	100/100	100/100	94.7/100	100/100
Mild	0/0	0/0	5.3/0	0/0

Table 2. Subject-reported intraoperative pain scores across modalities

	Deoxycholate injection	RF Coagulation	Alexandrite laser	980nm laser
Pain score (0-10 +/- standard deviation)	**1.9** +/- 1.8	**3.2** +/- 2.2	**1.9** +/- 2.0	**5.7** +/- 2.1

Table 3. Changes in height and volume across modalities

	Deoxycholate injection (mean % change from baseline +/- standard deviation)	RF Coagulation (mean % change from baseline +/- standard deviation)	Alexandrite laser (mean % change from baseline +/- standard deviation)	980nm laser (mean % change from baseline +/- standard deviation)
Height (3 months)	Treated: -23.9 +/- 30.3 Control: +7.1 +/-28.9	Treated: -23.8 +/- 41.8 Control: +4.7 +/-22.7	Treated: -26.6 +/-28.4 Control: +2.6 +/- 31.4	Treated: -18.8 +/-34.1 Control: +0.4 +/-23.2
Height (6 months)	Treated: -30.0 +/- 27.8 Control: +3.8 +/- 22.6	Treated: -21.8 +/- 39.1 Control: +3.5 +/- 22.8	Treated: -26.4 +/- 24.9 Control: -2.7 +/- 37.2	Treated: -12.9 +/- 30.6 Control: +1.6 +/- 24.9
Volume (3 months)	Treated: -21.4 +/- 35.0 Control: +3.6 +/- 31.8	Treated: -21.7 +/- 42.9 Control: -0.4 +/- 23.0	Treated: -31.2 +/- 24.2 Control: +1.8 +/- 25.9	Treated: -24.5 +/- 29.2 Control: -3.8 +/- 24.5
Volume (6 months)	Treated: -29.4 +/- 30.3 Control: -3.7 +/- 25.4	Treated: -23.3 +/- 39.3 Control: -0.8 +/- 21.2	Treated: -33.4 +/- 23.8 Control: -5.1 +/- 30.1	Treated: -24.9 +/- 27.8 Control: -9.2 +/- 23.0

Table 4. Physicians' assessment of tumor response across modalities

	Deoxycholate injection	RF Coagulation	Alexandrite laser	980nm laser
Improvement in tumor appearance at 6 months as compared to baseline (scale 0 [no change] to 6 [very large improvement] +/- standard deviation)	Treatment: **3.7** +/- 2.0 Control: 0.0 +/- 0.0	Treatment: **2.8** +/- 2.6 Control: 0.2 +/- 0.7	Treatment: **4.0** +/- 1.5 Control: 0.2 +/- 0.7	Treatment: **2.2** +/- 2.4 Control: 0.2 +/- 0.7

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Differentiation of Peripheral Nerve Sheath Tumors in NF1 - Intraindividual Comparison of the Diagnostic Accuracy of Diffusion-Weighted MRI and ^{18}FDG-PET/CT

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Purpose: The purpose of this study was to compare the diagnostic accuracy of diffusion-weighted MRI and fluorodeoxyglucose positron emission tomography (^{18}FDG-PET/CT) for the differentiation of benign vs. atypical and malignant peripheral nerve sheath tumors in patients with neurofibromatosis type 1 (NF1).

Methods: Thirty-four NF1 patients (20 male; 31 ± 11 years) underwent both diffusion-weighted 3T MRI (0-800 s/mm^2) and ^{18}FDG PET/CT. Two radiologists independently assessed the mean diffusion coefficient (ADC_{mean}) and maximum standardized uptake value (SUV_{max}) in 39 benign, 11 atypical, and 16 malignant nerve sheath tumors. The diagnostic reference standards included follow-up of ≥24 months for benign tumors or histopathological evaluation for atypical and malignant tumors. By comparing "benign vs. atypical/malignant tumors", ROC analysis was performed to determine whether the AUC of ADC_{mean} was non-inferior to the AUC of SUV_{max}. The non-inferiority margin was set at -10%. Diagnostic cut-off values were set considering best possible specificity while still maintaining good sensitivity.

Results: Compared with the BPNST, ANF and MPNST were characterized by lower mean ADC_{mean} and higher SUV_{max} values (ANF: ADC_{mean} 1.7x10^{-6} mm^2/s, SUV_{max} 5.9; MPNST: mean ADC_{mean} 1.2x10^{-6} mm^2/s, mean SUV_{max} 11.5; BPNST: ADC_{mean} 2.1x10^{-6} mm^2/s, SUV_{max} 2.9). The AUC for ADC_{mean} was 91.6% (95%-CI: 79.6-96.9%) and for SUV_{max} it was 94.0% (95%-CI: 84.0-97.9%). Non-inferiority of ADC_{mean} in comparison with SUV_{max} could not be shown because the lower limit of the confidence interval of the difference of both AUCs (-2.4%, 95%-CI: -12.9-8.2%) was below the non-inferiority margin of -10% (**Figure 1**). Using an ADC_{mean} cut-off value of 1.6 × 10^{-3} mm^2/s corresponded to a sensitivity of 85.3% and a specificity of 93.3%. Using a cut-off value of 5.0 for SUV_{max} achieved a sensitivity of 85.2% and specificity of 92.3%.

Conclusions: Both diffusion-weighted MRI and 18FDG-PET/CT prove to be helpful diagnostic tools for the differentiation of peripheral nerve sheath tumors in NF1. Nevertheless, diffusion-weighted MRI cannot be considered an equivalent method to FDG-PET/CT in this single-center study.

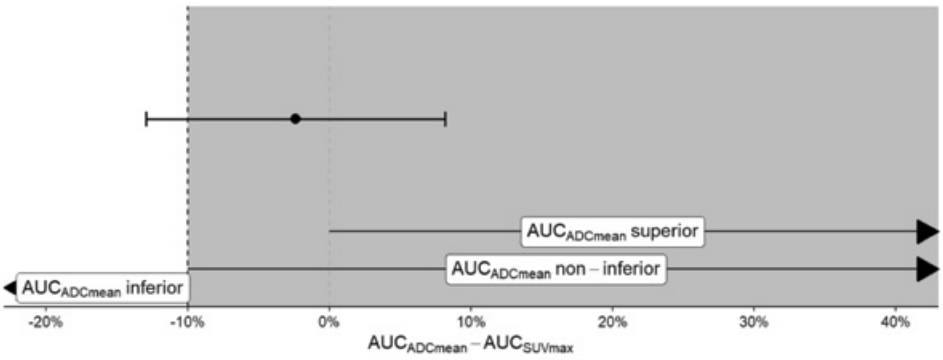

Fig. 1: Comparison of diagnostic performance of diffusion-weighted MRI and ^{18}FDG-PET/CT. The positive non-inferiority margin is 10% (grey marked area). The difference between ADC_{mean} and SUV_{max} is -2.37% (-12.91%-8.17%) (Box-and-Whiskers-Plot). We can't show non-inferiority of the AUC of ADC_{mean} in comparison with the SUV_{max} because the lower limit of the confidence interval is lower than the negative non-inferiority margin of -10%.

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Café Au-Lait Macules: Beyond What Meets the Eye

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Purpose of the study: CALM are common cutaneous findings and common clinical referral and concern for Neurofibromatosis 1 (NF1). However, NF1 has poor phenotypic expressivity in the early years and there are numerous conditions that can mimic its appearance[1].

Introduction: CALM can be isolated or a sign of a syndromic entity[2]. It is mostly NF1- associated. Lesions have been described as flat, ovoid, hyperpigmented birthmarks, with well-defined borders. CALM can be present since birth or increase in size and number during the first decade of life. However, they can be found in many other tumor predisposition syndromes that can mimic their appeareance[3].

Methods: A 7yo young female was assessed clinically for CALM concerning for NF1. Her medical records and laboratory tests results were reviewed.

Results: Patient presented with 5 significant CALM, ranging in size from >0.5cm to 4cm without other dermatological sign and no family history of NF1. She had a normal neurological examination. Patient had a history of recurrent fever, thrombocytopenia, leukopenia and positive ANA. She had a *NF1* genetic test, which reported VUS for *NF1:* c.5230A>G p. (Thr1744Ala) ((T1744A)). No conclusive and unlikely to be associated to NF1. Subsequently, WES was performed, reporting the presence of a compound heterozygous for two pathogenic variants in the *FANCA* gene: c.2278+1 G>A p.? and c.3763 G>T p.(E1255*), consistent with FANCA-related Fanconi Anemia (FA), for which she is currently being managed.

Conclusion: The case shown here highlights the importance of a full clinical assessment and knowledge of the differential diagnosis for CALMs. Importantly, this patient first presented with an important medical history concerning for hematological disorder with ANA (+). The VUS for NF1 created a diversion, however upon assessment, clinical criteria were not met for NF1. Therefore, differential diagnosis for a patient with CALM and hematological disorder prompted the request for WES. This will better help medical practitioners to recognize at earlier stages and start critical treatment, in potential life-threatening conditions. FA is a rare Autosomal Recessive disorder involved in DNA damage repair, with potential bone marrow failure, hematologic and solid organ malignancies. Most remarkable physical features include short stature and radial ray abnormalities. Cutaneous signs associated to FA can be found in >90% of patients, characterized as faint, ill-defined CALM, hypopigmented skin-fold freckle-like macules, hypo/hyperpigmented macules[4]. Therefore, NF1 clinical diagnostic efficiency in childhood could be difficult and should be confirmed with genetic testing.

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Post-Authorization Safety Study (PASS) of Pediatric Patients Initiating Selumetinib Treatment for Neurofibromatosis Type 1 with Symptomatic, Inoperable Plexiform Neurofibromas: A Multiple-Country Prospective Cohort Study

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Introduction: Selumetinib (ARRY-142886, AZD6244), a selective inhibitor of mitogen-activated protein kinase kinases 1 and 2 (MEK1/2), was approved by the European Medicines Agency (EMA) for the treatment of symptomatic, inoperable plexiform neurofibromas (PN) in pediatric patients (aged ≥3 years) with neurofibromatosis type 1 (NF1). EMA approval of selumetinib was based on results of the SPRINT trial.[1,2] However, at the time of approval, long-term safety of selumetinib had not been fully characterized. This European post-authorization safety study (PASS) is a non-interventional, multiple-country, prospective, cohort study (D1346R00004, NCT05388370), which aims to characterize the safety profile (including long-term safety) of selumetinib in pediatric patients with NF1 and symptomatic, inoperable PN. The study also aims to fulfill the EMA conditional marketing authorization.

Methods: The target population for this PASS is patients with NF1-associated symptomatic, inoperable PN in the European Union (EU) and the United Kingdom (UK), who have been prescribed at least one dose of selumetinib, and who are aged 3 to <18 years at the start of selumetinib treatment. Patients who received treatment with other MEK inhibitors, and those currently participating in a randomized trial are not eligible for the study. Two cohorts will be enrolled: the base cohort will include all patients aged 3 to <18 years and the nested prospective cohort (NPC) will include a subset of the base cohort of patients aged 8 to <18 years who have not reached Tanner Stage V at the start of selumetinib treatment. The primary objective is to characterize the occurrence of the safety outcomes of interest in the NPC. Safety outcomes of interest include left ventricular ejection fraction reduction, physeal dysplasia, myopathy, hepatotoxicity, ocular toxicity, and abnormal pubertal development. The secondary objective is to describe the demographics and clinical characteristics of patients in the base cohort. Baseline data will be retrospectively collected from medical records within one year prior to selumetinib initiation. Follow-up will be up to 5 years from enrollment date of the first patient. Patients in the NPC who discontinue selumetinib will remain in the study for follow-up safety assessments. This PASS aims to enroll 125 patients across the EU and UK; the study is ongoing and currently enrolling.[3,4] The enrollment period will last 2 years from date of first patient enrolled (May 23, 2022).

Conclusion: This PASS aims to characterize the long-term safety profile of selumetinib, including developmental effects on children with NF1 and symptomatic, inoperable PN.

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Disclosures: TR has received support for attending meetings and/or travel from Alexion, AstraZeneca Rare Disease, the German Society for Pediatric Neurology, and the German Society for Pediatrics; as well as payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or educational events from Alexion, AstraZeneca Rare Disease, the Taiwan Society for Pediatric Neurosurgery, and the LA-med Publishing Group. TR has participated on a Data Safety Monitoring Board or Advisory Board for Alexion, AstraZeneca Rare Disease and holds unpaid leadership roles at the German Society for Pediatric Neurology and the German NF Working Group. TR has also received consulting fees from Alexion, AstraZeneca Rare Disease, and TR's spouse owns Lonza Group stocks. JP received consulting fees, payment or honoraria and support for attending meetings and/or travel for lectures, presentations, speaker bureaus, manuscript writing or educational events from Alexion, AstraZeneca Rare Disease. JP has also participated on a Data Safety Monitoring Board or Advisory Board for Alexion, AstraZeneca Rare Disease. LM received consulting fees from Alexion, AstraZeneca Rare Disease. AM, VO, RA and CD report employment at Alexion, AstraZeneca Rare Disease. IB received consulting fees and support for an advisory board from Alexion, AstraZeneca Rare Disease. IB's institution also received support from Alexion, AstraZeneca Rare Disease during the conduct of this study.

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MEK Inhibition in NF1-Associated Plexiform Neurofibromas: 5-Years Experience in a Tertiary Treatment Center in Germany

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Purpose: In August 2021 the MEK inhibitor (MEKi) selumetinib was approved by the European Medicines Agency (EMA) as medical treatment for symptomatic, inoperable plexiform neurofibromas in NF1 patients aged 3-18 years. Here, we summarize our experience with this novel therapy in 25 NF1 patients treated at our institution.

Methods: NF1 patients were selected for MEKi therapy by a multidisciplinary team (MDT) consisting of neuropediatricians, pediatric oncologists, neurosurgeons and neuroradiologists. After informed consent was obtained patients received baseline laboratory tests, fundoscopy, echocardiography and a semi-automated 3-D-MRI of the plexiform neurofibroma. Oral selumetinib therapy was started at the recommended dose of 25 mg/m^2 BSA bid. Blood tests were repeated monthly, echocardiography and fundoscopy were performed every 2-3 months. After 6 months of continuous therapy a first 3-D-MRI was performed to evaluate tumor volume changes. Thereafter, MRI was repeated every 6-12 months. Patients presented in person in our NF center for physical check-up and evaluation of therapy related side effects every 6 months. In between, notification of therapy related issues was done by email or phone contact.

Results: 25 NF1 patients (17 male, 8 female) were treated with selumetinib or trametinib, respectively. Patients' age ranged from 3 months to 17 years (average 8.7 years). 8 patients were switched from a previous off-label treatment with trametinib, 15 patients started with selumetinib, 2 patients remained on trametinib. 21 patients were eligible for the first MRI evaluation after 6 months of therapy, data were available for 17 of these patients. 9/17 patients showed a partial response, tumor progression was observed in 2/17 patients. 6/17 patients showed a non-significant tumor volume change. Median tumor volume reduction was 26.8 $\pm$13.8 % after 6 months of therapy. Thereafter, one patient experienced tumor regrowth while on selumetinib and after significant previous tumor shrinkage. All other patients showed continuous tumor shrinkage or stable disease. Maximum volume reduction was 56%. All patients with tumor size reduction reported positive effects on individual patient related outcomes. Therapy related side effects were observed in 19/25 patients (76%) and mainly consisted of skin toxicity and mild gastrointestinal symptoms. It appeared that selumetinib was better tolerated in young children while severe side effects mainly occurred in adolescents.

Conclusions: Treatment of NF1-associated plexiform neurofibromas with selumetinib leads to robust tumor volume reduction in the majority of patients. Generally, therapy related side effects are mild and manageable and appear to occur less frequently in young children.

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Disclosures: TR received honoraria and travel reimbursement from Alexion for advisory boards and talks. PV received honoraria and travel reimbursement from Alexion for talks

Assessing the Economic Impact of a Multidisciplinary Neurofibromatosis Clinic in a Canadian Tertiary Care Hospital: A Protocol

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The main objective of this study protocol is to assess the economic impact of implementing a multidisciplinary neurofibromatosis clinic in a Canadian tertiary care hospital. The secondary objectives of the project are to assess the impact of our clinic on generic and disease-specific quality of life measures as well as employment.

We will perform a cost-utility analysis of the *Centre Hospitalier de l'Université de Montréal* (CHUM)'s *Centre d'expertise en neurofibromatose,* a newly established multidisciplinary neurofibromatosis clinic and the only of its kind in eastern Canada. Patients with neurofibromatosis type 1 (NF1) and type 2 (NF2) visiting our clinic will be filling out questionnaires every six months for a minimal duration of two years. Using administrative databases and scores from two questionnaires, an institutional one and the Costs for Patients Questionnaire (CoPaQ), we will first assess the costs associated with our clinic. Scores from the Short-Form Six-Dimension (SF-6D) questionnaire will be converted into quality-adjusted life years (QALYs). By dividing costs by QALYs gained over the two-year period, we will obtain the cost-utility ratio associated with our clinic. Changes in quality of life over time will be assessed using the SF-6D, Skindex 29, and the Penn Acoustic Neuroma Quality of Life (PANQoL). We will monitor employment using the Work Productivity and Activity Impairment General Health questionnaire (WPAI-GH).

To our knowledge, this is the first economic assessment of a multidisciplinary neurofibromatosis clinic. Calculating a favorable cost-utility ratio will help justify this clinic model in countries with publicly funded health care systems such as Canada. Using patient-reported outcomes, a longitudinal assessment of quality of life will inform us on the true impact of our clinic on patients' lives. However, a two-year period is too short to assess the impact of multidisciplinary and preventive care on long-term complications such as malignancies. We are currently building a comprehensive neurofibromatosis database which will allow us to answer these questions in the future.

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Usefulness of Photographic Register to Quantify Skin Neurofibromas in Individuals with Neurofibromatosis Type 1 (NF1)

Juliana Souza, MD, PhD, *Outpatient Neurofibromatosis Reference Center (CRNF), Federal University of Minas Gerais, Brazil*

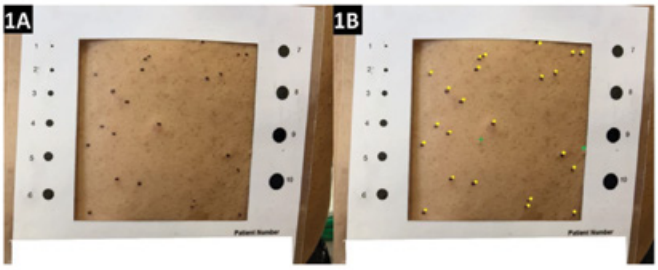
Background: Skin neurofibromas (SNF) are an esthetical and psychological burden for most individuals with NF1. Quantifying SNF in number and size is central for clinical studies, to follow up their natural history and possible effects of new drugs. A recent method validated the direct counting of SNF, using paper frames (PF), as an accurate predictor of the total number of these tumors in individuals with NF1. Our aim was to compare direct and photographic quantification of SNF, using PF, to verify the usefulness of an exclusive photographic recording method.

Methods: 54 individuals with NF1 were invited and agreed to participate. A 100 cm^2 PF was positioned at the back (just below the shoulder blades), upper left abdomen, and the middle anterior region of the left thigh and left forearm to delimit the analyzed area. All cutaneous and subcutaneous neurofibromas identified in the respective locations were marked with a washable pen, then counted and recorded. Each of the delimited locations was then photographed, with a high-resolution camera and in good lighting conditions. The images were then analyzed on a computer by the same researcher and recounted, accounting for any SNF that had not been verified at in person assessment (**Figures 1A and 1B**). SNF counts performed in person and by photograph were then compared using Pearson's correlation coefficient. Age, sex and skin color (Fitzpatrick scale) were also recorded.

Results: In this sample, 42 (77.8%) were female and 12 (22.2%) were male, with a median age of 28 years (ranging between 7 and 60 years), with a median age of 30 (18-39) years for women and 19.5 (11-35,5) years for men. Regarding skin color, volunteers were classified as follow by Fitzpatrick scale: FPI 0 (0%), FPII 7 (13%), FPIII 12 (22.2%), FPIV 28 (51.8%), FPV 6 (11.1%), FPVI 1 (1.9%). The median number of SNF/100 cm^2 of skin on all four locations was 26 (2-171) in women and 1(0-50) in men. Older individuals had a higher number of neurofibromas ($r = 0.72$; $R^2=0.52$) (**Graphic 1**). The quantification of SNF through photography proved to be consistent, showing a good intra examiner correlation with the direct in person count ($r=0.997$; $R^2=0.995$).

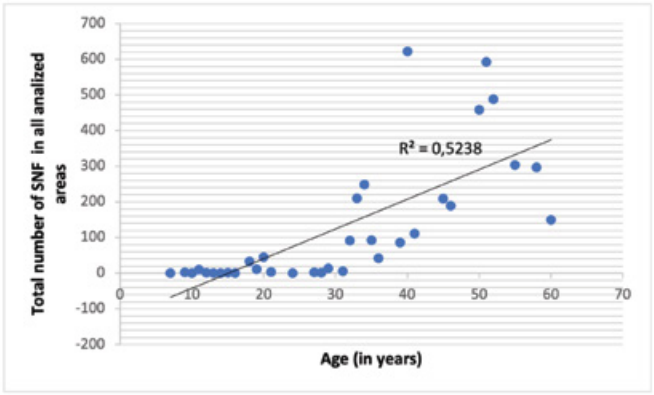
Conclusions: Exclusive photographic register method with PF has the potential to allow safe, comfortable, ethical, and more practical assessment and follow up of SNF for research purposes as for clinical assistance of individuals with NF1.

Figures 1A and 1B



Figures 1A and 1B: Quantification of SNF using a paper frame in the abdomen. 1A: SNF identified in the direct manual count (in person) marked with a black pen. 1B: SNF also counted through photographs on a computer marked in yellow. SNF identified only through photographs are marked in green.

Graphic 1



Graphic 1: Scatterplot of the total number of SNF counted on the paper frame area of the back, abdomen, thigh and forearm versus age. Abbreviation: R^2=coefficient of determination

Full List of Authors: Cotrim MA, Oliveira S de C, Souza JF, Rodrigues LO, Rezende NA, Cota BCL, Rodrigues LOC.

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Complete Resection of an Atypical Neurofibroma Prevents Further Progression to Malignancy

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Purpose: Plexiform neurofibromas are the hallmark of neurofibromatosis type 1 (NF1) and significantly contribute to the overall burden of disease in NF1 patients. While surgical excision has long been the only available therapy the MEK inhibitor (MEKi) selumetinib has been approved as non-surgical treatment option for these tumors in 2020 (US) and 2021 (Europe), respectively. However, selumetinib will result in tumor shrinkage only after several months of therapy and might not prevent malignant transformation of a plexiform neurofibroma that occurs with a frequency of 10-15%. Here, we demonstrate that surgical excision might be the therapy of choice in some plexiform neurofibromas despite the availability of MEKi therapy.

Methods: A 16 year old girl with NF1 and a plexiform neurofibroma of the neck was referred to our NF clinic with the specific request for a MEKi therapy. The plexiform neurofibroma had appeared two years ago and had slowly grown to its actual size. We describe the clinical, ultrasound and MRI findings that prompted us to recommend surgery instead of the requested MEKi therapy. Postoperative histopathological and molecular analysis of the neurofibroma confirmed that surgical excision was the therapy of choice in this case.

Results: The plexiform neurofibroma appeared as a tender and hard swelling with a diameter of 10 cm at the right neck. High resolution ultrasound showed a highly vascularized, inhomogeneous tumor with enhanced echogenicity. MRI confirmed the inhomogeneous structure of the neurofibroma. Since all these findings were suggestive of an ANNUBP (atypical neurofibromatous neoplasm of uncertain biological potential) surgical resection instead of MEKi therapy was recommended after discussion in a multidisciplinary team (MDT). After complete removal of the tumor histopathological and molecular workup demonstrated an increased cellularity in about 10% of high power fields (HPF) with loss of p16 but no CDKN2A/B deletion or p53 mutation. 90% of HPF showed typical neurofibroma tissue. Follow-up MRI 6 months after surgery confirmed complete tumor removal without signs of local recurrence.

Conclusion: Although MEKi therapy is now readily available as non-surgical treatment option for plexiform neurofibromas clinical, ultrasound and MRI findings suggestive of an ANNUBP might indicate that surgical excision is the recommended therapy in these cases. If feasible surgery will result in immediate relief and prevent further progression to malignancy.

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Development of a Large Crowdsourced International Database to Study How Variations in Cutaneous Neurofibromas Impact Quality of Life

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Neurofibromatosis type 1 (NF1) is a rare genetic disorder affecting 1 in 3000 individuals. It is characterized by a high propensity to develop skin, eye, nervous system tumors and cutaneous neurofibromas (cNFs), which occur in the majority of individuals with NF1. cNFs are a major cause of morbidity and social anxiety. While there is a vast array of phenotypic differences in individuals with cNFs, little is known about the factors that modulate cNF development or how differences in cNFs impact the quality of life (QoL) in people with NF1. Photo databases can play an integral role in understanding the specific effects of variability in NF1 and other skin diseases. We have developed a large crowdsourced international clinical and photographic platform, partnering with NF1 organizations and advocacy groups, to better understand how variations in NF1 manifestations affect QoL. Between May 2021 to July 2022, photos and surveys were collected from 366 adults with NF1 and photos were rated to analyze 12 skin characteristics, such as number, color, and size of cNFs. Most participants (37%) had between 101-500 cNFs, 57% reported itching, and 62% reported pain. The cohort highlighted the serious impact of cNFs on QoL with an average cNF-Skindex score of 48.9 (out of 108). Increasing number of total cNFs and facial cNFs were associated with decreasing QoL, affecting emotions, symptoms, and function, and thus increasing cNF-Skindex score. Individuals with 10 or fewer cNFs had an average score of 20, individuals with 11-100 cNFs had an average score of 48, and individuals with 101-500 cNFs had an average score of 52. However, individuals with a cNF number greater than 500 did not report a lower QoL, proposing that negative impact of cNFs may reach its maximum effect on QoL at 500 cNFs. Individuals with globular or pedunculated cNFs reported increased itch compared to those with sessile or flat cNFs, suggesting that the subtype of cNF is also related to symptoms. These data highlight the features of adults with NF1 and cNF tumors that impact QoL, providing insight into which individuals and cNF tumors may benefit most from therapies. The data also underscore the significance of photo databases to the field of dermatology and its contribution to data sharing in the future.

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A Phase 1 Study to Assess the Effect of Food on the Pharmacokinetics (PK) and Gastrointestinal (GI) Tolerability of Selumetinib in Adolescents with Neurofibromatosis Type 1 (NF1)-Related Plexiform Neurofibromas (PN)

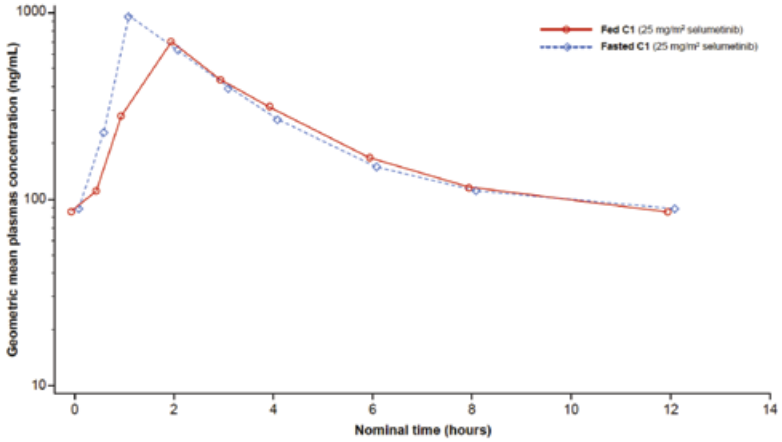
David Viskochil, MD, PhD, *Department of Pediatrics, University of Utah, Salt Lake City, Utah*

Purpose: Selumetinib (ARRY-142886, AZD6244) is a MEK1/2 inhibitor approved for pediatric patients with NF1 and symptomatic, inoperable PN in regions including Europe and the USA (EMA, aged ≥3 years; FDA, aged ≥2 years). Based on single-dose food-effect (high-fat and low-fat meal) studies in adults,[1–3] selumetinib is dosed in a fasted state (2 hours pre- and 1 hour post-dosing twice daily). This Phase 1 study (NCT05101148) evaluated the effect of a low-fat meal on steady-state exposure and GI tolerability in adolescents with NF1 and inoperable PN to confirm a recommendation for dosing selumetinib with food.

Methods: Eligible patients aged ≥12 to <18 years received selumetinib 25 mg/m^2 twice daily for 1 cycle (28 days) with a low-fat meal according to FDA guidance[4] (T1; fed C1), then in a fasted state for 1 cycle (T2; fasted C1) following 1-week washout. T2 continued beyond 1 cycle until study end or dose-adjusted selumetinib in a fed state (T3), if required. Primary endpoints were $AUC_{0–12,ss}$, GI adverse events (AEs; CTCAE v5.0), GI patient-reported outcomes (PROs), and GI concomitant medications in fed vs fasted state. Secondary endpoints included other PK parameters and safety.

Results: Overall, 24 participants received selumetinib (safety population); 19 were evaluable for fed vs fasted paired PK comparison. Mean duration of actual exposure for fed and fasted states was 28.3 (standard deviation [SD] 1.1) and 28.3 (SD 3.0) days, respectively. Steady-state extent of absorption was similar for fed vs fasted ($AUC_{0-12,ss}$ geometric mean ratio [GMR] 0.919; lower 1-sided 90% confidence interval [CI] bound 0.841). Fed-state maximum serum concentration (C_{max}) was reduced (GMR 0.763; lower 1-sided 90% CI bound 0.667) and time to C_{max} was delayed (34 minutes; 90% CI 28, 60; **Figure**) vs fasted state. Similar findings were observed for N-desmethyl selumetinib metabolite. Similar proportions of participants experienced GI AEs (fed C1, 29.2%; fasted C1, 33.3%). GI PROs were consistent between fed C1 vs fasted C1. Overall use of concomitant GI medications was low. No new safety signals were identified. No patients in either state experienced grade ≥3 or serious AEs.

Figure. Geometric mean plasma concentration–time profiles following administration of selumetinib 25 mg/m^2 twice daily in Cycle 1 of fed and fasted states



Conclusions: At steady state, dosing selumetinib with a low-fat meal delayed absorption rate and lowered C_{max} but had no clinically significant effect (<30% $AUC_{0–12,ss}$ reduction) on the extent of absorption. There was no clinically significant impact on GI tolerability. Therefore, a dose adjustment is unlikely required. This study shows that dosing selumetinib with a low-fat meal has no significant impact on selumetinib AUC nor GI tolerability.

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- FDA recommendations for a low-fat meal are 400–500 calories with 25% calories from fat (e.g. 8 oz milk, a boiled egg, and instant oatmeal)

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Observational Registry Study of Treatment Practices and Long-Term Outcomes of Children with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PN) Initiating Selumetinib in Real-World Practice in the United States (US): Study Design and Methodology

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Purpose: Selumetinib (ARRY-142886, AZD6244) is an oral, allosteric MEK1/2 inhibitor approved in the US for children aged ≥2 years with NF1 and symptomatic, inoperable PN, based on results from the pivotal SPRINT study. Aims of the US Selumetinib Registry study [NCT05683678] include understanding treatment practices and assessing short- and long-term safety and effectiveness outcomes of selumetinib treatment in children with NF1-PN in real-world US practice. Clinical and non-clinical factors affecting outcomes will also be explored.

Methods: This observational registry study of pediatric patients with NF1-PN will be conducted in up to 22 US centers. Eligible patients will be 2–18 years old at the time of selumetinib initiation (on/after April 10, 2020), and not currently participating in a clinical trial. Patients will be divided into three cohorts: Cohort 1 – treatment discontinued before enrollment, Cohort 2 – treatment initiated before enrollment and currently on treatment, Cohort 3 – treatment initiation intended within 3 months of enrollment. Patients will be followed for ≥36 and up to 60 months. Primary objectives are to describe patient demographics and disease characteristics (including diagnosis criteria and related manifestations, diagnostic tests and results, and PN-related morbidities), selumetinib treatment course, short- and long-term effectiveness and safety, and disease course and treatment following discontinuation. Key secondary objectives include measures of quality of life, pain and physical functioning before, during, and after selumetinib. Target enrollment is 200 patients with a 24-month enrollment period and will begin in 2023.

Conclusion: The US Registry study will facilitate understanding of treatment practices and assess short and long-term outcomes of selumetinib for NF1-PN in a real-world setting.

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Disclosures: AW received consulting fees for scientific advisory boards from Alexion, AstraZeneca Rare Disease and Day One Biopharmaceuticals and has participated on a data safety monitoring board or advisory board for the Lurie Cancer Center DSMC without compensation. AW received payment or honoraria for a grant review from the National Institutes of Health and has received support for travel to a meeting from Minderoo. AW also holds a paid leadership or fiduciary role in the Children's Brain Tumour Network. KY holds a leadership or fiduciary role in the Department of Defense Congressionally Directed Medical Neurofibromatosis Research Program and has received payment for a consulting or advisory role from Alexion, AstraZeneca Rare Disease. SF and RR report employment at Alexion, AstraZeneca Rare Disease as well as ownership of AstraZeneca stocks. SW reports employment at AstraZeneca and owns AstraZeneca stocks. MB received payment for a consulting or advisory role from Alexion, AstraZeneca Rare Disease, and received payment or honoraria for lectures or educational events from AstraZeneca. MB has also received support for attending meetings and/or travel from Bionano Genomics. JM received payment for a consulting or advisory role from Alexion, AstraZeneca Rare Disease; payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing or educational events from the American Society of Pediatric Oncology and support for attending meetings and/or travel from the Children's Oncology Group. KV received payment for a consulting or advisory role from Alexion, AstraZeneca Rare Disease and received payment or honoraria for a speaker bureau from Alexion, AstraZeneca Rare Disease.

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MPNST Clinical and Immunohistochemistry Data: 33 Years' Experience at the University of Florida

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The goal of this study was a retrospective clinical analysis of MPNST cases (1988 - 2021) seen at the University of Florida College of Medicine, along with an immunostaining study of sections from these cases.

Past MPNST cases were identified (1988 - 1921), and clinical data were obtained from paper and electronic chart records as approved by the UF Institutional Review Board (IRB). The data included demographics, presence/absence of NF1, tumor grade, anatomical location, use of chemotherapy and/or radiation therapy (before and/or after any surgery), type of surgery, whether there was local recurrence and/or metastasis (and time until such, after diagnosis), and survival (months from diagnosis to last follow-up or death). In addition, archived FFPE sections were obtained from nearly all of the cases. These were stained for H&E, and immunostained for S100B (the loss of which correlates with higher tumor grade), H3K27me3 (absence of which is thought to correlate with higher tumor grade), and HMGA2. This last marker is a non-histone chromatin factor whose increased expression has recently been reported in a number of tumor types, and has been hypothesized to be a potential therapeutic target in MPNST and correlating with survival (Yang et al., 2019). Immunohistochemistry controls included neurofibromas. Statistical analysis is underway, to test for clinical variables' relationship to survival, and for relationship of immunostaining data with tumor characteristics. Survival analysis will exclude cases diagnosed less than 5 years ago.

A total of 57 MPNST cases were included in the study. There were 33 males, and 24 females. Clinical records reported that 37 were white, 13 Black/African-American, and 7 Hispanic. 34 patients had an NF1 diagnosis (average age at MPNST diagnosis early 40s), 21 did not have NF1 (average diagnosis mid 50s), and there was insufficient NF1-related information for 2 patients. 75% of patients were cared for by the Orthopaedics Oncology group. We previously reported 70% five-year survival on a subset of 20 patients (Knewitz et al., 2021), with 60% five-year survival for patients who developed metastases. Here we will present our complete analysis of this much larger cohort, including relationship of clinical variables to survival, and any correlations with the immunostaining data, in particular HMGA2.

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Cognitive and Quality of Life Functioning of Adults with NF1: An Integrative Review

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We conducted an integrative review that synthesized studies of neuropsychological and quality of life (QoL) functioning of patients with Neurofibromatosis type 1 (NF1) across the lifespan. The goal of this project was to inform a framework and guidelines for the cognitive and QoL assessment and investigation of adults with NF1.

Literature search keywords included: "Neurofibromatosis type 1," "NF1," "Cognition," "Neuropsychology," "Neurocognitive," "Functioning," "Quality of Life," "Adult," "Child," "Adolescent," "Parent," "Aging." Databases searched included PubMed, Wiley Online Library, ScienceDirect, and Google Scholar. We applied the following inclusion criteria to found studies: a) focused on the cognitive and quality of life functioning of patients with NF1; b) included outcome measures; c) published in peer reviewed journals; d) published in 1990 or later; e) available in English. 63 journal articles met inclusion criteria.

Our primary finding was a lack of comprehensive research with adult NF1 samples, especially with regard to cognitive functioning studies ($N = 9$ adult; $N = 41$ child). Importantly, there was little consistency in cognitive and QoL measures across studies, with very few overlapping measures between child and adult studies. Among the included studies, the review revealed a largely heterogenous cognitive profile across the lifespan, and that cognitive functioning did not tend to improve with age. Results further implicated reduced health-related QoL (e.g., appearance, pain, sleep disturbance, social skills, role-functioning difficulties), and increased psychological symptoms (e.g., depression, anxiety) across the lifespan when compared to controls. Findings also indicated significantly fewer supportive services for adults with NF1 compared to children and adolescents, affecting the long-term management of NF1-associated cognitive and psychological symptoms that affect daily functioning and overall QoL.

There is great need to expand neurocognitive and QoL research with adult patients with NF1 in order to develop targeted interventions and supportive care. Informed by this review, we recommend that future studies adopt a lifespan approach to characterize the transition of care from adolescence well into adulthood. Further, given the heterogeneity of measures used thus far, we propose assessment guidelines for future studies, including relevant cognitive domains, measures per domain, and utilizing measures that are widely available and culturally appropriate.

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Primary Analysis of a Phase 1 Study of Selumetinib in Chinese Pediatric and Adult Patients with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PN)

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Purpose: Selumetinib (ARRY-142886, AZD6244) is approved for pediatric patients aged ≥2 (US) or ≥3 (EU) years with NF1 and symptomatic, inoperable PN. For the first time, we report primary results of an open-label Phase 1 study evaluating selumetinib in Chinese pediatric and adult patients with NF1 and inoperable PN.

Methods: Patients received oral selumetinib 25 mg/m^2 twice daily continuously in 28day cycles until progressive disease or unacceptable toxicity. Primary endpoints were safety, tolerability, and pharmacokinetics (PK). Secondary endpoints included efficacy (objective response rate [ORR], complete or confirmed partial response as per Response Evaluation in Neurofibromatosis and Schwannomatosis [REiNS]), and change from baseline in pain, physical functioning, and health-related quality of life (HRQoL). Data were analyzed following post-baseline response assessment of the last-dosed patient (Cycle 10, Day 28).

Results: Overall, 16 pediatric (range 4–16 years, 56% male) and 16 adult (range 18–51 years, 56% male) patients were treated. All pediatric patients remained on selumetinib at primary data cut-off (8/16/2022); one adult patient (6.3%) had discontinued (patient decision). The two most common PN-related morbidities at baseline were pain (88%) and disfigurement (31%) in pediatric patients, and pain (25%) and reduced range of motion (19%) in adult patients. All patients experienced ≥1 adverse event (AE). Pyrexia (38%) and dermatitis acneiform (81%) were the most common AEs, respectively, in pediatric and adult patients (both AEs have been reported in previous selumetinib studies). No Grade 4 or 5 AEs were reported. Selumetinib was rapidly absorbed (median t_{max} of 1.0–1.5 hours) and metabolized into its active metabolite (N-desmethyl selumetinib) in both pediatric and adult patients. Geometric means of $C_{max,ss}$ (ng/mL) and $AUC_{(0–12),ss}$ (h*ng/mL) of selumetinib were 1032 and 2961 in pediatric, and 1168 and 3932 in adult patients, respectively. ORR and PN volume versus baseline are given in the Table. Median duration of response and progression-free survival were not reached per investigator assessment by primary data cut-off. When compared to baseline, pain and HRQoL measures generally remained stable or improved in both pediatric and adult patients. In pediatric patients, overall improvement from baseline in physical functioning was reported; there was slight deterioration in adult patients, but individual improvements were observed.

Conclusions: At primary analysis, selumetinib at the 25 mg/m^2 twice daily dose approved in other countries showed acceptable safety, sufficient PK exposure, PN shrinkage, and improvements in multiple patient-reported outcomes in Chinese pediatric and adult patients with NF1 and inoperable PN.

Table. Response assessed by ICR and investigator as per REiNS criteria at primary data cut-off (after the last-dosed patient completed Cycle 10, Day 28)

	Pediatric n=16		Adult n=16	
Outcome	**Investigator**	**ICR**	**Investigator**	**ICR**
Response, n (%)				
Complete	0	0	0	0
Partial	14 (88)	12 (75)	5 (31)	8 (50)
Confirmed*	10 (63)	5 (31)	4 (25)	3 (19)
Unconfirmed†	4 (25)	7 (44)	1 (6)	5 (31)
ORR, n (%)	10 (63)	5 (31)	4 (25)	3 (19)
Stable disease, n (%)	2 (13)	3 (19)	11 (69)	7 (44)
Progressive disease, n (%)	0	1 (6)	0	1 (6)
Not evaluable, n (%)	0	0	0	0
Best % change from baseline in target PN volume, median (min, max)	−36 (−61, −7)	−34 (−68, 10)	−14 (−41, −3)	−21 (−47, 11)
Patients with volume reduction in target PN, n (%)	16 (100)	14 (88)	16 (100)	14 (88)

*≥20% tumor shrinkage (REiNS), unconfirmed at the first detection until observed again within 3–6 months; †Partial response achieved but either no confirmation assessment performed, or a confirmation assessment performed but response not confirmed.
ICR, independent committee review; ORR, objective response rate, PN, plexiform neurofibroma; REiNS, Response Evaluation in Neurofibromatosis and Schwannomatosis.

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High Costs, Impaired Productivity and Low Health-Related Quality of Life: Burden of Disease of Adults with Neurofibromatosis Type 1 in China

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Objectives: To estimate the economic and humanistic burden of adults with Neurofibromatosis type 1(NF1) in China.

Methods: Cross-sectional survey was conducted between Nov 2022 and Jan 2023 employing a one-on-one online interview system developed by China Alliance for Rare Diseases. NF1 patients over 18 years old were recruited through Neurofibromatosis Shenzhen Care Center, a nationwide patient network. Economic burden was estimated as direct (inpatient, outpatient, pharmacy and other out-of-pocket costs related to NF1) and indirect (productivity loss) costs in 2021. Work Productivity and Activity Impairment Questionnaire: General Health V2.0 (WPAI-GH) were administered to measure the productivity and activity impairment of NF1 patients in the last 7 days. EQ-5D-5L and PedsQL™ 3.0 Neurofibromatosis Module (PedsQL NF Module) were to measure humanistic burden.

Results: A total of 226 adults (age 31.54±7.60 years, female 63.72%, height 159.91±9.42 cm, weight 55.84±11.35 kg, body surface area 1.57±0.19 m2) with NF1 from 29 provinces of China were recruited, providing a relatively nationally representative sample. Annual NF1- related average direct cost was CNY 24,531 [10 to 435,000] (USD 3,560 [1 to 63,135], n=107), and employed patients' and caregivers' productivity loss were 33 [1 to 365] (n=34) and 39 [1 to 365] (n=37) days, respectively. Among patients working for pay (n=111), average absenteeism of 8.54% and average presenteeism of 21.62% were reported, contributing to 24.03% work productivity loss in the last 7 days. Among the study population (n=226), average activity impairment was 25.80%. EQ- 5D-5L utility was 0.84±0.17 and EQ-5D VAS was 72.32±23.49, which were lower than the EQ-5D-5L norms of China, 0.912-0.971 and 82.9-88.3 respectively. Percentage of patients reported having problems in pain/discomfort and anxiety/depression were 58.85% and 74.34%, respectively. PedsQL NF Module total score was 68.40±15.57. A lower score of PedsQL scale indicated a worse health state. Females had a lower total score than males (66.45±15.41 vs. 71.83±15.35, P=0.011). Among the 18 dimensions of PedsQL NF Module, extremely low scores were reported in worry, perceived physical appearance, and communication (31.63±26.33, 33.67±31.09, 47.03±31.49 respectively). Females had lower scores in skin itch bother (P=0.014), pain (P=0.004), cognitive functioning (P=0.010), perceived physical appearance (P=0.006), worry (P=0.010), treatment (P=0.025), stomach discomfort (P=0.017) and constipation (P=0.002).

Conclusions: Economic burden of NF1 patients varied widely. Productivity and activity loss was considerable. Adults with NF1 in China had a significant burden in health-related quality of life, especially in psychological and psychosocial distress, indicating an unmet need for more effective disease management.

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Long-Term Distress Throughout Childhood and Teens: Economic, Humanistic and Caregiver Burden of Underage Patients with Neurofibromatosis Type 1 in China

M.M. Wanxian Liang, *Beijing University of Chinese Medicine*

Objectives: To estimate the economic, humanistic, and caregiver burden of children with neurofibromatosis type 1(NF1) in China.

Methods: Cross-sectional survey was conducted between Nov 2022 and Jan 2023 employing a one-on-one online interview system developed by China Alliance for Rare Diseases. Caregivers of NF1 patients aged≤18 were recruited through Neurofibromatosis Shenzhen Care Center, a nationwide patient network. Economic burden was estimated as direct (inpatient, outpatient, pharmacy, and other out-of-pocket costs related to NF1) and indirect (productivity loss) costs in 2021. EQ-5D-Y proxy version and PedsQL™ 4.0 Generic Core Scales proxy version were administered to measure humanistic burden. Zarit Burden Interview (ZBI) was to measure burden of caregivers for pediatric patients.

Results: A total of 223 caregivers of pediatric patients (age 6.26±4.25 years, female 54.26%, height 115.88±27.53 cm, weight 23.95±14.19 kg, body surface area 0.87±0.34 m^2) from 29 provinces of China were recruited, providing a relatively nationally representative sample. Among the patients, 47.09% were preschoolers, 50.67% were in school, and 2.24% had suspended schooling. Mothers filled 80.27% of the survey. Annual NF1-related direct cost in 2021 was CNY33,614 [30 to 390,000] (USD 4,879 [4 to 56,604], n=152), and employed caregivers' annual productivity loss was 91 [2 to 415] days (up to 5 caregivers per patient, n=113). For patients in school, 50 reported NF1-related sick leave from school in 2021 and the average of days off was 58 days. EQ-5D-Y utility and VAS scores were 0.88±0.13 (n=154) and 75.38±20.67 (n=154), respectively. The EQ-5D dimension that patients had the most problems with was pain/discomfort (52.60%), followed by anxiety/depression (42.86%). The total scores of PedsQL for different age ranges were 68.47±19.42(age 2-18), 77.38±19.13 (age 2-4), 64.79±18.22 (age 5-7), 72.08±14.26 (age 8-12), and 56.84±24.50 (age 13-18). Physical health scores of PedsQL for different age ranges were 71.64±22.13 (age 2-18), 76.74±20.31 (age 2-4), 66.00±21.19 (age 5-7), 79.28±17.10 (age 8-12), and 59.14±28.03 (age 13-18). Psychosocial health scores of PedsQL were 66.83±19.52 (age 2-18), 77.35±18.87 (age 2-4), 64.14±17.93 (age 5-7), 68.25±15.09 (age 8-12), and 55.62±24.81 (age 13-18). ZBI scores demonstrated that 39.46% of caregivers had moderate-to-severe or severe burden, and caregiver burden of mothers was higher than that of fathers (36.34±16.17 vs. 29.95±17.04, $P<0.05$).

Conclusions: Economic burden of NF1 patients varied widely due to diversified clinical manifestations and limited choice of treatments. Children with NF1 in China had a low health-related quality of life, especially in psychological dimension.

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Discrimination of Benign, Atypical and Malignant Peripheral Nerve Sheath Tumors in Patients with Neurofibromatosis Type 1 Using ^{18}FDG-PET/CT

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Purpose: The purpose of this study was to assess the diagnostic accuracy of fluorodeoxyglucose positron emission tomography (^{18}FDG-PET/CT) in distinguishing benign, atypical, and malignant peripheral nerve sheath tumors in neurofibromatosis type 1 (NF1) patients, and to identify clinically relevant diagnostic cut-off values for tumor differentiation.

Methods: Thirty-four NF1 patients (20 male; 31±11 years) underwent ^{18}FDG-PET/CT between 2014 and 2021. Two radiologists independently assessed the maximum and mean standardized uptake value ($SUV_{max/mean}$) in 38 benign (BPNST), 11 atypical (ANF), and 17 malignant peripheral nerve sheath tumors (MPNST) by semiautomatic measurement. The diagnostic reference standards included follow-up of ≥24 months for benign tumors or histopathological evaluation for atypical and malignant tumors. Receiver operating characteristic (ROC) analysis was conducted to determine the area under the curve (AUC) of SUV_{max} and SUV_{mean} by comparing "BPNST vs. MPNST" and "BPNST vs. ANF/MPNST". Diagnostic cut-off values were determined based on good sensitivity and optimal specificity.

Results: Mean SUV_{max} and SUV_{mean} were higher in MPNST than in BPNST and ANF (mean SUV_{max}/SUV_{mean} MPNST: 11.49/6.92; ANF: 5.87/3.38; BPNST: 2.93/1.95). Mean SUV_{max} and SUV_{mean} differed significantly between groups (<0.005). The AUC for SUV_{max} and SUV_{mean} was 93.2% (95%-CI: 85.6-100%) and 93.3% (95%-CI: 86.3-100%), respectively, when comparing "BPNST vs. MPNST" (**Figure 1**). Using a cut-off value of 5.1 for SUV_{max} and 3.3 for SUV_{mean}, sensitivity and specificity were high (SUV_{max}: 82.4% and 94.7%; SUV_{mean}: 82.4% and 92.1%). When atypical tumors were included in the comparison, the AUC decreased to 81.9% (95%-CI: 71.4-92.4%) for SUV_{max} and to 82.1% (95%-CI: 71.9-92.3%) for SUV_{mean} (**Figure 2**). The sensitivity and specificity remained high for SUV_{max} (82.4% and 94.7%), but decreased for SUV_{mean} (66.7% and 90.9%).

Conclusions: ^{18}FDG-PET/CT allows identification of MPNST in NF1 with high levels of sensitivity and specificity. However, inclusion of atypical neurofibromas results in a decrease in discriminatory power for PET-based differentiation of peripheral nerve sheath tumors in NF1.

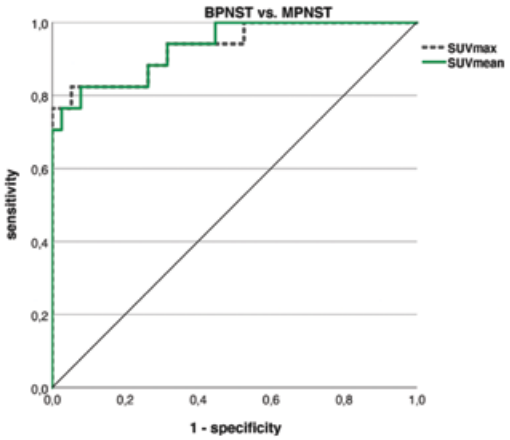

Fig. 1) Receiver operating characteristic (ROC) curve for the comparison "BPNST vs. MPNST". BPNST = benign peripheral nerve sheath tumor, MPNST = malignant peripheral nerve sheath tumor, SUV_{max} = maximum and mean standardized uptake value, SUV_{mean} = mean standardized uptake value.

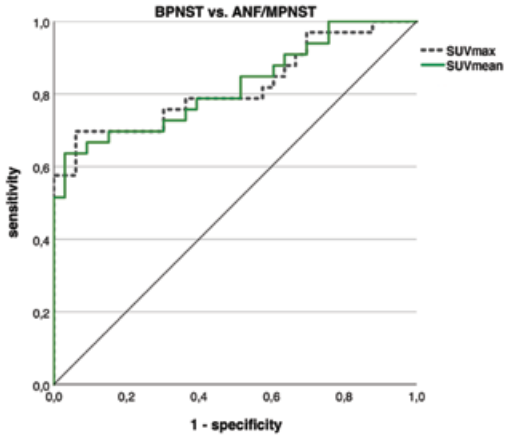

Fig. 2) Receiver operating characteristic (ROC) curve for the comparison "BPNST vs. ANF/MPNST". BPNST = benign peripheral nerve sheath tumor, MPNST = malignant peripheral nerve sheath tumor, SUV_{max} = maximum and mean standardized uptake value, SUV_{mean} = mean standardized uptake value.

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Development of Patient-Reported Outcome (PRO) Measures and a Mobile App to Assess Tumor-Related Pain in Children and Adults with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (pNFs) for Clinical Trials

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Background: Clinical trials for the treatment of pNFs in individuals with NF1 require endpoints that assess changes in symptoms, such as pain, in addition to tumor reduction. No validated PRO measures exist that assess pNF-related pain and its effect on daily life across the lifespan. Thus, we conducted qualitative research with children and adults with NF1 and pNFs. Findings are presented that guided the modifications of PRO pain tools and development of a mobile app to administer these measures at home.

Methods: Individuals with NF1, ages ≥5 years, and pNF-related pain were eligible. The Numeric Rating Scale-11, assessing pain intensity, and Pain Interference Index, evaluating impact of pain on daily life, were selected based on REiNS consensus recommendations and accepted into the rigorous FDA Drug Development Tool Qualification Program. Investigators conducted focus groups and individual interviews in six age groups for concept elicitation. Topics of investigation included types of pNF pain, ability to rate specific tumor pain, ways to document the location of pain, length of recall period (e.g., "past 24 hours"), areas of daily life affected by pNF pain, specific wording of items, interest for daily electronic ratings from home, and the youngest age that could understand the measures. After initial modifications, 5 subsequent waves of cognitive debriefing interviews asked patients about the measures, and additional changes were made after each wave. Audio recordings were transcribed, and thematic analysis was conducted until content saturation was reached; problem codes were used to evaluate cognitive debriefing interviews to further adapt the tools.

Results: Eleven concept elicitation focus groups and 26 individual interviews were completed (n=56; mean age=25.3 years; range 6-68; 59% female); in addition, 52 cognitive debriefing interviews about the new measures were completed across 5 waves. Recurrent themes included: two distinct types of pain (chronic/episodic), variability of pain, ability to distinguish between pains of different pNF tumors and rate the pain intensity of specific tumors, preference for using a figure to show the tumor pain location, need for shorter recall period, openness to completing daily electronic ratings from home, new items to assess pain interference specific to pNFs in 3 domains (physical, physiological, social-emotional), and difficulties in using these tools with young children. The final tools for ages ≥8 years included a 2-item measure of tumor pain intensity (PAINS-pNF) and 12-item measure of tumor pain interference (PII-pNF). These measures were developed into a clinical trial platform consisting of a web-based portal for researchers to enroll patients and manage data, a mobile app for completing the measures, text-to-speech audible instructions, and blue/green NF awareness colors.

Conclusions: Qualitative research identified critical information from children and adults with NF1 that was used to design the first electronic PRO pain measures and mobile app assessing pNF-specific pain intensity and pain interference in daily life. This technology will allow home-based evaluation of pNF pain specifically for use in NF1 clinical trials. Next steps include evaluating the psychometric properties of these new pain tools used at home and sensitivity to change in future pNF trials.

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DITTO4NF: Classification and Prioritization of Likely Pathogenic Variants in *NF1* using Explainable Machine Learning

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Purpose of study: More than 20,000 rare and more common variants have so far been identified within the NF1 gene. Despite ongoing curation and functional testing efforts, more than 41% of the variants seen in patients with Neurofibromatosis or Hereditary cancer-predisposing syndrome are classified as Variants of Uncertain Significance (VUS) or have conflicting classifications. Lack of a definitive classification can hamper medical management, family member testing, and the ability to provide prognostic information. The purpose of this study was to test our machine learning based methods to determine whether they could 1) be used to accurately and rapidly classify *NF1* variants and 2) prioritize variants for functional impact studies.

Methods: We developed a deep learning-based model (DITTO) for variant deleteriousness prediction that integrates genomic, transcriptomic, and proteomic data. DITTO was trained and tested on 696,546 pathogenic and benign variants from ClinVar. Each variant was annotated with hundreds of data points spanning frequency, impact, damage predictions, conservation, and disease association using OpenCravat and protein folding, stability and molecular interaction predictions. We validated DITTO based on variant classifications from the NF1 Leiden Open Variation Database (LOVD) database.

Results: DITTO Precision, Recall, and Accuracy scores were 0.99, 0.98, and 0.98 respectively using a deleterious probability score cutoff of >0.8. 877/901 variants were classified in agreement with LOVD. Further integration of experimentally derived data points as well as protein predictions allowed us to go beyond pathogenicity classification to prioritize variants based on their potential functional impact. For example, by integrating our methods with predictions from these existing tools we predicted that the p.G848R NF1 variant would be deleterious and reduce protein stability (SAAFEC-seq = -1.09). Further, we were able to show that the variants impact on stability differed between open and closed protein conformations (closed = 0.47; open = -1.75); transition between these is critical for NF1 function.

Conclusions: We showed that DITTO is able to rapidly and accurately classify NF1 variants. We identified a few cases pointing towards potential misclassification in LOVD thereby prioritizing these for expert curation efforts. We were also able to classify hundreds of VUSs in to likely pathogenic or likely benign categories. We are integrating additional functional and structural knowledge to better predict specific variant effects. These advances are of critical importance for rapid, sensitive, and accurate diagnosis and for generation of prognostic insights. We will discuss our methods, applications, and findings.

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A Retrospective Analysis of Treatment Sequence and Outcomes in Patients with Relapsed MPNST

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Background: Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas that arise from nerve cellular components within nerve sheath. Currently, the only curative treatment is surgical resection with wide margins, although this is often not feasible due to the location or size of the tumor or the presence of metastasis. Despite many clinical trials, there has been little advancement in treatment outcomes and survival for those with advanced disease remains poor. Advances in the understanding of MPNST molecular biology has led to pre-clinical studies of targeted therapies that appear promising in model systems. However, tailored chemotherapy and novel targeted therapies have yet to achieve the anticipated clinical successes. Following failure of typical first-line chemotherapy, an evidence-based choice of chemotherapy for recurrent/ refractory MPNST remains elusive. Therefore, we conducted a retrospective analysis of our single-institutional experience in the treatment of relapsed MPNST with the goal to describe patient outcomes based on salvage chemotherapy regimens.

Methods: We conducted a retrospective electronic health record analysis of patients with MPNST who were treated at Johns Hopkins Hospital from January 2010 to June 2021.

Results: We analyzed the treatment sequences and overall outcome of 65 patients. Upfront therapy included single or combined modalities of surgery, chemotherapy, or radiotherapy and these did not have apparent correlation to outcomes. Patients received a median of three lines of therapy. Forty-eight patients received at least one line of chemotherapy, which included 23 different regimens (excluding active clinical studies). Most patients received a combination of doxorubicin, ifosfamide, or etoposide as first-line chemotherapy. Salvage chemotherapy regimens and their time to progression varied greatly. Interestingly, patients who received vincristine/ irinotecan/ temozolomide had a longer average time to progression (316.5 days, n=3) followed by irinotecan/ temozolomide (313 days, n=1) compared to other regimens.

Conclusions: Patients with MPNST often succumb to their disease despite multiple lines of therapy. Here, we retrospectively investigated the clinical outcomes following physician-selected chemotherapy regimens for patients treated for recurrent MPNST. These data provide a description of the regimens used and may be used as comparative information in decision-making for future patients and clinical trials.

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NF2-Related Schwannomatosis (NF2): Basic Science
(The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Bornhorst	Miriam	141	Identification of Structural Variants in Cases with Neurofibromatosis Type 2 Using Optical Genome Mapping
Duhon	Bailey	143	Matrix Metalloproteinase 9 (MMP-9) is a Biomarker in NF2-Associated and Aggressive Sporadic Vestibular Schwannomas
Hardin	Haley	145	Evaluation of BRD4 Inhibitors in Combination with Kinase Inhibitors in Human Schwannoma Model Cells
Hass	Ethan	147	Dual HDAC/PI3K Inhibitor Fimepinostat Slows Growth of Murine Model Schwannoma Cells in an *In Vivo* Allograft
Logan	Isabelle	149	Nitrated Proteins are a Novel Category of Therapeutic Targets in *NF2*-Related Schwannomatosis
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NF2-Related Schwannomatosis (NF2): Basic Science

Identification of Structural Variants in Cases with Neurofibromatosis Type 2 Using Optical Genome Mapping

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Purpose of the Study: Neurofibromatosis Type 2 is a genetic syndrome associated with the development of tumors, including meningiomas and schwannomas. While many patients with NF2 will present with their initial tumor during adult years, some patients develop multiple tumors in early childhood, impacting quality of life and overall prognosis. Structural variants (SVs) including large deletions, duplications, inversions and translocations play an important role in tumorigenesis and growth. Genetic studies have shown that most tumors in patients with NF2 will have chromosome 22 loss, along with a germline NF2 mutation. However, a more complete SV profile of NF2-associated tumors is not well characterized. Optical genome mapping (OGM) utilizes ultra-long DNA molecules to construct and evaluate long genomic fragments, making it effective in identifying large SVs. In this study, we used OGM, to investigate the SV profile of NF2-associated meningiomas and schwannomas in children, adolescents and young adults.

Methods: Ultra high molecular weight DNA was extracted from a cohort of NF2 associated meningiomas and schwannomas and matched normal samples when available (n=10). OGM was performed using nanochannel chip arrays on a Saphyr instrument and SVs were identified. We then used *nanotatoR* to subclassify SVs into functional pathways and determined which genes impact specific cell developmental functions.

Results: Tumor-associated, low population frequency SVs were identified in all samples. The most common chromosome aberration was loss of chromosome 22 (as expected). Additional chromosome gains and losses were most common in young children and tumors with an aggressive clinical behavior. Additional SVs (inversions, deletions, duplications, translocations) were also identified in all samples, many of which had not been previously reported in NF2-associated tumors before, and some overlapping with genes known to have tumorigenic potential.

Conclusion: In this small study, NF2-associated tumors in young children or with an aggressive clinical behavior had a more complex SV phenotype (particularly chromosome abnormalities) than slow growing tumors in adult patients. Future studies will expand testing to a larger cohort of samples to better understand patterns of SVs in different tumors and age groups

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Matrix Metalloproteinase 9 (MMP-9) is a Biomarker in NF2-Associated and Aggressive Sporadic Vestibular Schwannomas

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Introduction: Neurofibromatosis type 2 (NF2) and sporadic vestibular schwannoma (VS) share common NF2 mutations and tumor microenvironment (TME) compositions. However, distinguishing between aggressive and non-aggressive VS, such as tumors that are highly adherent to the brainstem requiring subtotal resections, remains challenging. In this study, we aim to identify potential biomarkers of aggressive VS by investigating changes in the TME using murine and human NF2 models as well as human primary VS.

Methods: A mouse model of NF2 was established by implanting mouse merlin-deficient Schwann cells (genetic deletion of NF2 using Cre-lox, MD-MSC) in nude mice. Wild-type murine Schwann cells (WT-MSC) were used as a syngeneic control. The expression of genes involved in extracellular matrix remodeling, protease activation, and immune cell infiltration was analyzed using multiplex transcriptomic gene expression profiling. Top candidates were validated in mouse and human NF2 cell lines and in fresh VS obtained from tumor surgery. Immunohistochemical staining of candidate genes was performed in fresh and formalin-fixed sporadic VS tissues. Biomarker expression was correlated with the degree of tumor adhesions found intraoperatively.

Results: Thirty-eight genes were significantly upregulated and 25 were downregulated ($p=0.05$). Several metalloproteases were enriched in MD-MSC tumor allografts compared to WT-MSCs, with MMP-9 being the most upregulated by over 5-fold ($p=0.05$). This was further validated by qRT-PCR in MD-MSC cells compared to WT-MSC (over 15-fold increase). In an established human NF2 tumor cell line (HEI-193), MMP-9 was significantly enriched in tumor secretions compared to human Schwann cell controls (1750 vs. 470 pg/mL, $p=0.05$). In aggressive VS highly adherent to the brainstem, MMP-9 expression was elevated in tumor parenchyma compared to non-adherent VS. In freshly resected tumors matched in size and gender (n=4 in each cohort), tumor secretion of MMP-9 was 10-fold higher in adherent VS compared to non-adherent VS (46.5 ng/mL vs.4.6 ng/mL, $p=0.015$).

Conclusions: The TME of NF2-associated VS is characterized by the dysregulation of multiple genes, including matrix metalloproteinase 9 (MMP-9. In a murine NF2 mouse model, a human NF2 tumor cell line, and primary VS obtained from surgical resection, MMP-9 expression was consistently and significantly upregulated. Furthermore, aggressive, adherent VS exhibited significantly elevated levels of MMP9 secretion compared to non-adherent VS.

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Reference: Petrilli, A M, and C Fernández-Valle. "Role of Merlin/NF2 inactivation in tumor biology." *Oncogene* vol. 35,5 (2016): 537-48.

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Evaluation of BRD4 Inhibitors in Combination with Kinase Inhibitors in Human Schwannoma Model Cells

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Neurofibromatosis Type 2 (NF2) is a nervous system tumor disorder that predisposes individuals to benign schwannomas, meningiomas, and ependymomas. Current treatment for NF2 is limited to surgery, radiosurgery, or enrollment in clinical trials for a small number of kinase inhibitors. When used for cancer, these kinase inhibitor monotherapies are often successful for a short period of time until patients experience disease progression. Activation of one or multiple alternative survival and/or proliferation signaling pathways enables tumor cells to overcome inhibition of the targeted pathway.

Our previous research evaluated MEK inhibitors in NF2 mouse and human schwannoma cell models and found efficacy for trametinib, cobimetinib and PD0325901 (mirdametinib) but not selumetinib. In a mouse nerve allograft model, trametinib (but not cobimetinib or mirdametenib) led to re-expression of phospho-ERK after a two week treatment consistent with development of drug resistance. Ingenuity pathway analysis of proteomic data from trametinib-treated versus DMSO treated mouse schwannoma cells predicted activation of bromodomain-containing protein 4 (BRD4). BRD4 is an epigenetic chromatin reader that binds acetylated lysines on histones and non-histones and regulates gene transcription.

Here we confirmed in two human primary schwannoma model cells (HS01, HS05) that phospho-ERK is re-expressed following a 72hour exposure to trametinib that coincides with increased BRD4 protein levels. Conversely, human schwannoma model cells treated for 48hours with JQ1, a BRD4-inhibitor had increased p-ERK levels compared to the vehicle control. Thus, BRD4 inhibitor monotherapy also activates compensatory escape pathways. Several BRD4 inhibitors are in clinical trials in combination with other approved cancer drugs. We compared efficacy of JQ1 and six other BRD4 inhibitors in clinical trials for their ability to reduce viability of HS02 and HS05 schwannoma model cells. The most effective BRD4 inhibitors were tested for synergy in combination screens with multiple kinase inhibitors already evaluated in NF2 or cancer patients. Two combinations including one with brigatinib, were advanced for additional studies using live-imaging, cell cycle analysis, and cell death assays to assess for superiority of the combination versus monotherapies.

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Dual HDAC/PI3K Inhibitor Fimepinostat Slows Growth of Murine Model Schwannoma Cells in an *In Vivo* Allograft

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Introduction: Neurofibromatosis type 2 (NF2) and schwannomatosis are neurocutaneous disorders characterized by predisposition to developing tumors, predominantly bilateral vestibular schwannomas (VS) and peripheral nerve schwannomas, respectively. No pharmacologic treatments are approved by the FDA for these tumors. Fimepinostat (CUDC-907) is an investigational dual-inhibitor of HDACs and PI3K currently in phase 1 clinical trial for pediatric brain tumors that promotes apoptosis in model Schwann cells (SCs) possessing *NF2* deficiency which defines the molecular diagnosis of NF2.

Objectives: This study investigates the *in vivo* efficacy of fimepinostat using a murine schwannoma allograft model.

Methods: *NF2*-deficient SCs expressing firefly luciferase were implanted into the sciatic nerves of immune deficient NSG mice. Tumor growth was monitored using bioluminescent imaging, and tumors were dissected after three weeks of treatment with 75mg/kg fimepinostat, or vehicle and their weights were compared.

Results: Treatment with fimepinostat resulted in a statistically significant reduction of 43% ($p<0.02$) in average final tumor weights, and a reduction of 41% ($p<0.1$) in average fold increase in tumor bioluminescent flux over the treatment period.

Conclusions: This study provides preliminary data for future studies comparing the differential effects of fimepinostat on schwannomas that arise in individuals with NF2 and in schwannomas arising in patients with other genetic subtypes of schwannomatosis.

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Nitrated Proteins are a Novel Category of Therapeutic Targets in *NF2*-Related Schwannomatosis

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Purpose: Determine the mechanism behind nitrated Heat Shock Protein 90 (Hsp90) proliferative activities and identify novel nitrated targets that support schwannoma cell proliferation.

Methods: We used three-dimensional and monolayer cell culture models of normal and merlin deficient (MD)-Schwann cells together with human site-specific nitrated proteins produced by genetic code expansion technology. We delivered the proteins intracellularly and performed *in vitro* and in culture assays and extracellular flux analysis to study the effect of the nitrated proteins on cell proliferation and metabolism.

Results: We previously showed that merlin inactivation in Schwann cells leads to production of the powerful oxidant peroxynitrite and subsequent protein tyrosine (Y) nitration, and that one or more nitrated proteins induce a metabolic reprogramming to support cell proliferation. We identified 9 promising nitrated targets in vestibular schwannoma resections from NF2-related schwannomatosis patients, including the molecular chaperone Heat shock protein 90 (Hsp90), RhoA, and galectin 1 (Gal1). Here, we identified nitration of Hsp90 on Y33 ($Hsp90_{NY33}$) or Y56 ($Hsp90_{NY56}$) was enough to induce normal and MD-Schwann cell proliferation. Further, $Hsp90_{NY33}$ associated with mitochondria and decreased mitochondrial metabolism, while $Hsp90_{NY56}$ increased glycolysis, suggesting that *in the absence of merlin nitrated Hsp90 acts as a metabolic switch to support cell proliferation.* We previously showed that Hsp90 nitrated on Y56 activates the purinergic receptor P2X7 (P2X7R) in motor neurons, inducing cell death. However, P2X7R activation in tumors has been shown to increase glycolysis and tumor growth. Targeting P2X7R using the specific inhibitor KN62 prevented the increase in glycolysis induced by $Hsp90_{NY56}$ and abrogated the proliferative activities of $Hsp90_{NY33}$ and $Hsp90_{NY56}$, suggesting that P2X7R mediates $Hsp90_{NY}$ effects. Next we investigated the role of nitrated RhoA and Gal1 on proliferation in normal Schwann cells. RhoA nitrated at Y34, and Gal1 nitrated at Y120 but not at Y105 increased cell proliferation. We are currently starting screens for drugs that bind to nitrated Hsp90 selectively and specifically inhibit its proliferative activity and continue to investigate the role of nitrated RhoA and Gal1 in MD-Schwann proliferation, metabolism, adhesion, and in the regulation of signaling pathways.

Conclusions: Nitrated proteins are not present in any normal tissue or cell type we have tested so far, including brain, spinal cord, and specifically Schwann cells. Together, these results support *nitrated proteins and particularly nitrated Hsp90 as a novel category of tumor-direct targets for therapeutic development in NF2-related schwannomatosis that could be translated to other solid tumors.*

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PI3K Inhibitor, Pictilisib Combined with PAK Inhibition Reduce Tumor Size and Induce Apoptosis in Neurofibromatosis Type 2 Orthotopic Allograft Mouse Model

Anna Nagel, PhD, *University of Central Florida*

Neurofibromatosis Type 2 (NF2) is a genetic disorder that causes various nervous system tumors. The primary tumor type associated with this condition is bilateral vestibular schwannomas, and patients with NF2 often develop multiple tumors. There is no cure for NF2. Recommended treatments include surgical resection and radiation, which can leave patients with severe neurological deficits. Therefore, the risks and potential benefits must be carefully considered before making the decision. The search for highly needed drug candidates has identified phosphoinositide 3-kinase (PI3K) inhibitors as strong candidates due to the merlin loss in NF2 tumors.

We explored the synergistic effect of three PI3K inhibitors in combination with inhibitors targeting other kinases. The best synergy was presented by combining pictilisib (pan-PI3K inhibitor) and PF-3758309 (PAK inhibitor) in human and mouse merlin-deficient Schwann cells (MD-SCs). Both single and combination therapies significantly reduced the growth of mouse MD-SCs in an orthotopic allograft mouse model with reduced levels of P-Akt and YAP and with positive Cleaved Caspase 3 staining induced by pictilisib treatment. We demonstrated cell cycle arrest and apoptosis in mouse MD-SCs and cell proliferation inhibition in human MD-SCs using flow cytometry, life Cleaved Caspase 3/7 staining and apoptosis arrays.

This study demonstrates that simultaneous inhibition of PI3K and PAK causes tumor cell inhibition and identifies critical pathways that could be targeted in developing therapies for NF2 or other merlin-deficient cancer types. Understanding these mechanisms can improve the identification of new targets and inhibitors to slow NF2 schwannoma disease progression. Furthermore, these results support the use of combinatorial treatments to take advantage of the cooperative properties of targeted inhibitors.

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Phagocytosis Gone Awry: Exploring Macrophage Dysfunction in Neurofibromatosis Type 2 (NF2)

Michael Reuter, PhD, *Leibniz Institute on Aging, Fritz Lipmann Institute, 07745 Jena, Germany*

Purpose: The study aimed to investigate the role of macrophages in NF2 disease, a condition characterized by faulty nerve repair leading to Schwannoma formation, and to identify potential molecular mechanisms underlying the observed phagocytosis defect.

Methods: The study utilized a nerve injury approach with a conditional NF2 knock-out (ko) mouse model, targeting macrophages, neurons, and Schwann-cells. Macrophage number and cytokine release were evaluated, and myelin phagocytosis was assessed both *in vivo* and *in vitro.* The proteome of sciatic nerves was analysed to identify potential molecular mechanisms involved in the defective phagocytosis, and a RhoA specific inhibitor was used to test the hypothesis that RhoA signalling is involved in myelin phagocytosis.

Results: The study found that the number of macrophages and cytokine release were not affected in conditional NF2-ko mice compared to healthy wildtype mice, but myelin phagocytosis was reduced both *in vivo* and *in vitro.* This defect was not specific to myelin, as a more general phagocytosis defect for bioparticles derived from different pathogens was observed in NF2-deficient macrophages. Proteomic analysis revealed that RhoA signalling was a potential cause of the defective phagocytosis, and a RhoA specific inhibitor was able to reduce myelin phagocytosis in healthy wildtype macrophages to the level observed in NF2-ko macrophages.

Conclusions: The study suggests that macrophages play an important role in nerve regeneration and emphasizes the significance of myelin debris clearance to create a regeneration permissive microenvironment, particularly in the context of the NF2 disease. The study also identifies a phagocytosis defect in NF2-deficient macrophages and provides evidence for a direct link between RhoA signalling, merlin, and phagocytosis. These findings provide potential targets for future therapies aimed at improving nerve regeneration in NF2 patients.

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Neuregulin 1 Beta Protein Replacement Therapy for Treatment of NF2-Associated Schwannomas – Insights from a Multicenter Preclinical Confirmatory Drug Trial

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

The NRG1-PRT project is conducting a randomized, blinded, preclinical confirmatory multicenter study in mice following human clinical trial standards to validate the benefit of treatment with soluble recombinant human Neuregulin 1 beta (rhNRG1beta) to prevent Neurofibromatosis type 2 (NF2)-associated schwannoma growth.

Our previous work has revealed a multifactorial schwannoma induction process involving both Schwann cells and neuronal cells as well as additional cues from the nerve microenvironment. Depletion of the *NF2* gene product Merlin causes downregulation of Nrg1 expression and presentation on axons and thereby a lack of (re-)differentiation signals for repair Schwann cells during nerve regeneration. In this environment Merlin-deficient Schwann cells are unable to support normal nerve regeneration and continue to proliferate, subsequently giving rise to Schwannoma. Replacing the missing differentiation cue by injecting soluble rhNRG1beta significantly reduces Schwannoma formation and improves nerve regeneration and functional recovery.

In the present study we are aiming to confirm our previous pilot data (n=8 per treatment) in a randomized, blinded, preclinical confirmatory multicenter study design following human clinical trial standards as well as a correspondingly increased sample size of mice (n=108 per treatment). To this end, we have established a preclinical study framework and analysis pipeline consisting of: Preregistration of the study protocol and detailed standard operating procedures (SOPs), three centrally trained but independently operating experimental sites, comprehensive documentation in a REDCap database, a dedicated quality control structure including study monitoring and data management as well as independent analysis of the primary study outcome by clinical biostatisticians.

The study design employs our previously established mouse model (Nf2flox; Nefh-Cre; P0-Cre) deleting Nf2 in both Schwann cells and neurons, a defined sciatic nerve crush injury inducing reproducible schwannoma formation, blinded treatment of mice for 13 weeks with rhNRG1beta1 versus vehicle control, a user-guided, semiautomatic ImageJ-macro-based quantification of sciatic nerve schwannoma size (primary outcome) as well as immunohistochemistry, immunoblotting and (phospho-)proteome analysis by mass spectrometry (secondary outcomes).

While the study is still in progress and data collection, analysis and unblinding of results will not be concluded before mid-2024, interim project results comprise a translatable preclinical study framework and analysis pipeline as well as lessons learned from planning and conducting a preclinical confirmatory multicenter trial expediting translation from basic to clinical research.

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Role of *Nf2* in Peripheral Nervous System Maintenance and Aging

Marina Zahid, *Leibniz institute on aging – Fritz Lipmann Institute, Jena*

Purpose: Aim of this study is to investigate the role of Merlin, encoded by the *Nf2* gene, in peripheral nervous system maintenance and to identify pathways associated with non-tumor related pathologies upon *Nf2* mutation.

Methods: We use *Nf2* knockout (KO) mice with cell specific mutation in Schwann Cells (SCs), axons and a combination of both to investigate the role of merlin in nerve maintenance during aging. Electron microscopy and proteomics analyses are primarily used in this study for detailed insights into the morphological and molecular changes upon *Nf2* mutation.

Results: We observe changes in myelination, axon size and number in KO mice compared to WT counterparts. Moreover, upon homozygous KO mutation in both SC and axonal compartment, we also observe formation of collagen pockets, abnormal non-myelination SCs (nmSCs) and onion bulbs already at young age. These changes are normally found in an aged nerve.

Conclusion: We show that *Nf2* mutation in the peripheral nervous systems leads to morphological changes in myelin thickness, axon diameter and axonal numbers. Additionally, frequent presence of onion bulbs, collagen pockets and abnormal nonmyelinating SCs was observed in homozygous *Nf2* KO in both SCs and axons. We speculate that Merlin plays an important role in maintenance of nerve and its loss has deleterious effects leading to abnormal aging-like morphology.

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NF2-Related Schwannomatosis (NF2): Clinical Science
(The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Buono	Frank	154	The Development of an Auto-Segmentation Tool for the 3D Volumetric Analysis of Vestibular Schwannomas
Chang	Long-Sheng	156	Single-Cell Transcriptomic Analysis of NF2-Asscoiated Schwannomas Reveals Novel Changes in Tumor and Immune Cell Subpopulations After Bevacizumab Treatment

NF2-Related Schwannomatosis (NF2): Clinical Science

The Development of an Auto-Segmentation Tool for the 3D Volumetric Analysis of Vestibular Schwannomas

Frank D. Buono, PhD, *Department of Psychiatry, Yale School of Medicine*

Introduction: *NF2*-related schwannomatosis (formerly Neurofibromatosis Type 2) affects the myelination of nerves throughout the body and is characterized by growth of Vestibular Schwannomas (VS), which can lead to significant hearing loss and vestibular dysfunction.[1,2] Current progression of VS is monitored with volumetric analysis however this is based on ellipsoid measurements and is inefficient and inaccurate as it does not portray the actual 3D shape.[2] 3D volumetric analysis, using AI, can be a novel solution to show the accurate 3D shape of the VS in relation to surrounding structures.

Methodology: Brain MRIs, from patients with VS, were collected from the NF2 database at Yale New Haven Hospital and from patient recruitment through NF2 BioSolutions. A total of 37 patients were eligible for inclusion, providing 143 MRIs, which could be successfully mapped. Ground truth dataset creation was undertaken manually using an image processing software (Simpleware ScanIP, Synopsis, Mountain View, CA) The ground truth datasets were subdivided into train (80%), validation (10%) and test (10%) groups for model training. The initial ground truth data set consisted of 25 high quality MRIs. The remaining 118 MRIs were used for model training and testing. To visualize the shape, size, and growth pattern of the tumors, a segmentation of the pons was also included at the levels of the tumors.

A visualization tool was developed by writing Python code to organize bilateral tumors in chronological order and sort them into left and right categories. Sorted tumors and their volumes were displayed in a table within ScanIP, under the “Mask Statistics” tab

Results: The DICE score (Dice Coefficient = 2 * the Area of Overlap / by the total number of pixels in both images) was used to calculate accuracy of the model in comparison with the initial ground truth data set and a mean DICE score of 0.88 (standard deviation 0.052) was achieved in a proof-of-concept. No tumors identified within the ground truth segmentation were missed by the helper.

Discussion: The results show that our initial AI prototype is an accurate tool for 3D volumetric analysis, providing a far more accurate visualization of tumor size and growth than other current methods. Although the model training and testing with the remaining images is yet to be concluded, tumor recognition and the DICE score show excellent foundations, on which further training can be assembled. Introduction of the system into radiological practice could potentially be applied to any pathological tumors.

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Single-Cell Transcriptomic Analysis of NF2-Asscoiated Schwannomas Reveals Novel Changes in Tumor and Immune Cell Subpopulations After Bevacizumab Treatment

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Vestibular schwannomas (VS) are Schwann cell (SC) tumors originating from the 8th cranial nerve and cause significant morbidities, including hearing loss, facial paralysis, and brainstem compression. VS can occur sporadically or are commonly seen in patients with neurofibromatosis 2 (NF2), a debilitating tumor predisposition syndrome in which afflicted patients can also develop multiple meningiomas and other nervous system tumors. Invasive surgery and radiation have been the treatment options; however, due to multiple tumors in NF2 patients and complications from these treatments, drug therapy may be more desirable for some patients. Recent studies show that the anti-VEGF monoclonal antibody bevacizumab improves hearing and slows tumor growth in ~30-40% of patients with NF2 and progressive VS. To better understand the effects of bevacizumab treatment, we analyzed a right VS surgically excised from an NF2 patient with bevacizumab treatment for five years and a left VS from the same patient after 12 years of treatment due to tumor growth. Next-generation sequencing shows that these NF2-related VS maintain low mutational burdens and stable microsatellite status with no new gene mutations. Comparing with the right VS from this patient prior to bevacizumab treatment, the bevacizumab-treated right VS and left VS have a greatly increased number of blood vessels. In addition, the 12-year bevacizumab-treated left VS shows extensive extravasation and hemosiderin-laden macrophages, as well as vessels with obstruction and recanalization. Consistently, the patient experienced significant blood loss during recent surgical removal of this left VS. Single-cell RNA-sequencing was performed to comprehensively analyze the transcriptional landscape of the bevacizumab-treated left VS and three other naïve NF2-associated schwannomas from patients that have not received any drug therapies. Analysis of cellular diversity via unsupervised clustering in Seurat reveals that these VS harbor several tumor SC subpopulations with gene signatures resembling repair SCs in peripheral nerve injury, along with various immune subsets and stromal cells, including fibroblasts, within their tumor microenvironment (TME). Macrophages were highly represented along with a small population of T cells and NK cells. Macrophages can be further divided into subpopulations, including those expressing different immune signatures. Both M2 and M1 macrophages were present as corroborated by immunostaining. Importantly, we identified three new or highly enriched tumor SC subpopulations with high VEGF expression, mesenchymal, or immune phenotype, respectively, in the bevacizumab-treated VS. In addition, NF2-related VS appear to contain an exhausted immune ME as they express little immunostimulatory IL-2 but high levels of TGFβ, TNFα, and interferon-γ in the immune cell subsets. Also, we detected robust expression of several immune checkpoint receptors, including TIM3 in macrophage and T cell subpopulations and VISTA and LAG3 in T cells within the TME of both naïve and bevacizumab treated VS. Together, our results suggest that targeting these negative immune checkpoints may be a viable treatment strategy for VS. The emergence of a high VEGF-expressing tumor SC subpopulation in bevacizumab-treated VS supports the idea of a novel treatment-related resistance mechanism.

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Schwannomatosis: Basic Science

A Community-Driven and Open-Source Pipeline to Annotate and Prioritize Germline Variants in Schwannomatosis

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Purpose: Schwannomatosis is a rare genetic disorder that causes the development of nerve sheath tumors. Germline mutations in *SMARCB1* and *LZTR1* are causal in a subset of cases, but in some cases the cause of the condition is unknown. The goal of the Schwannomatosis Open Research Collaborative (SORC) is to uncover genomic variants and other genomic features that contribute to disease etiology and heterogeneity in schwannomatosis.

Methods: Germline variants from 33 whole exome sequencing and 6 whole genome sequencing samples from the Synodos for Schwannomatosis initiative (Mansouri et al., 2021) are available to SORC researchers via Synapse and the NF Data Portal (nfdataportal.org). These samples were processed using the nf-core sarek 2.7.1 pipeline which called germline variants using GATK HaplotypeCaller, DeepVariant, and Strelka.

Results: In a collaboration between SORC and the Bioinformatics Research Network, we developed a pipeline to annotate and prioritize germline single nucleotide variants in schwannomatosis patients in order to identify variants for further study. The pipeline takes a VCF file as input and annotates variants with scores from 9 different tools using OpenCRAVAT. To better capture splicing variants, we also annotated variants with scores from SpliceAI v1.3.1 and SQUIRLS v2.0.0, which are both advanced machine learning methods that together offer a complimentary approach for predicting splice-disrupting variants. Next, we integrated all of the annotated scores to identify variants that are most likely to impact gene expression.

Conclusions: Our pipeline is meant to be a first step in filtering low-impact variants before proceeding with additional genomic analyses, thus increasing the power of downstream analyses compared to genome-wide analyses. The pipeline will be applied to the schwannoma germline variants that are available to SORC researchers in order to generate a list of likely expression-altering variants that may be used to identify novel genes or druggable pathways in schwannomatosis. Future SORC analyses will utilize these variants as part of integrated analyses with available gene expression and DNA methylation data. Information about the SORC and information on joining this initiative can be found at www.synapse.org/swnts.

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Dominant-Negative Pathogenic Variants in *LZTR1*-Related Schwannomatosis

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Background: Loss-of-function (LoF) and dominant-negative (DN) pathogenic variants in the *LZTR1* gene cause autosomal recessive and autosomal dominant Noonan syndrome (NS), respectively. LoF pathogenic variants are also associated with *LZTR1*-related schwannomatosis, which is inherited in an autosomal dominant manner. However, an association between DN pathogenic variants and schwannomatosis remains unknown, primarily due to a paucity of affected individuals with DN pathogenic variants. Only one presumably DN variant (p.S247N) has been identified in a patient who had both NS and schwannomas[1]; another DN variant (p.R284C) has been reported in autosomal dominant NS[1,2] and schwannomatosis[3] separately.

Methods: To delineate a relationship between DN pathogenic variants and schwannomatosis, we reviewed clinical details of two different cohorts with one of three DN (likely) pathogenic variants (p.G248R, p.P281L, and p.R284C) for the presence of schwannomas. The first cohort included children with clinical features consistent with autosomal dominant NS (age 0-17 years). The second cohort consisted of adults who underwent routine multi-gene cancer panel testing (mean age 55 years). In addition, the frequency of schwannomas was compared between the three DN and five most common LoF variants (p.Q10Afs*24, p.Q10Rfs*15, p.R210*, p.F258Lfs*93, and p.R362*) in the cancer cohort.

Results: None of the children with NS were reported to have schwannomas. In the cancer cohort, a personal history of schwannomas was reported in two of 33 patients (6.06%) with DN (likely) pathogenic variants, whereas only two of 311 patients (0.64%) with LoF pathogenic variants reported schwannomas. There was borderline statistical significance in the frequency of schwannomas between DN and LoF variants (Fisher's exact test $p=0.047$). Six variants of unknown significance (p.A116V, p.Y119H, p.Y136H, p.R283Q, p.G286R, and p.H287Y) have evidence of autosomal dominant inheritance in the literature or unpublished data. When these variants were combined with (likely) pathogenic variants, the results were similar – three of 55 patients with schwannomas (5.45%, $p=0.026$ compared to LoF).

Conclusions: To our knowledge, this is the first study to investigate an association between DN pathogenic variants and *LZTR1*-related schwannomatosis. It remains unknown whether patients with autosomal dominant NS develop schwannomas. However, our data suggests that DN pathogenic variants can cause schwannomas in some individuals and may have a higher risk of schwannomatosis, compared to LoF variants.

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Deciphering the Pathogenicity of *LZTR1* Variants in Schwannomatosis

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Purpose of study: We have previously shown that leucine zipper-like transcription regulator 1 (LZTR1) functions as an adaptor protein for cullin-3 mediated proteasomal degradation of small guanosine triphosphate hydrolases, RIT1 and MRAS. Germline variants of the *LZTR1* gene are commonly associated with schwannomatosis. Many different *LZTR1* variants have been identified in this rare genetic disorder, including splice, frameshift, nonsense, and single nucleotide variants (SNV). Although many of these are considered loss-of-function, the significance of SNV variants is unknown and many of these remain classified as variants of unknown significance (VUS). These variants span along different domains of LZTR1, which play important functional roles. For instance, it has been suggested that the Kelch and BTB-BACK domains are required for substrate and cullin-3 binding, respectively. Therefore, it is possible that VUS located in these different domains can ultimately impair the function of LZTR1. To understand the underlying molecular mechanism of schwannomatosis in the context of LZTR1, we established a biochemical pipeline to characterize a large panel of *LZTR1* schwannomatosis-associated SNV to determine their pathogenicity.

Method: Using site-directed mutagenesis, we produced a catalog of schwannomatosis-associated *LZTR1* variants and these were expressed ectopically in mammalian cells. We developed different biochemical assays that assessed the interaction between LZTR1 variants and their substrates, such as RIT1 and MRAS, their ability to degrade these substrates, as well as their capacity to interact with the ubiquitin ligase, cullin-3. We quantitatively analyzed the effect of these VUS in our assays and used a structural model of LZTR1 developed with AlphaFold to assess the effects of each variant.

Result: Characterization of the different *LZTR1* variants using our experimental pipeline demonstrated that most VUS result in their inability to bind and/or degrade the substrates: RIT1 or MRAS, or fail to engage with cullin-3.

Conclusion: Our experimental approach suggests that the majority of germline *LZTR1* VUS found in schwannomatosis act as loss-of-function variants through mechanisms consistent with the role of LZTR1 in the degradation of RIT1 and MRAS substrates.

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Drug Screening Using Patient Primary Schwannoma Cells in Three-Dimensional Spheroid Assays

Anna Nagel, PhD, *University of Central Florida*

A goal of research in this lab is to develop schwannoma patient-centric drug screening assays that can inform their clinical care. Two schwannomas were obtained with consent to participate in a precision medicine study (approved by the Institutional Review Board of the University of Central Florida). A paraspinal schwannoma was obtained from a patient with a severe *NF2* germline mutation. A peripheral schwannoma was obtained from a clinically diagnosed schwannomatosis patient with no germline or somatic mutation in *NF2, SMARCB1* or *LZTR1.* Tumor chunks were prepared for immunohistochemistry, primary schwannoma cells were isolated from freshly resected tumor, and tumor pieces were frozen in culture medium for future isolations. Schwannoma cells were used in two dimensional (2D) and three dimensional (3D) drug screening assays, immunofluorescence staining, and western blot analysis. Cells from both schwannomas grew surprisingly well as primary cells without the need for immortalization.

Schwannoma cells were isolated using digestion enzymes and cultured in Schwann cell medium (SCM, ScienCell) for one passage before seeding on standard Cellbind (Corning) plates, and ULA round-bottom plates (S-Bio) with and without Matrigel. Sphere formation was observed for 0-3 days and on day 3 cell spheres were treated with a panel of selected drugs. Drug efficiency was measured as the changes in sphere diameter and area using IncuCyte live imager. Schwannoma cells formed spheres independent of a Matrigel substrate addition, however Matrigel allowed cells to exhibit invasive phenotype characterized by extension of cellular processes beyond the outer sphere margin. Several drug treatments inhibited sphere enlargement. As a comparison, we simultaneously performed a 2D dual cell proliferation and apoptosis assay that yielded comparable results to the 3D assay.

Future validation using *in vivo* patient derived xenograft models and/or clinical data is needed to reliably assess the translatability of results obtained with the 3D spheroid drug screening assay and primary patient schwannoma cells.

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The Schwannomatosis Open Research Collaborative: A Community-Based Approach to Genomics Research

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Purpose: Schwannomatosis is a rare, understudied syndrome that causes the development of nerve sheath tumors. Recent efforts to study the genomics of schwannomatosis patients have increased our understanding of the disorder, though most studies focus only on select genes of interest. We have developed the Schwannomatosis Open Research Collaborative (SORC) with the goal of uncovering noncoding variants, coding variants in under-studied genes, and other genomic features that contribute to disease etiology and heterogeneity in schwannomatosis.

Methods: We used the nf-core sarek 2.7.1 pipeline to process 86 whole exome sequencing (WES) and 13 whole genome sequencing (WGS) samples from schwannomatosis patients, generated as part of the Children's Tumor Foundation Synodos for Schwannomatosis initiative (Mansouri et al., 2021). Germline and somatic variants, including structural variants, were detected using GATK HaplotypeCaller, DeepVariant, Mutect2, Strelka, and Manta. RNA sequencing data from these same samples were processed with the nf-core rnaseq pipeline. Processed data is available to researchers via Synapse and the NF Data Portal (nfdataportal.org).

Results: SORC decentralizes computational biology research by engaging and collaborating with researchers from around the world, building on the experiences of other community research projects such as the Open Pediatric Brain Tumor Atlas. To facilitate SORC, we use the Synapse collaborative workspace (synapse.org/swnts), Slack (tinyurl.com/schwannomatosis), and GitLab (gitlab.com/nf-community/swnts). These tools enable asynchronous research collaboration, contain guidelines for project contributions, and host code and data generated as part of the project. SORC researchers have developed a pipeline to prioritize genomic variants of interest for additional studies, compared variant calling algorithms to help determine best practices in data processing and filtering for sequencing samples, applied algorithms to detect viral signatures in schwannomatosis data, and evaluated differential gene expression signatures between schwannoma specimens and normal tibial nerve specimens from the GTEx project.

Conclusions: As a community-based project, we always welcome feedback, ideas for analyses, and additional data. To learn more about SORC or to contribute ideas or data to SORC, please scan the QR code.

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Schwannomatosis: Clinical Science (The Poster Sessions are a Non-CME Activity)

LAST	FIRST	POSTER	TITLE
Belling	Elizabeth	158	Survey of Current Approaches and Opinions of Non-Specialist and Specialist Clinicians to Unexpected Findings in Neurofibromatosis and Schwannomatosis-Related Genes on Oncology Gene Panels
Powers	Kelly	160	Clinical and Prognostic Implications of *LZTR1* Molecular Genetic Overlap in Noonan Syndrome and Schwannomatosis

Schwannomatosis: Clinical Science

Survey of Current Approaches and Opinions of Non-Specialist and Specialist Clinicians to Unexpected Findings in Neurofibromatosis and Schwannomatosis-Related Genes on Oncology Gene Panels

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Neurofibromatosis (NF) and schwannomatosis (SWN) are conditions caused by pathogenic/likely pathogenic (P/LP) variants in *NF1, NF2, SMARCB1,* and *LZTR1.* Recently, these genes were added to multigene hereditary cancer panels, leading to a chance of unexpected P/LP variants and variants of uncertain significance (VUS) being identified. The purpose of the study was to investigate how providers referred and managed patients with unexpected variants based on the gene, the variant classification and presence or absence of clinical features. Anonymous online surveys were distributed through NSGC, ABGC, and LIU alumni listservs to genetic counselors who order oncology panels, and to NF-specialists through the NFCN listserv. 38 genetic counselors (non-specialists) and 7 NF-specialists completed the surveys. Most non-specialists (97.4-100%) and NF-specialists (85.7-100%) agreed that they would refer or accept a referral of a patient with a P/LP variant identified on an oncology panel regardless of clinical features, or a VUS with clinical features in any of the genes. However, they disagreed on referrals for asymptomatic patients with a VUS. Most non-specialists (92.1-100%) would not refer these patients for further evaluation, differing from the 71.4% of NF-specialists who indicated they would accept a referral in *NF2* ($p<0.001$) and the approximately half of NF-specialists (42.9%-57.1%) that would accept a referral in *NF1, SMARCB1,* or *LZTR1* ($p<0.001$-0.039). Statistically significant variables impacting providers' choices were identified for specific genes and variant classifications including practice length, setting, awareness of an NF clinic, familiarity with NF and SWN conditions, and the percentage of pediatric patients seen by an NF-specialist. However, no variable globally impacted providers' choices across all genes or variant classifications. 64.4% of participants reported previous experiences with unexpected *NF1, NF2,* and/or *LZTR1* variants, mostly in *NF1* (73.5%). This pilot study highlights discrepancies between specialists and non-specialists for an asymptomatic patient with an unexpected VUS found on an oncology panel, and brings attention to the need for further investigation of factors impacting referral patterns and care of patients with unexpected variants. It also highlights the differences in opinion on how to care for patients with unexpected variants, particularly between specialty and non-specialty providers, suggesting the need to develop and disseminate standard guidelines.

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Clinical and Prognostic Implications of *LZTR1* Molecular Genetic Overlap in Noonan Syndrome and Schwannomatosis

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Noonan syndrome (NS) is a common genetic condition characterized by congenital heart defects, developmental delays, short stature and distinctive facial features. It is caused by pathogenic variants in multiple genes involving the RAS-MAPK pathway, including *LZTR1* which has also been associated with LZTR1-associated schwannomatosis. Schwannomatosis predisposes affected individuals to the development of benign nerve sheath tumors throughout the nervous system. The recent advent of more frequent genomic testing and the use of diagnostic panels for disorders considered to be Rasopathies have led to the detection of *LZTR1* variants in many patients but the clinical long-term implications of this finding are poorly understood.

We identified 4 children with varying phenotypes, all of whom were found to have pathogenic variants in *LZTR1* on Rasopathy, neurodevelopmental disorders and exome panels. Patient 1 presented at 3 years of age with bilateral hearing loss as well as gross motor and speech delays. On exam, he was noted to have epicanthal folds, mildly low set ears and pectus excavatum but he did not meet the clinical criteria for NS. Patient 2 presented with a ventricular septal defect and pulmonic stenosis shortly after birth and later developed short stature and facial features consistent with NS including hypertelorism and a broad forehead. Patient 3 presented at 18 years of age with autism, intellectual disability and new tonic seizures but did not have full NS clinical features. Patient 4 presented as a neonate with an atrial septal defect and later developed classic facial features, short stature and developmental delays consistent with NS. An MRI of the brain in patients 1 and 2 showed no evidence of schwannomas. Patient 4 had a normal lumbar spine MRI.

As the majority of genetic testing under these circumstances is done in young children and schwannomatosis typically does not appear until the later decades of life, interpretation of the identification of an *LZTR1* variant presents a challenge to clinicians. Peripheral nerve sheath tumors have been reported in NS and clinical overlap between these 2 conditions clearly exists. Further research is needed to better understand this phenotypic heterogeneity and help develop clinical management guidelines.

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