
CHILDREN'S TUMOR FOUNDATION
ENDING NF
THROUGH RESEARCH
2020 VIRTUAL
NF CONFERENCE
JUNE 15 - 16, 2020
Keeping the NF community connected

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Dear NF Conference Attendees,

I am thrilled to welcome you to the 2020 virtual NF Conference on behalf of the Children's Tumor Foundation. For most of the world, 2020 is mainly only a sad year characterized by isolation, separation, and disease. However, for the NF community, this year already has a few major first-ever wins: the first-ever drug approval for NF1, the first-ever platform trial for NF2, and the first-ever clinical trials for schwannomatosis, and now the first-ever virtual NF Conference. For CTF and the NF community, I hope 2020 will be remembered as a year of innovation, creativity, and the strength of a community that is not disrupted by a virus, but keeps standing united to end NF.

It is, therefore, our absolute honor to bring the work of our ever-strong community together virtually. Even if COVID has tried to stop us from coming together, this year's amazing co-chairs, Drs. Matthias Karajannis, Conxi Lazaro, and Nicole Ullrich, collaborated to create a shortened but powerful virtual event. Since we all hope that we will be able to be united in person at the NF conference in Rotterdam in December, for this meeting, we have decided to bring the 'hottest' NF advancements to your living room! We want to make sure that all the new exciting discoveries are shared so that the field is not slowed down by this virus. The agenda is shorter than usual, but it is maximally inclusive to reach all the time zones. It will be a truly global event.

We will celebrate the 30th anniversary of the discovery of the *NF1* gene with no one less than Dr. Francis Collins, who is the only keynote and opening speaker this year. He has taken the time to make an amazing opening video for you.

This year's conference will be one track only. Following the clinical care symposium, all platform presentations covering all forms of NF are a nice mix of basic, translational, and clinical science, carefully selected by the reviewers. Moreover, all posters will be available as e-posters, so there are still ample opportunities to interact.

We will also take a moment to celebrate: the 30th anniversary of the *NF1* gene discovery and the Koselugo approval.

I wish you a short but still enriching, inspiring, and fun days. Enjoy and be inspired by great successes and discoveries.

While we all hope to be together again soon, in the meantime, let's never stop our efforts to end NF!

ANNETTE BAKKER
President

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CHILDREN'S TUMOR FOUNDATION
ENDING NF
THROUGH RESEARCH

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Georg Terstappen (DDIRR) - member
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Members of the RAB are also the 50+ experts and patient representatives that agreed to conduct rigorous peer-review of scientific proposals

CHILDREN'S TUMOR FOUNDATION FUNDING OPPORTUNITIES IN 2021

The Children's Tumor Foundation has always striven to fund innovative NF research through its investigator-initiated award programs, namely the Young Investigator Award (YIA), Drug Discovery Initiative (DDI), Drug Discovery Initiative Registered Reports (DDIRR), and Clinical Research Award (CRA). In 2019, CTF instituted the Discovery Fund, a $8 million fundraising campaign that will support all the investigator-initiated award programs for the next 3-5 years. Towards this, we are very happy to announce that we have successfully completed the campaign and secured the $8 million of funding for all the above-mentioned programs.

All CTF award programs are currently under a revision of terms and conditions, eligibility, award amounts, and overall structure to broaden accessibility and opportunity to apply. We expect that this change will increase the number of applications for all programs and allow for more studies to be funded. This process will not delay the announcement of all the 2020 award cycles that will happen as described below. Please refer to our website on or after July 20th, 2020 for a complete list of changes to our award programs – https://www.ctf.org/research/awards-applications for more information.

YOUNG INVESTIGATOR AWARDS (YIA)

The YIA program will once again be expanded to include all manifestations of all types of NF (NF1, NF2, schwannomatosis). Furthermore, a subset of the available funds will be dedicated exclusively to funding NF2 and schwannomatosis-focused studies, and such proposals will be evaluated separately from the NF1-focused proposals to ensure that at least a minimum number of NF2/schwannomatosis studies can be initiated. We hope that these changes will encourage all early-stage NF researchers, especially those interested in NF2 and schwannomatosis, to consider applying to the YIA program!

AWARD AMOUNTS:
Award amounts vary depending on pre-doc or post-doc status of the applicant and level of training. WE ARE REVIEWING THE YIA AWARD AMOUNTS. Please refer to our website on or after July 20th, 2020 https://www.ctf.org/research/awards-applications for more information.

IMPORTANT DATES TO NOTE
The next cycle for YIAs will start in **December 2020.** Exact dates for submission of application materials will be provided on the CTF website shortly.

DRUG DISCOVERY INITIATIVE REGISTERED REPORTS (DDIRR)

DDIRR Awards is a funder-publisher partnership that integrates the Registered Reports model in the grant application process. This model will allow for more rigorous, reproducible and transparent science, guaranteeing its awaradees with an in-principle acceptance (IPA) to publication in the journal PLOS ONE, regardless of study outcome.

AWARD AMOUNTS:
up to $40,000 for in-vitro studies
up to $85,000 for in-vivo studies

IMPORTANT DATES TO NOTE
The next round of DDIRRs will begin in the **fall of 2020.** Exact dates for submission of application materials will be provided on the CTF website shortly.

CLINICAL RESEARCH AWARDS (CRA)

To accelerate NF research, CTF launched the Clinical Research Award (CRA) program in 2007 to support clinical studies focusing on interventions which improve care and quality of life in individuals with NF, including early stage pilot clinical trials of candidate therapeutics or interventions for treatment of manifestations of NF1, NF2 and schwannomatosis. In addition, these awards support innovative clinical trial enabling studies, for example, biomarker development, patient-reported outcome measures, and studies to better characterize the clinical manifestations of NF.

AWARD AMOUNTS:
Funding of up to $150,000 is offered for these two-year awards.

IMPORTANT DATES TO NOTE
The next award cycle for CRAs will begin in the **fall of 2020.** Exact dates for submission of application materials will be provided on the CTF website shortly.

Please connect with any of the CTF staff for any questions. We look forward to receiving your applications!

The Friedrich von Recklinghausen Award: Neurofibromatosis Tradition and Progress

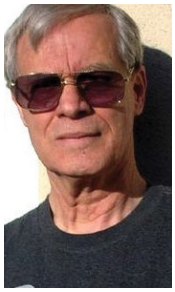
The Children's Tumor Foundation's Friedrich von Recklinghausen Award is given to individuals in the professional neurofibromatosis community who have made significant contributions to neurofibromatosis research or clinical care. It is named after Friedrich Daniel von Recklinghausen (1833-1910), the German physician who first described 'von Recklinghausen's disease' – what we now know as neurofibromatosis type 1.

2020 Friedrich von Recklinghausen Award Recipient

It is with great pleasure that the Children's Tumor Foundation announces the 2020 recipient of the Friedrich von Recklinghausen Award, Prof. Dr. D. Wade Clapp, of the Indiana University School of Medicine, where he serves in multiple capacities. He is the Richard L. Schreiner Professor and Chairman of the Department of Pediatrics, physician-in-chief for Riley Hospital for Children and a Professor of Microbiology & Immunology/Biochemistry and Molecular Biology. Dr. Clapp is one of the rare physician-scientists, who is making significant breakthrough contributions to all forms of NF. From his contributions to the basic understanding of NF to the creation of key preclinical models, he is considered by many to be the global gold standard in this broad role. Dr. Clapp's list of accomplishments in NF is very long, and he truly stands out in his commitment to unique collaborations. He was a principal investigator in the NF preclinical consortium and Synodos for NF2. They are two transformational team science efforts that have been successfully building a preclinical pipeline that front-load, accelerate and diversify the drug candidates for clinical trials. Dr Clapp also has a long track-record as an out of the box thinker when it comes to leveraging previous investments. The most recent one being the National Cancer Institute's Specialized Program of Research Excellence (SPORE), a 5-year, $12 million national research project, leveraging his work in the NF preclinical consortium and aimed at efficiently bringing new treatments for pediatric cancers, with the focus being on NF1. We are very proud and delighted that the community honored Dr. Clapp's major achievements to NF by selecting him as the 2020 FVR award winner.

Please join us in congratulating Dr. Clapp for this well-deserved award!

The following are the most recent recipients of the Award:



2008
Vincent 'Vic' Riccardi, MD
The Neurofibromatosis Institute

2009
Luis Parada, PhD
University of Texas Southwestern

2010
Nancy Ratner, PhD
Cincinnati Children's Hospital Medical Center

2012
David Gutmann, MD, PhD
Washington University

2013
Brigitte Widemann, MD
National Cancer Institute

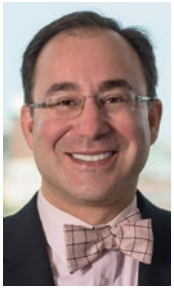
2014
Gareth Evans, MD
St. Mary's Hospital, University of Manchester, UK

2015
Eric Legius, MD, PhD
University of Leuven, Belgium

2016
David Viskochil, MD, PhD
University of Utah

2017
Karen Cichowski, PhD
Harvard Medical School

2018
Ludwine Messiaen, PhD
University of Alabama at Birmingham



2019
Scott Plotkin, MD, PhD
Massachusetts General Hospital
Harvard Medical School

Schedule At-A-Glance

	TIME		EVENT
MONDAY JUNE 15	9:45 AM	10:00 AM	Welcome - Conference Co-Chairs
	10:00 AM	10:10 AM	Opening Remarks - Francis S. Collins, MD, PhD, Director, National Institutes of Health
	10:10 AM	11:40 AM	**CLINICAL CARE SYMPOSIUM**
	11:40 AM	12:00 PM	Break
	12:00 PM	2:00 PM	**COMBINED PLATFORM SESSION**
	2:00 PM	2:15 PM	Break
	2:15 PM	2:45 PM	Recognition and Reflection: 30th Anniversary of the Cloning of the *NF1* Gene
	2:45 PM	3:15 PM	The Long Journey to the Clinic: First-Ever FDA Approved Treatment for NF1
TUESDAY JUNE 16	9:30 AM	10:00 AM	Poster Competition Winners
	10:00 AM	12:00 PM	**COMBINED PLATFORM SESSION**
	12:00 PM	12:15 PM	Break
	12:15 PM	12:30 PM	INTUITT - Brigatinib Trial for NF2
	12:30 PM	12:45 PM	European Initiatives - EU PEARL
	12:45 PM	1:15 PM	Revision of the Diagnostic Criteria - Update
	1:15 PM	1:30 PM	NF Clinical Trials Consortia - Update
	1:30 PM	1:45 PM	Presentation of the 2020 Friedrich von Recklinghausen Award
	1:45 PM	2:00 PM	Final Remarks and Meeting Adjournment

THE MEETING TIMES LISTED ARE EASTERN DAYLIGHT TIME

BIOS

2020 NF Conference Co-Chairs

Matthias Karajannis, MD, MS, *Chief of the Pediatric Neuro-Oncology Service, Memorial Sloan Kettering Cancer Center*

Dr. Karajannis has been serving as Chief of the Pediatric Neuro-Oncology Service at Memorial Sloan Kettering Cancer Center since 2017. Previously, he directed the NF Clinical Research Program and the Pediatric Hematology/Oncology Fellowship Training Program at NYU Langone Medical Center. He received his MD from the Free University Berlin, Germany, completed residency training at Duke University Medical Center, and fellowships in pediatric hematology/oncology, as well as pediatric neuro-oncology, at Memorial Sloan-Kettering Cancer Center. In addition to treating children and young adults with brain and spinal cord tumors including patients with neurofibromatosis, Dr. Karajannis has a special interest in caring for patients with NF2. His clinical and translational research aims at developing novel diagnostic tools and therapies for patients with nervous system tumors, including NF2 related tumors.

Conxi Lázaro, PhD, *Head of the Molecular Diagnostic Unit, Hereditary Cancer Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain*

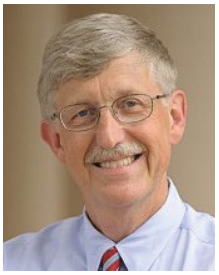
Conxi Lázaro studied biology in the University of Barcelona. Her PhD thesis was completed in the Medical Genetics Department of the Institut de Recerca Oncològica (IRO) under the supervision of Dr. Estivill. She worked as a molecular geneticist at the Genetics Service of Hospital Clínic and after that she returned to IRO to lead the Neurofibromatosis 1 group as well as to participate in several research projects related to common multifactorial disorders (asthma and psoriasis). During 2003/04 she was a visiting scientist in the Cancer Center of the Massachusetts General Hospital (Harvard, Boston).

In 2006 she obtained her current position as Director of the Molecular Diagnostics Unit for Hereditary Cancer at ICO. In addition to the routine clinical work, Dr. Lázaro leads two lines of research, one focused on deciphering the genetic basis underlying the Hereditary Cancer Syndrome participating in large consortia such as CIMBA and ENIGMA, and another focused on the development of new therapeutic strategies for Neurofibromatosis Type 1. Last year she was a visiting scientist at Mount Sinai Hospital in Toronto (Canada).

Nicole J Ullrich, MD, PhD, *Associate Professor of Neurology, Boston Children's Hospital*

Nicole J Ullrich, MD, PhD is currently Associate Professor of Neurology at Boston Children's Hospital, where she serves as Associate Director and Director of Clinical Trials in the Multidisciplinary Neurofibromatosis Program at Boston Children's Hospital and Director of Neurologic NeuroOncology at Dana-Farber/Boston Children's Cancer and Blood Disorders Center. She completed her medical and graduate degrees at Yale University followed by residency in Pediatrics and Neurology at Boston Children's Hospital, where she then did her fellowship in NeuroOncology before joining the faculty in the Department of Neurology. Her research focuses on neurologic and oncologic complications of NF1 and the long-term neurologic complications of childhood systemic cancer and brain tumors. Dr. Ullrich has been involved in the design and execution of several clinical trials for complications of NF1 through the Neurofibromatosis Clinical Trials Consortium, where she is currently the site principal investigator for the Harvard site that includes Boston Children's Hospital, Dana-Farber Cancer Institute and Massachusetts General Hospital. Within the consortium, she is former chair of the Low Grade Glioma committee and currently co-chair of the Neurocognitive Committee. She serves as co-Chair of the Clinical Research Award committee and is a member of the Clinical Care Advisory Board for the Children's Tumor Foundation. She also serves on the board of NF Northeast. Most recently, she participated in the working groups to revise and update the diagnostic criteria for the neurofibromatosis and in the updated AAP health supervision guidelines for children with NF1.

2020 NF Conference Keynote Speaker



Francis S. Collins, MD, PhD, *Director, National Institutes of Health (NIH)*

Francis S. Collins, MD, PhD was appointed the 16th Director of the National Institutes of Health (NIH) by President Barack Obama and confirmed by the Senate. He was sworn in on August 17, 2009. On June 6, 2017, President Donald Trump announced his selection of Dr. Collins to continue to serve as the NIH Director. In this role, Dr. Collins oversees the work of the largest supporter of biomedical research in the world, spanning the spectrum from basic to clinical research.

Dr. Collins is a physician-geneticist noted for his landmark discoveries of disease genes and his leadership of the international Human Genome Project, which culminated in April 2003 with the completion of a finished sequence of the human DNA instruction book. He served as director of the National Human Genome Research Institute at NIH from 1993-2008.

Before coming to NIH, Dr. Collins was a Howard Hughes Medical Institute investigator at the University of Michigan. He is an elected member of the National Academy of Medicine and the National Academy of Sciences, was awarded the Presidential Medal of Freedom in November 2007, and received the National Medal of Science in 2009. In 2020, he was elected as a Foreign Member of the Royal Society (UK) and was also named the 50th winner of the Templeton Prize, which celebrates scientific and spiritual curiosity.

AGENDA

Monday · June 15, 2020

9:45 AM	**10:00 AM**	**WELCOME – Conference Co-Chairs**
10:00 AM	**10:10 AM**	**OPENING REMARKS – Francis S. Collins, MD, PhD, Director, National Institutes of Health**
10:10 AM	**11:40 AM**	**CLINICAL CARE SYMPOSIUM**
10:10 AM	10:20 AM	**Mission of the Clinical Care Advisory Board** Scott Plotkin, MD, PhD, *Massachusetts General Hospital, Harvard Medical School, Chair CCAB*
10:20 AM	10:30 AM	**Quality Guidelines for NF Care** Justin Jordan, MD, MPH, *Massachusetts General Hospital, Harvard Medical School*
10:30 AM	10:40 AM	**The Impact of COVID-19 on NF Care and Research** Heather Radtke, MS, CGC, *Children's Tumor Foundation*
10:40 AM	10:50 AM	**Telemedicine: Promises and Challenges** Jaishri Blakeley, MD, *Johns Hopkins University Medical Center*
10:50 AM	**11:40 AM**	**CASE STUDIES**
10:50 AM	11:15 AM	**Case Presentation - NF2** Presenter: Oliver Adunka, MD, *The Ohio State University*
11:15 AM	11:40 AM	**Case Presentation - NF1** Presenter: Kaleb Yohay, MD, *NYU Langone Medical Center* Discussants: Laura Klesse, MD, *UT Southerwestern Medical Center;* Christopher Weldon, MD, PhD, *Boston Children's Hospital*
11:40 AM	12:00 PM	**BREAK**
12:00 PM	**2:00 PM**	**COMBINED PLATFORM SESSION**
12:00 PM	12:15 PM	**SWN Basic Platform: Genomic and Epigenomic Hallmarks of Schwannomatosis Schwannomas** Sheila Mansouri, PhD, *University of Toronto*
12:15 PM	12:30 PM	**NF1 Clinical Platform: Liquid Biopsy Detection of MPNST vs. Plexiform Neurofibroma in NF1 Patients Using Cell-Free DNA Ultra-Low-Pass Whole-Genome Sequencing** Paul Jones, BS, *Washington University*
12:30 PM	12:45 PM	**NF2 Basic Platform: YAP-Mediated Recruitment of YY1 and EZH2 Represses Transcription of Key Cell Cycle Regulators** Joseph Kissil, PhD, *Scripps Research Institute*
12:45 PM	1:00 PM	**NF1 Basic Platform: Stepwise Tumor Progression of Plexiform Neurofibroma to Atypical Neurofibroma and then MPNST in a Mouse Model with CDKN2a Alteration** Melissa Perrino, MD, *Cincinnati Children's Hospital Medical Center*
1:00 PM	1:15 PM	**NF1 Clinical Platform: The Impact of MEK Inhibitor Therapy on Neurocognitive Functioning in Children and Adults with NF1** Karin Walsh, PsyD, *Children's National Medical Center*
1:15 PM	1:30 PM	**NF1 Basic Platform: Minipig Models of Neurofibromatosis Type 1 for Therapeutic Development** Adrienne Watson, PhD, *Recombinetics, Inc.*
1:30 PM	1:45 PM	**NF2 Clinical Platform: Evaluating the Performance of the NF2 Genetic Severity Score and the Potential Impact of Using Functional Assays to Improve Prognosis Prediction in a Cohort of Spanish Patients** Nuria Catasus-Segura, MS, *Germans Trias i Pujol Research Institute*
1:45 PM	2:00 PM	**NF1 Basic Platform: Oncolytic Adenovirus as a Therapy for Malignant Peripheral Nerve Sheath Tumors** Julia Nikrad, BA, *University of Minnesota*
2:00 PM	2:15 PM	**BREAK**
2:15 PM	**2:45 PM**	**RECOGNITION AND REFLECTION: 30TH ANNIVERSARY OF THE CLONING OF THE *NF1* GENE** Speakers: David Viskochil, MD, PhD, *University of Utah;* Margaret (Peggy) Wallace, PhD, *University of Florida*
2:45 PM	**3:15 PM**	**THE LONG JOURNEY TO THE CLINIC: FIRST-EVER FDA APPROVED TREATMENT FOR NF1** Speakers: Andrea Gross, MD, *National Cancer Institute/NIH;* Brigitte Widemann, MD, *National Cancer Institute* **NF Community celebrates the first-ever FDA-approved treatment for NF1 inoperable plexiform neurofibromas**

AGENDA

Tuesday · June 16, 2020

9:30 AM	**10:00 AM**	**POSTER COMPETITION WINNERS** Oral presentations of top three winners of both the basic and clinical science poster competition. This competition focuses on the work of the young researchers and clinicians in the NF field.
10:00 AM	**12:00 PM**	**COMBINED PLATFORM SESSION**
10:00 AM	10:15 AM	**NF1 Basic Platform: Use of Nf1-KO Mice for Exploring Efficacy of Binimetinib and Selumetinib in Cutaneous Neurofibroma Prevention** Piotr Topilko, PhD, *Institut Mondor de Recherche Biomedicale (IMRB), France*
10:15 AM	10:30 AM	**NF2 Basic Platform: A Molecular Testing Summary of Sporadic Vestibular Schwannoma** Miriam Smith, PhD, *University of Manchester, UK*
10:30 AM	10:45 AM	**NF1 Clinical Platform: Selumetinib in Children with Clinically Asymptomatic Inoperable Neurofibromatosis Type 1 Related Plexiform Neurofibromas** Brittany Glassberg, BS, *NIH*
10:45 AM	11:00 AM	**NF1 Basic Platform: Social Behavioral Deficits in NF1 Emerge from Peripheral Chemosensory Neuron Dysfunction** Emilia Moscato, PhD, University of Pennsylvania
11:00 AM	11:15 AM	**NF2 Clinical Platform: Long-Term Follow-Up of Schwannoma Growth Behavior an Adult Neurofibromatosis Type 2 and Schwannomatosis Patients Using Whole-Body MRI** Ina Ly, MD, *Massachusetts General Hospital*
11:15 AM	11:30 AM	**NF1 Basic Platform: Severe Phenotype-Linked NF1 Substitution Mutations in Codons 844-848 of Neurofibromin Act in a Dominant Negative Manner by Destabilizing Wild-Type Protein** Lucy Young, PhD, *University of California San Francisco*
11:30 AM	11:45 AM	**NF1 Clinical Platform: Supporting NF Education and Parent Advocacy, Pandemic and Beyond** Meredith Chambers, MS, *Boston Children's Hospital*
11:45 AM	12:00 PM	**NF1 Clinical Platform: Development of Topical MEK Inhibitor, NFX-179, for the Treatment of Cutaneous Neurofibromas in Neurofibromatosis Type 1** Kavita Sarin, MD, PhD, *Stanford University Medical Center*
12:00 PM	12:15 PM	**BREAK**
12:15 PM	**12:30 PM**	**INTUITT – BRIGATINIB TRIAL FOR NF2** Speaker: Scott Plotkin, MD, PhD, *Massachusetts General Hospital* **INTUITT-NF2, an innovative platform trial which will evaluate multiple treatments simultaneously in order to minimize potential negative impact on patients while at the same time maximize response opportunities to specific drugs within the same multi-arm trial.**
12:30 PM	**12:45 PM**	**EUROPEAN INITIATIVES – EU PEARL** Speaker: Rianne Oostenbrink, MD, PhD, MSc, *Erasmus MC, Rotterdam, Netherlands* • **The EU Patient-cEntric clinicAl tRial pLatforms (EU-PEARL) is a unique public-private strategic partnership focused on creating a framework to set up adaptive clinical trial platforms of the future** • **EU-PEARL aims to define a novel enabling infrastructure where pharmaceutical companies and healthcare providers can work together and integrated research platforms will become an obtainable alternative** • **EU-PEARL hopes to transform clinical trials into a cross-company collaborative, multi-compound platform focused on patients**
12:45 PM	**1:15 PM**	**REVISION OF THE DIAGNOSTIC CRITERIA – UPDATE** Speakers: Gareth Evans, MD, *University of Manchester, UK;* Susan Huson, MD, *St. Mary's Hospital, Manchester, UK;* Eric Legius, MD, PhD, *University of Leuven, Belgium;* Ludwine Messiaen, PhD, *University of Alabama at Birmingham;* Scott Plotkin, MD, PhD, *Massachussetts General Hospital;* Pierre Wolkenstein, MD, PhD, *Hôpital Henri-Mondor Ap-Hp, France*
1:15 PM	**1:30 PM**	**NF CLINICAL TRIALS CONSORTIA – UPDATE** Speaker: Michael Fisher, MD, *Children's Hospital of Philadelphia*
1:30 PM	**1:45 PM**	**PRESENTATION OF THE 2020 FRIEDRICH VON RECKLINGHAUSEN AWARD**
1:45 PM	**2:00 PM**	**FINAL REMARKS AND MEETING ADJOURNMENT – Conference Co-Chairs**

Speaker Abstracts

Platform: Genomic and Epigenomic Hallmarks of Schwannomatosis Schwannomas

Monday, June 15, 12:00pm – 12:15pm

Sheila Mansouri, PhD, *MacFeeters-Hamilton Center for Neuro-Oncology Research, Princess Margaret Cancer Center, Toronto, Ontario, Canada*

Schwannomatosis (SWNTS) is a tumor predisposition syndrome that manifests largely as numerous extremely painful peripheral schwannomas. Although germline mutations in *SMARCB1* and *LZTR1,* somatic mutations in *NF2,* and loss of heterozygosity (LOH) in chromosome 22q have been identified in a portion schwannomas that arise in SWNTS, recurrent epigenetic and genetic alterations that contribute to tumorigenesis and pain in majority of the cases remain unknown. Furthermore, genomic and epigenomic signatures that differ between SWNTS and the histologically similar non-syndromic schwannomas, are not well understood. In this study, we performed epigenetic, genetic, and transcriptomic profiling of the largest cohort of 165 SWNTS examined to date. We identified specific DNA methylation profiles associated with the anatomic location of the tumors. Our whole exome sequencing showed that SWNTS harbour a low tumor mutation burden. Comparison with non-syndromic schwannomas demonstrated that SWNTS had more extensive chromosomal copy number alterations, particularly cases with germline mutations in *LZTR1* and painful SWNTS. Through whole genome sequencing, we identified several novel recurrent non-22q structural variants and our transcriptome analysis detected the *SH3PXD2A-HTRA1* gene fusion in 14% of SWNTS. This fusion was more prevalent in tumors with germline mutations in *LZTR1* and familial SWNTS. Copy number variation, germline *LZTR1* mutations, *SH3PXD2A-HTRA1* gene fusion, gender, and tumor location were among attributes associated with painful SWNTS. This is the first study to demonstrate the comprehensive genomic landscape of SWNTS and molecular alterations associated with pain in this disease.

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Platform: Liquid Biopsy Detection of MPNST vs. Plexiform Neurofibroma in NF1 Patients Using Cell-Free DNA Ultra-Low-Pass Whole-Genome Sequencing

Monday, June 15, 12:15pm – 12:30pm

P. A. Jones, BS, *Washington University School of Medicine, Department of Radiation Oncology, Saint Louis, MO*

Purpose: Plexiform neurofibromas (PN) are common, benign tumors in patients with neurofibromatosis type I (NF1) but have the risk for transformation into malignant peripheral nerve sheath tumors (MPNST). It is critical to detect the transformation of PN to MPNST early, as early detection improves long-term survival. Unfortunately imaging unreliably distinguishes MPNST from PN, thus the malignancy may progress before being identified. To address this, we developed and applied liquid biopsy cell-free DNA (cfDNA) technology based on ultra-low- pass whole-genome sequencing (ULP-WGS). We hypothesized that patients with MPNST have significantly higher circulating-tumor DNA (ctDNA) levels in their cfDNA than those with PN.

Methods: Blood was collected from NF1 MPNST patients (n=8) and NF1 PN patients (n=19) at several time points, along with age-matched healthy donors (n=6). cfDNA was isolated from 2-8 mL of plasma and sequenced on an Illumina Hiseq X10. After mapping sequencing reads, the number of reads per sample was downsampled to 10 million and informatically selected for short fragment lengths between 95 and 150 bp. The proportion of ctDNA within the cell-free compartment was calculated based on copy number alterations (CNAs) using ichorCNA (Broad). Genome-wide CNAs were segmented using CNVkit v0.9, after which NF1- and transformation-associated mutations were queried. In an exploratory analysis, an additional set of PN patients (n=38) from the National Cancer Institute (NCI) was included.

Results: Following sequencing, mapping and downsampling of plasma cfDNA, the median depth-of- coverage was 0.6x; sequencing depth did not correlate with clinical status or ctDNA tumor- fraction (Fisher's $p>0.1$). Focal copy number loss in *SUZ12, TP53* and *CDKN2A* was observed in MPNST patient plasma (n=3;38%). As expected, focal copy number loss in NF1 was observed in all PN (n=19;100%) and MPNST (n=8;100%) plasma but not in healthy donor plasma (n=0;0%). A ctDNA cutoff of 2.5% stratified PN (low tumor-fraction) from MPNST (high tumor-fraction) effectively (chi-squared $p=0.05$). Healthy donor, PN and pre- treatment MPNST groups showed significant differences in ctDNA levels by one-way ANOVA ($p=0.006$), with MPNST patients harboring significantly higher levels of tumor-derived cfDNA compared to PNs and healthy controls (chi-squared $p=0.002$; 0.038%vs.0.018; 2.1-fold).
These differences persisted when 38 PN patients from the NCI (n=38) were added for analysis (chi-squared $p=0.037$; 0.038%vs.0.026%; 1.5-fold).

Conclusion: Our results suggest that ctDNA analysis by ULP-WGS can distinguish MPNST from PN, thus potentially enabling early cancer detection in NF1 patients.

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Platform: YAP-Mediated Recruitment of YY1 and EZH2 Represses Transcription of Key Cell Cycle Regulators

Monday, June 15, 12:30pm – 12:45pm

Joseph L. Kissil, PhD, *Department of Molecular Medicine, The Scripps Research Institute, Jupiter, FL*

The Hippo pathway mediates contact inhibition of cell proliferation through control of the transcriptional regulators YAP (yes-associated protein) and TAZ. Upon extracellular stimuli such as cell-cell contact, the pathway negatively regulates YAP through cytoplasmic sequestration. Under conditions of low cell density, YAP is nuclear and associates with enhancer regions and gene promoters. YAP is mainly described as a transcriptional activator of genes involved in cell proliferation and survival. Using a genome-wide approach, we show here that, in addition to its known function as a transcriptional activator, YAP functions as a transcriptional repressor by interacting with the multifunctional transcription factor Yin Yang 1 (YY1) and Polycomb repressive complex (PRC2) member EZH2. YAP co-localized with YY1 and EZH2 on the genome to transcriptionally repress a broad network of genes mediating a host of cellular functions, including repression of the cell-cycle kinase inhibitor p27, whose role is to functionally promote contact inhibition. This work unveils a broad and underappreciated aspect of YAP nuclear function as a transcriptional repressor and highlights how loss of contact inhibition in cancer is mediated in part through YAP repressive function.

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Platform: Stepwise Tumor Progression of Plexiform Neurofibroma to Atypical Neurofibroma and then MPNST in a Mouse Model with CDKN2a Alteration

Monday, June 15, 12:45pm – 1:00pm

Melissa Perrino, MD, *Cincinnati Children's Hospital Medical Center*

Neurofibromatosis Type 1 (NF1) is a monogenic, inherited disorder that predisposes affected individuals to tumors of the peripheral nervous system. Plexiform neurofibromas (PN) are benign nerve sheath tumors, characterized by biallelic Schwann cell mutations in the *NF1* gene, which can demonstrate significant morbidity due to their involvement in large nerves. Atypical neurofibromas (ANF) show additional frequent loss of *CDKN2A*, (*Ink4a*/*Arf* in mouse), and may be precursor lesions to malignant peripheral nerve sheath tumors (MPNST), highly aggressive sarcomas contributing to early mortality in NF1 patients. We created a mouse model of NF1 tumor progression that combines loss of *Nf1* in developing Schwann cells with global *Ink4a*/*Arf* loss (+/- or -/-). Loss of *Ink4a*/*Arf* correlates with decreased senescence in paraspinal nerves before tumors are present. By six months of age, each mouse had 3-5 paraspinal tumors. Of the paraspinal tumors, 63% were histologically classified as PN and 37% as ANF. Also, 40% of mice had superficial tumors, all of which were GEM-PNST containing regions of nerve-associated PN or ANF surrounded by high grade tumor.

All superficial GEM-PNST tumors allografted into immunodeficient mice formed GEM- PNST. Of paraspinal tumors allografted into immunodeficient mice, 8/20 (40%) grew and developed histology of GEM-PNST. This result confirms the susceptibility of ANF to transformation and is consistent with the incidence of ANF in this model. Paraspinal tumors allografted into immunocompetent mice also formed GEM-PNST, but with significant delay and reduced incidence (2/9, 22.2%) in the presence of an intact immune system. Mechanistically, *Ink4a*/*Arf* loss of heterozygosity was absent in paraspinal tumors and in allografts derived from paraspinal tumors and occurred in only one third of GEM-PNST. However, all allografted tumors showed reduced p16 protein expression, suggesting posttranscriptional regulation of expression. We performed preliminary RNA sequencing analysis of paraspinal tumors versus PN and GEM-PNST. Paraspinal tumors sub-clustered into clusters #1 (similar to PN) and #2 (similarities to GEM-PNST), giving insight into possible sequence of transcription alterations acquired after neurofibroma formation and prior to transformation into GEM-PNST. Thus, we recapitulate peripheral nerve tumor progression in NF1 and provide preclinical platforms for therapeutic testing in an intact nervous system at each tumor grade.

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Platform: The Impact of MEK Inhibitor Therapy on Neurocognitive Functioning in Children and Adults with NF1

Monday, June 15, 1:00pm – 1:15pm

Karin S. Walsh, PsyD, *Children's National & The George Washington School of Medicine*

Background: Cognitive impairments in NF1 are a significant life-long morbidity and the lack of effective treatments remains a significant unmet need. The aim of this study was to examine changes within the first 48 weeks of treatment in learning and working memory in children and young adults with NF1 treated with a MEK inhibitor.

Methods: The study included 59 patients with NF1 ages 5-27 enrolled on a MEK inhibitor clinical trial for the primary treatment of plexiform neurofibroma. Pre-treatment cognitive assessment and three additional assessments were administered over the first 48-weeks of treatment. Performance based (Cogstate) and observer reported outcomes (BRIEF) were used to assess the primary outcomes of working memory and executive function. We evaluated changes in performance and ratings with a traditional group-level analysis (repeated measures ANOVA) and an individually-based reliable change (RCI) framework.

Results: Statistically significant improvements were found on BRIEF MCI and BRI at 24- and 48 weeks compared to baseline (24-weeks $F_{(1, 58)} = 5.79$, $p = .02$, $d = 0.18$; 48-weeks $F_{(1,39)} = 7.29$, $p = .01$, $d = 0.27$). Reliable change statistics indicated a higher proportion of patients with significant improvement at 48-weeks than expected by chance (*Chi Square* $= 4.89$, $p = .09$). There was stable performance on Cogstate that supports a lack of potential neurotoxicity over 48-weeks of MEK inhibitor therapy. Patients with baseline cognitive functional impairments (MCI) were more likely to show significant improvement than non-impaired patients at 24-weeks (50% v. 9%; *Chi Square* $= 10.36$, $p = .006$) and 48-weeks (71% v. 16%; *Chi Square* $= 9.45$, $p = .009$).

Conclusions: Our data does not indicate any significant cognitive deterioration in functioning over the first 48-weeks of treatment with a MEK inhibitor that might suggest drug neurotoxicity. There is some evidence of real-world functional improvement in executive functioning that emerges by 24 weeks on a MEK inhibitor and continues up to 48 weeks of treatment, particularly for patients with baseline cognitive dysfunction. Based on these initial findings, future research in this area should be a focus for the NF1 community.

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Platform: Minipig Models of Neurofibromatosis Type 1 for Therapeutic Development

Monday, June 15, 1:15pm – 1:30pm

Adrienne L. Watson, PhD, *Recombinetics Inc., Eagan, MN*

Neurofibromatosis Type 1 (NF1) is a genetic disease caused by mutations in *Neurofibromin 1 (NF1)*. NF1 patients present with a variety of clinical manifestations and are predisposed to cancer development. Many NF1 animal models have been developed, yet none display the spectrum of disease seen in patients and the translational impact of these models has been limited. In fact, the FDA has emphasized the need for development and testing of new therapies in large animal disease models prior to human studies. To this end, we have employed gene-editing technology to develop a minipig model of NF1 that exhibits clinical hallmarks of the disease, including café au lait macules, neurofibromas, and optic pathway glioma. We have conducted pharmacological studies in our NF1 swine to look at the pharmacokinetic and pharmacodynamic properties MEK inhibitors, in clinical trials for NF1. We have demonstrated that oral administration of the MEK inhibitors results in clinically relevant plasma levels of the drug and inhibition of Ras signaling, and that certain MEK inhibitors can cross the blood brain barrier and have a pharmacodynamic effect, suggesting that they may be effective in treating NF1-associated brain tumors. In addition, because over 20% of NF1 patients harbor *NF1* nonsense mutations, we are also assessing safety and efficacy of a new class of drugs, known as nonsense mutation suppressors. Nonsense mutation suppressors are currently in clinical trials for Cystic Fibrosis and Duchenne muscular dystrophy, however, the impact these drugs could have on NF1 patients has yet to be explored. We evaluated six drugs known to induce nonsense mutation suppression in several primary cell types isolated from our NF1 minipig model and show that several of these drugs have the propensity to induce the production of full length Neurofibromin protein, leading to a subsequent reduction in MAPK signaling. Information acquired from this large animal preclinical model will be leveraged towards initiating a clinical trial in NF1 patients with a nonsense suppression therapy that is safe, tolerable, and effective in restoring *NF1* gene function. By addressing the underlying genetic and biochemical cause of NF1 and restoring the function of *NF1,* nonsense mutation suppression therapies have the potential to ameliorate both the malignant and non-malignant features of NF1. The NF1 minipig provides an unprecedented opportunity to study the complex biology and natural history of NF1 and could prove indispensable for development of imaging methods, biomarkers, and evaluation of safety and efficacy of NF1 therapies.

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Funding for this work was provided by the Gilbert Family Foundation through their Gene Therapy Initiative and by The Children's Tumor Foundation through their NF1 Synodos Program.

Platform: Evaluating the Performance of the NF2 Genetic Severity Score and the Potential Impact of Using Functional Assays to Improve Prognosis Prediction in a Cohort of Spanish Patients

Monday, June 15, 1:30pm – 1:45pm

Núria Catasús, Ms, *Germans Trias i Pujol Research Institute (IGTP)*

Introduction: Neurofibromatosis Type 2 (NF2) is an autosomal dominant disorder characterized by the development of multiple schwannomas and meningiomas, particularly, at vestibular nerves[1]. A genotype-phenotype association concerning the type of NF2 mutation is well established. A Genetic Severity Score (GSS) for NF2 mutations was developed to help predicting the disease course[2]. In the present study, we evaluated the performance of the GSS in our Spanish cohort and analyzed the potential added value of using functional molecular information.

Methods: Patients: GSS was validated using the Spanish Reference Center on the NF2 Phakomatoses HUGTiP-ICO cohort. Functional assays: Merlin, ERK and their phosphorylated forms were measured by Western Blot using patients' fibroblasts. Association analysis: Mann-Whitney U test and regression model analysis.

Results: The application of the GSS to the Spanish NF2 cohort confirmed the identification of significant correlations across patient's phenotype and NF2 mutation: Spanish patients that harbor a truncating mutation in the NF2 gene were associated with a most severe disease. However, for some mosaic patients and moderate phenotypes, GSS did not predict their clinical outcome as expected. We identified a correlation between the phenotype and the degree of activation of ERK pathway. We developed a lineal regression model to predict NF2 prognosis from functional and molecular data.

Conclusions: We validated the GSS in the Spanish cohort although a significant phenotypic variability was identified. To improve the performance of GSS, we evaluated the additional use of functional assays that allowed a better classification of mosaics and patients with moderated phenotypes by applying a new statistical model.

References: (1) Evans DG, Orphanet J Rare Dis. 2009 Jun 19;4:16. (2) Halliday D. Et al. J Med Genet 2017;54:657–664.

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The authors declare no conflict of interest.

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Platform: Oncolytic Adenovirus as a Therapy for Malignant Peripheral Nerve Sheath Tumors

Monday, June 15, 1:45pm – 2:00pm

Julia Nikrad, *University of Minnesota*

Oncolytic adenoviruses are cytocidal agents that demonstrate anti-tumor activity versus tumor cell lysis following intratumoral replication. These agents have been used as therapeutics in a variety of cancers but are not yet explored for MPNST and NF1-associated tumors. Oncolytic adenovirus may represent a novel therapeutic option for *NF1*-associated tumors and require further characterization. The ultimate goal of this project is to develop oncolytic adenovirus to be used for the treatment of unrespectable tumors by direct intratumoral injection.

To this end we have explored Conditionally Replicative Adenoviruses (CRAds) as a novel treatment for MPNST. Conditionally Replicative Adenoviruses are replication-competent vectors provided that a certain host cell "condition" is satisfied. These vectors are used to achieve preferential replication in the tumor cells, while leaving normal, non-tumor cells relatively unharmed. The conditionality of the virus replication can be achieved in a number ways, such as control the viral tropism through the modification of the fiber-knob domain of the adenovirus, a viral surface protein responsible for the attachment and entry of the virus into the human cell, and engineering of tumor-specific promoters (TSP) to encourage virus replication in those cells where the chosen TSP is active.

Here we explore cyclooxygenase 2 (*COX2*) promoter as a TSP. *COX2* promoter-driven CRAds are designed to preferentially replicate in tumor cells with high *COX2* expression. This is achieved by designing adenovirus vector with the adenoviral E1 gene under the control of Cox2 promoter. Since E1 gene is crucial for adenovirus replication and failure to transcribe E1 renders adenovirus replication deficient, the virus replication is preferentially restricted to cell with high *COX2* expression. We also investigated the effect of adenoviral fiber-knob structure modification on infectivity of the CRAd vectors in MPNST cells. We found high levels of *COX2* transcript characterizes most MPNSTs. We demonstrate that *COX2* promoter-driven CRAds are able to both bind and replicate in MPNSTs significantly better than in immortalized human Schwann cells. Finally, immunodeficient mice with MPNST cell xenografts survive significantly longer following intratumoral injection of *COX2* promoter-driven CRAds.

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Funding: National Institutes of Health, 5R01NS086219 and American Cancer Society, ACS Research Professor Award #123939 (to DAL)

Platform: Use of Nf1-KO Mice for Exploring Efficacy of Binimetinib and Selumetinib in Cutaneous Neurofibroma Prevention

Tuesday, June 15, 10:00am – 10:15am

Topilko Piotr, PhD, *Institut Mondor de Recherche Biomédicale, Inserm U955-Team 9, Créteil, France*

Neurofibromatosis type 1 (NF1) is a human genetic disorder caused by heterozygous loss-of-function of the tumour suppressor gene *NF1,* encoding neurofibromin. It predisposes patients to develop benign nerve sheath tumours, termed neurofibromas (NFs). All NF1 patients develop cutaneous NFs (cNFs) localised at nerve terminals in the skin. Although of benign character, these tumours are disfiguring and often itching, thus significantly affecting quality of life. We have developed a novel *Nf1*-KO mouse model, which fully recapitulates the human disease at the tumor level, including cNFs. In this line, *Nf1* loss is restricted to the boundary cap (BC) cells and their derivatives and is accompanied by constitutive expression of fluorescent reporter in the mutant cells making easy the follow up of tumor growth in the living animals. Despite the presence of numerous micro cNFs in the young mutant skin, *Nf1*-KO mice spontaneously develop mature cNFs at 1 year and beyond. Recently, we observed that skin trauma accelerates maturation of the micro-tumors into cNFs at the site of injury but also at the distance from it, supporting the implication of diffusible inflammatory factors as key players in this process. Using this *Nf1*-KO mice, we have performed pilot studies with two MEK inhibitors, Binimetinib and Selumetinib, by treating 6-month-old *Nf1*-KO mice either systemically or topically for 1 month. In all treated animals we observed significant reduction in the number of mutant SCs compared to placebo-treated group at the end of the treatment and 6 months later. Importantly, no cNFs developed in MEK-treated skin, in contrast to placebo controls which carried multiple cNFs from 12 months of age. No significant differences were reported between 2 drugs. Altogether, we consider that Binimetib and Selumetinib are promising drugs for treatment of cNFs and *Nf1*-KO mice constitute a valuable animal model for testing drugs to impede development of those tumors.

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Granting Agencies: INSERM, ANR, NTAP, Pierre Fabre Dermatology

Platform: A Molecular Testing Summary of Sporadic Vestibular Schwannoma

Tuesday, June 15, 10:15am – 10:30am

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

Cases of sporadic vestibular schwannoma (sVS) have a low rate of association with germline pathogenic variants. However, some individuals with sVS can represent undetected cases of neurofibromatosis type 2 (NF2) or schwannomatosis. Earlier identification of patients with these syndromes can facilitate more accurate familial risk prediction and prognosis.

We ascertained 394 cases of sVS from a local register at the Manchester Centre for Genomic Medicine. Genetic analysis of *NF2* was conducted on blood samples for all patients, and tumour DNA samples when available. *LZTR1* and *SMARCB1* screening was also performed in patient subgroups.

We found that age at genetic testing for VS presentation was young in comparison to previous literature, a bias resulting from updated genetic testing recommendations. Mosaic or constitutional germline *NF2* variants were confirmed in 2% of patients. Pathogenic germline variants in *LZTR1* were found in 3% of all tested patients, with a higher rate of 5% in patients <30 years. No pathogenic *SMARCB1* variants were identified within the cohort. Within 188 individuals who received tumour DNA analysis, 69% of patients were found to possess two somatic pathogenic *NF2* variants, including those with germline *LZTR1* pathogenic variants.

In conclusion, undiagnosed schwannoma predisposition may account for a significant minority of apparently sVS cases, especially at lower presentation ages. Loss of *NF2* function is a common event in VS tumours and may represent a targetable common pathway in VS tumourigenesis. These data also support the multi-hit mechanism of *LZTR1*-associated VS tumourigenesis.

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Platform: Selumetinib in Children with Clinically Asymptomatic Inoperable Neurofibromatosis Type 1 Related Plexiform Neurofibromas

Tuesday, June 15, 10:30am – 10:45am

Brittany Glassberg, BS, *National Institutes of Health, Bethesda, MD*

Background: Neurofibromatosis type 1 (NF1) is a genetic disorder characterized by hyperactivation of the RAS pathway. Up to 50% of people with NF1 develop histologically benign tumors called plexiform neurofibromas (PN). PN-related symptoms such as pain and airway obstruction can be debilitating, and often worsen with increasing PN volume. A phase II trial of selumetinib (AZD6244, ARRY142668), a MEK1/2 inhibitor, demonstrated PN shrinkage, growth inhibition and clinical improvement in children with baseline PN-related symptoms (stratum 1, NCT01362803).

Objective: We characterized the effect of selumetinib in patients without clinically significant baseline PN-related morbidity (stratum 2) and determined if PN-related morbidity developed during the course of treatment.

Design/Method: Children 2-18 years-old with NF1 and inoperable PN but without clinically significant PN related morbidity were eligible. Potential PN-related morbidities and associated functional assessments (motor, airway, vision) were determined based on tumor location. Patients received selumetinib orally 25 mg/m^2/dose BID for continuous 4-week cycles, with volumetric MRI analysis and functional assessments at baseline and then after every 4 cycles.

Results: Twenty-five patients (64% male) had a median baseline age of 12.3 years (range 4.5-18.1) and median baseline tumor volume of 381 mL (range 12-3159). Eleven patients had progressive PN growth at baseline (≥20% increase in PN volume within 15 months prior to enrollment). After 12 cycles of treatment, PN volume decreased by a median of 29% (range -37.9% to -2.5%), with 18 patients (72%) achieving a partial response (≥20% shrinkage) and no patients with progressive disease. At baseline, functional evaluations of strength (n=12), range of motion (n=8), exophthalmometry (n=2) and pulmonary function (n=8) were within normal limits, excluding patients with non-PN related comorbidities limiting their functional status (e.g. scoliosis). There were no statistically significant changes (improvement or worsening) in any of these measures after 12 cycles ($p > 0.05$). Selumetinib was well tolerated, with only 3 patients experiencing dose limiting toxicity, one of which came off treatment due to toxicity (asymptomatic elevation of lipase). After 12 cycles, 23 (92%) patients remain on treatment.

Conclusion: Selumetinib shrinks inoperable NF1-related PN in the majority of pediatric patients enrolled on this study presenting without clinically significant morbidity. In addition, none of the patients developed PN-related morbidity during one year of treatment, and selumetinib was generally well tolerated. This suggests that treatment with MEK1/2 inhibition may provide benefit by preventing the development of PN-related morbidity, though follow-up is relatively short and additional prospective studies are needed.

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Platform: Social Behavioral Deficits in NF1 Emerge from Peripheral Chemosensory Neuron Dysfunction

Tuesday, June 15, 10:45am – 11:00am

Emilia H. Moscato, PhD, *Department of Psychiatry, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA*

Neurofibromatosis type 1 (NF1) is a neurodevelopmental disorder characterized by neurofibromas and other tumors of the nervous system. In addition, a substantial number of patients have broad deficits in neurobehavioral function that manifests in social, learning, and communicative disabilities. In particular, impaired social behaviors are widespread in NF1 and among the greatest contributors to disease morbidity. Children with NF1 experience increased isolation and bullying, decreased friendships, and poorer social outcomes. No tractable animal model has been harnessed to study the neurobiological processes that underlie social behavioral dysfunction in NF1, and thus little is known about the cellular and circuit mechanisms by which loss of neurofibromin 1 (Nf1) function results in social deficits. The fruit fly *Drosophila* has increasingly become an animal model of choice for investigating biological mechanisms of social behaviors due to their genetic accessibility, high conservation of molecular pathways in the brain, and stereotyped social behaviors. Here, we identify social behavioral dysregulation in a *Drosophila* model of NF1. *Nf1* mutant male flies demonstrate impairments in sex-specific social behaviors. These deficits map to primary dysfunction of a small group of peripheral sensory neurons, rather than central brain circuits. Specifically, Nf1 regulation of Ras signaling in ppk23+ chemosensory cells is required for normal social behaviors in flies. Restoring Nf1 expression only during adulthood rescues courtship behavior of *Nf1* mutants, suggesting Nf1 has an ongoing role in coordinating social functions in the adult. *In vivo* monitoring of neural activity demonstrates that *Nf1* mutants show decreased ppk23+ neuronal activation in response to specific pheromonal cues, giving rise to disinhibition of brain neurons that direct social decisions. Circuit-specific manipulation of Nf1 expression or neuronal activity in ppk23+ neurons rescues mutant social impairments. Unexpectedly, this disrupted sensory processing in *Nf1* mutants gives rise to persistent changes in behavior lasting beyond the social interaction, indicating a sustained effect of an acute sensory misperception. Together our data identify a specific circuit mechanism in which Nf1 acts in peripheral sensory processing to regulate social behaviors. Our work supports a new understanding of how social deficits in NF1 may arise from propagation of sensory misinformation, potentially opening up new lines of clinical research.

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Platform: Long-Term Follow-Up of Schwannoma Growth Behavior an Adult Neurofibromatosis Type 2 and Schwannomatosis Patients Using Whole-Body MRI

Tuesday, June 15, 11:00am – 11:15am

K. Ina Ly, MD, *Pappas Center for Neuro-Oncology, Massachusetts General Hospital, Boston, MA*

Background: Neurofibromatosis type 2 (NF2) and schwannomatosis (SWN) are related genetic tumor predisposition syndromes caused by distinct gene mutations on chromosome 22, and are characterized by the presence of cranial, peripheral, and/or spinal nerve schwannomas. The long-term growth behavior of schwannomas is unknown but knowledge thereof would help guide patient surveillance and selection for treatment. Whole-body MRI (WBMRI) can detect whole-body schwannoma burden.

Methods: 12 NF2 and 10 SWN patients who underwent a WBMRI between 2007-2010 underwent a repeat WBMRI between 2018-2019. Schwannomas were segmented on short tau inversion recovery (STIR) sequences. Tumor volume was calculated using a three-dimensional tumor quantification software (3DQI). Tumor growth and shrinkage were defined as a volume change ≥20% over the entire study period.

Results: Median time between scans was 10 years. A total of 103 schwannomas (46 NF2-associated, 57 SWN-associated) were analyzed. In both NF2 and SWN, 50% of tumors grew. Median growth was 88.3% in NF2 and 100.4% in SWN. All growing NF2-associated schwannomas grew in the setting of exposure to systemic therapy whereas only one growing SWN-associated tumor had been treated systemically. Excluding resected tumors, 19.4% of schwannomas shrank. Median shrinkage was 48.5% in NF2 and 37.4% in SWN. All shrinking NF2-associated tumors had been treated with systemic therapy whereas none of the shrinking SWN-associated tumors had been. 19 new tumors (7 NF2-associated, 12 SWN-associated) developed in 8 patients.

Conclusions: Half of NF2- and SWN-associated schwannomas grow significantly over a decade. In NF2 patients, growth occurs despite systemic treatment whereas, in SWN patients, schwannomas may shrink spontaneously without treatment. These findings suggest a more aggressive tumor phenotype in NF2 patients. Continued patient enrollment and correlation of MRI findings with functional outcomes and hormone exposure history are ongoing.

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Platform: Severe Phenotype-Linked NF1 Substitution Mutations in Codons 844-848 of Neurofibromin Act in a Dominant Negative Manner by Destabilizing Wild-Type Protein

Tuesday, June 15, 11:15am – 11:30am

Lucy C. Young, PhD, *Helen Diller Comprehensive Cancer Center, University of California, San Francisco, CA*

Only a few of over 3000 known *NF1* mutations have been linked to specific symptoms and disease severity. The majority of *NF1* mutations are predicted to reduce neurofibromin protein expression through premature truncation or aberrant splicing, but the mechanism of action of most missense mutations is less clear. Patients with a missense mutation in codons 844-848 display severe phenotype disease characterized by a higher incidence of major plexiform and symptomatic spinal neurofibromas, optic pathway gliomas and other malignant neoplasms[1].Our biochemical analysis shows that mutations in these residues have no effect on the GTPase activating function of neurofibromin, but do have profound effects on protein stability. Neurofibromin exists as a high affinity dimer[2] and through this, the mutant allele causes destabilization of the wild-type protein. In this study we demonstrate for the first time in NF1, a dominant-negative mechanism of the mutation which is predicted to be the underlying cause of the severe phenotype. Since GAP activity is retained in 844-848 mutants, preventing protein instability is likely to restore regulation of RAS signaling. Our insights into the mechanisms of genotype-phenotype correlation are critical to developing new targeted therapies aimed at restoring protein expression and emphasize the importance of genotyping in the clinical setting.

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[2]Sherekar, M. *et al.* Biochemical and structural analyses reveal that the tumor suppressor neurofibromin (NF1) forms a high-affinity dimer. *J Biol Chem* 295, 1105–1119 (2019).

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NIH: 5R35CA197709 McCormick (PI)

Platform: Supporting NF Education and Parent Advocacy, Pandemic and Beyond

Tuesday, June 15, 11:30am – 11:45am

Meredith Chambers, MEd, *Boston Children's Hospital, Boston, MA*

Introduction: Children with NF1 are at increased risk for cognitive, academic and social impairments. The frequency of specific learning disabilities in children with NF1 ranges between 30 and 75 percent (Krab, et al., 2008; North et al, 1995). Educators rarely understand the unique issues of children with NF1; therefore, academic needs are often insufficiently addressed.

The Neurofibromatosis School Liaison Program (NF SLP) is a model of care for children with complex medical and educational issues. The program provides cost-free, collaboration with and education of school-based teams to assist in understanding the effects of NF1 and associated medical conditions on learning and social functioning. (Chambers et al, 2018) The NF-SLP clinician also teaches families to advocate for their child's learning needs.

The NF SLP assists approximately 250 families yearly. Approximately 87% receive special education services or accommodations. Since its inception, the SLP has informed >3,000 team members about the social and academic impacts of NF and successfully advocated for more than 1,500 additional services and accommodations on patients' educational plans.

The COVID-19 pandemic has quickly changed the delivery of education to children from the school building to remote learning at home. Schools, families and students have all had to make immediate adaptations to ensure learning continues. Parents who often require support around school advocacy now find themselves in the unfamiliar role of 'teacher' requiring even more support. The NF SLP also has made adaptations now providing supports via virtual meetings and an online webcast learning library.

Parent training opportunities are an integral piece of the NF School Liaison Program. With the onset of the Covid-19 pandemic, the NF SLP pivoted from in-person trainings to creating an online learning library to assist not only our families with NF but any family on how to best support and advocate for their children. Families can now access SLP developed webcasts on helpful topics of their choosing, in their own time and on any device; e.g., *Home to School Advocacy, Understanding the Individual Education Program (IEP) Document and Executive Function.*

Conclusion: Meeting with school teams, advocating for services and providing parent training provided by the School Liaison Program has adapted since the onset of Covid-19. Providing delivery of services remotely and developing an online training library during the pandemic has allowed us to successfully continue to meet patient and family needs in a population that requires ongoing services and will likely continue well beyond the pandemic.

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Platform: Development of Topical MEK Inhibitor, NFX-179, for the Treatment of Cutaneous Neurofibromas in Neurofibromatosis Type 1

Tuesday, June 15, 11:45am – 12:00pm

Kavita Y. Sarin, MD, PhD, *Stanford University Medical Center*

Neurofibromatosis Type 1 (NF1) is a genetic disorder caused by a mutation in the neurofibromin (*NF1*) gene and characterized by the development of multiple nervous system tumors. Cutaneous neurofibromas (cNFs) are benign nerve sheath tumors that form in the dermis and are the most common manifestation in NF1. The majority of individuals with NF1 develop hundreds of cNF tumors which can cause irritation, pain and social anxiety. Thus, there is a pressing need for new therapies for cNFs. cNFs arise due to uncontrolled activation of the Ras/MEK pathway due to loss of heterozygosity in neurofibromin, representing a compelling therapeutic target pathway. Recently, systemic delivery of the MEK1/2 inhibitor, selumetinib, demonstrated benefit in plexiform neurofibromas, another Ras/MEK driven tumor in NF1. Unfortunately, systemic toxicities of MEK inhibitors limit use for benign cNFs. Here we report the development of a topically formulated, novel MEK inhibitor, NFX-179, designed to potently and selectively suppress the Ras/MEK pathway in target cNF tissue while limiting systemic exposure and toxicity. NFX-179 was identified from a targeted drug discovery effort based on its potency, selectivity, and rapid metabolism upon systemic absorption. NFX-179 is highly potent in suppressing Ras/MEK signaling in cell lines, mice and minipig models. Franz cell testing in excised human skin of topically applied NFX-179 demonstrated the ability to concentrate into the dermis. Twenty-eight days of dermal application to minipigs demonstrated high levels of NFX-179 concentration in the epidermis and dermis and a dose dependent suppression of phosphorylation of ERK (p-ERK), a key downstream component of the Ras/MEK signaling pathway. p-ERK suppression was also observed in ex-vivo human neurofibroma explants. Plans are underway to conduct human clinical trials in cNF in 2020.

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Funding: NFlection Therapeutics, Inc.

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NF1: Basic Science

Rescue of NF1 Cryptic Splice Site in Exon 13 mRNA with Antisense Morpholino Treatment

Elias K. Awad, MD, *Department of Genetics, University of Alabama at Birmingham, Birmingham, AL*

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disease with almost 3000 different pathogenic mutations within the NF1 gene identified. Approximately 27% of these mutations cause splicing errors to occur within pre-mRNA. A recurrent mutation in exon 13, c.1466A>C, forms a cryptic splice site leading to aberrant splicing resulting in a 62 base pair deletion from the mRNA and subsequent frameshift. To model this mutation, we have developed a human iPS cell line homozygous for the mutation. We have also generated a humanized mouse containing c.1466A>C along with the entire human exon 13 and 50 base pairs of flanking intronic sequence on each side replaced using CRISPR/Cas9 gene editing.

Methods: iPS cells homozygous for the mutation were treated for 48 hours with an antisense morpholino designed to mask the cryptic splice site mutation and restore normal splicing. RNA was extracted and reverse transcribed followed by qPCR to evaluate the response to treatments. We established a Taqman assay to distinguish between wild type and mutant alleles.

Results: Our initial testing in human iPSCs indicated that the morpholino is able to repress the cryptic splice site and restore normal splicing. Treatment with 10 micromolar morpholino concentration showed 25.1 fold increase in wild type allele expression and a 10.4 fold decrease in mutant expression, whereas 20 micromolar treatment showed 36.3 fold increase in wild type allele expression and a 48.5 fold decrease in mutant allele expression compared to controls.

Conclusion: Morpholino treatment masks the cryptic splicing site and restores normal splicing function in c.1466A>G iPS cells in our initial trials. Our next steps will be to analyze GTP-Ras levels and use the morpholino in our mouse model.

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PTCH1 and *APC* Regulated Pathways Contribute to Malignant Peripheral Nerve Sheath Tumorigenesis

Minu Bhunia, BS, *University of Minnesota, Twin Cities*

Neurofibromatosis Type 1 syndrome (NF1) is an autosomal, dominant cancer predisposition syndrome caused by inheritance of one loss of function allele of the *NF1* gene. *NF1* encodes a GTPase activating protein (GAP) for Ras proteins. Most NF1 patients develop benign Schwann cell tumors, including dermal neurofibromas and deep nerve plexiform neurofibromas (PNs). PNs cause significant morbidity and even worse can progress to malignant peripheral nerve sheath tumors (MPNST), a deadly soft tissue sarcoma. PNs and MPNSTs develop after somatic loss of the wild-type NF1 allele, resulting in an increase in Ras-GTP activated signaling[1]. How these neurofibromas become malignant is still incompletely understood, however loss of *TP53* or *CDKN2A/2B* function and the polycomb repressor complex 2 (PRC2) are common events during the transition to MPNST[2–4]. Our recent 'omics studies have revealed that MPNSTs may form two distinct molecular subgroups, SHH and WNT pathway-like. Our lab has performed a large scale forward genetic *Sleeping Beauty* screen for malignant Schwann cell tumors in mice. The screen resulted in a common insertion sites list including various SHH and WNT pathway regulatory genes consistent with the findings in human MPNST[5]. In order to validate these findings, we have edited human Schwann cells to be deficient in *PTCH1* or *APC,* important tumor suppressor genes and negative regulators of SHH and WNT signaling respectively. We find that loss of *PTCH1* and *APC* each lead to malignant transformation of human Schwann cells based on increased anchorage-independent growth, migration, and xenograft tumor formation. These findings reveal more druggable targets for MPNST treatment and help confirm the concept that MPNST progression involves at least major molecular subtypes.

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Modeling the Development of NF1 Peripheral Nerve Sheath Tumors with Induced Pluripotent Stem Cells

Garrett Draper, BS, *University of Minnesota*

The tumor predisposition syndrome Neurofibromatosis type 1 (NF1) results from the inheritance of a mutant copy of *NF1*, a Ras-GAP tumor suppressor gene. Subsequent loss of the remaining wildtype *NF1* allele in peripheral nerve Schwann lineage cells leads to complete functional loss of the encoded protein neurofibromin. Schwann cells lacking neurofibromin exhibit hyperactive Ras signaling, cell proliferation, and can contribute to the formation benign plexiform neurofibromas (PNFs) in deep peripheral nerves of patients. Through additional mutations in canonical tumor suppressor genes *TP53* or *CDKN2A,* these PNFs can overcome cellular senescence and progress to premalignant atypical PNFs. Furthermore, loss of function mutations in the histone modifying Polycomb Repressive Complex 2 (PRC2) genes *EED* or *SUZ12,* have been strongly implicated in the progression of atypical PNFs to lethal malignant peripheral nerve sheath tumors (MPNSTs). Although these and other genetic alterations have been associated with MPNST formation, their temporal dependence during Schwann cell development and contribution to malignant transformation using a human cell model has not been studied. To model the effects of these mutations in MPNST development, we utilized human induced pluripotent stem cells (iPSCs) and specific differentiation protocols to obtain cells with a Schwann cell identity. Cells were subjected to targeted mutations using CRISPR/Cas9 at different stages of development to mimic potential malignant tumorigenesis. Our preliminary results show that multiple iPSC lines can reliably be differentiated to neural crest cells (NCCs), an intermediate cell type in Schwann cell development, and further to mature Schwann cells. Using CRISPR/Cas9 and short guide RNA ribonucleoproteins (RNPs), the *NF1* locus in all three developmental stages could be edited at high levels (>70%) resulting in loss of functional neurofibromin protein. NCCs lacking neurofibromin showed an increase in proliferation and migration. Further refinement of this induced-MPNST (iMPNST) model will allow for better understanding of the timing at which these transforming mutations are necessary during Schwann cell development to result in MPNST formation. Also, the results of these studies could validate or uncover *de novo* gene mutations and pathways that are necessary or sufficient for the transformation of benign PNFs to MPNSTs and improve the effectiveness of future targeted therapeutics.

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Hexokinase 2 Displacement from Mitochondria-Associated Membranes as a New Antineoplastic Approach in NF1-Related Tumors

Ciscato Francesco, PhD, *Department of Biomedical Sciences (DSB), University of Padova, Padova, Italy*

Metabolic rewiring is a key feature of cancer cells, as they funnel metabolites through different anabolic branches in order to obtain "building blocks" needed for cell survival and proliferation. We have shown that metabolic changes also occur in cells lacking neurofibromin and play an important role in their neoplastic growth (Masgras et al, Cell Rep, 2017). Targeting these pathways discloses selective and effective therapeutic opportunities. A crucial component of tumor-related metabolic changes is induction of hexokinase 2 (HK2), the most efficient isozyme of the hexokinase family, which primes glucose utilization for glycolysis and anabolic and antioxidant pathways. HK2 (over)expression associates with unfavorable prognosis and radiotherapy/chemotherapy resistance, whereas its silencing or genetic ablation abrogates neoplastic growth and restores sensitivity to anti-tumor treatments. HK2 expression is enhanced by HIF-1 and RAS signalling. These pathways are hyperactivated in NF1-related tumors, such as plexiform neurofibroma (PN) or malignant peripheral nerve sheath tumor (MPNST), where we found that HK2 is induced. We observed that HK2 is expressed in NF-related tumor cell models as well by NF1 mouse tumor models and in human NF1 tumor samples. We found that HK2 displays anti-apoptotic properties that rely upon its binding to mitochondria-associated membranes (MAMs), the interface domains between endoplasmic reticulum and mitochondria, in diverse tumor cells, including PN and MPNST cell models. Taken together, these observations indicate that targeting HK2 is a promising approach for the development of antineoplastic strategies directed towards NF1-related neoplasms. However, the use of pharmacological HK2 inhibitors has proven arduous due to toxicity caused by side effects on other HKs. Here, we have used a Cell Penetrating Peptide (CPP) that mimics the N-terminal tail of HK2 to displace it from MAMs without targeting its catalytic activity. This HK2 relocation elicits massive Ca^{2+} fluxes to mitochondria and the ensuing death of tumor cells. We have developed a modular CPP (HK2pep) for *in vivo* HK2 targeting. HK2pep is composed by: 1) an active sequence mimicking the N-terminal tail of HK2; 2) a polycation stretch allowing HK2pep cell entrance; 3) a sequence cleavable by extracellular proteases (over)expressed by tumor cells for the specific release of the active moiety inside tumor masses; 4) a polyanion sequence that prevents non-specific cell uptake, shielding the polycation stretch in the absence of protease cleavage. Intraperitoneal administration of HK2pep in mice bearing aggressive breast or colon xenografted tumors halts their growth without noxious effects. This strategy is efficient and very rapid in killing NF1-derived tumor cells *in vitro.* The modular structure of HK2pep opens the possibility to tailor this approach on NF1-tumors that display heterogeneous microenvironments and extracellular proteases. HK2pep is an unprecedent approach against PN and MPNST and can represent a first step towards an innovative anti-neoplastic treatment for NF1 patients.

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A High Frequency of Chromosomal Translocations Affecting CDKN2A Demonstrates its Necessary Loss for Malignant Peripheral Nerve Sheath Tumor Formation

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

Malignant peripheral nerve sheath tumors (MPNST) are aggressive and highly metastatic soft-tissue sarcomas with limited sensitivity to chemotherapy and radiation. MPNST develop sporadically or in persons with Neurofibromatosis Type 1 (NF1), who have a lifetime risk of 10% of developing them. Like other sarcomas, these tumors contain hyperploid and highly rearranged genomes with a low mutation burden. In the context of NF1, a common path of MPNST progression is the development of a nodular atypical neurofibroma (aNF) from a pre-existing plexiform neurofibroma (pNF). aNFs are considered premalignant lesions at risk of progression. In addition to the loss of *NF1* necessary for pNF formation, aNF also lose the CDKN2A/B locus. However, genomic analysis evidenced the loss of the CDKN2A/B locus in only 70-80% of MPNST, raising the question of whether there is more than one molecular path to MPNST or a limitation in the analysis of CDKN2A/B inactivation.

To investigate this issue, we analyzed whole-exome sequencing (WES) and SNP-array data from 8 established MPNST cell-lines (5 NF1-associated and 3 sporadic). In addition to 3 *CDKN2A* homozygous deletions and one point mutation, we unexpectedly identified inter-chromosomal translocations disrupting *CDKN2A* in 4 MPNST cell-lines. Translocation-breakpoints were validated by PCR amplification and sequencing. All translocations had different partners and their breakpoints clustered in a small hotspot region. Since *CDKN2A* was completely inactivated in all MPNST cell lines, many due to translocation events, we further investigated *CDKN2A* status in primary MPNSTs by whole genome sequencing. The analysis of 10 primary MPNSTs (5 NF1-associated and 5 sporadic) revealed several CDKN2A deletions and a point mutation but also translocations and inversions disrupting *CDKN2A* in 4 of them, with most breakpoints in or near the translocation hotspot region. All breakpoints were validated by PCR and Sanger sequencing. Altogether, we identified the complete inactivation of *CDKN2A* in all MPNST and MPNST cell-lines except for the partial inactivation of a sporadic MPNST.

Our results show a previously unappreciated high frequency of chromosomal translocations involving *CDKN2A* and the presence of an intronic translocation hotspot next to exon 2. We demonstrate that *CDKN2A* bi-allelic inactivation is a necessary molecular event for MPNST formation both in sporadic and NF1-related contexts.

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Tumor Pleiotropy in Heterozygous Rats with a Neurofibromatosis Type I Missense Mutation

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Background: Rat models of neurofibromatosis type I (NF1) were created to fulfill a need for additional preclinical models to allow examination of the efficacy and safety of pharmacological modulators and more readily assess cognition and behavior. The pathogenic patient missense allele c.3827G>A, p.R1276Q knockin (KI), associated in humans with spinal NF1, as well as a 14 base pair deletion c.3661_3674del, p.P1220fs*1223 knockout (KO) model were generated.

Methods: KO and KI rat models were created using two CRISPR guides and a single- stranded DNA repair template designed to target exon 28 of rat *Nf1* gene. Affected animals were euthanized upon tumor mass ulceration or inhibition of normal movement, and tissues were collected for histology, immunostaining and Western blot analysis. Males were analyzed for cognitive and behavioral defects at six months of age by elevated plus maze, open field test, novel object recognition and Morris water maze (n= 6-11) and euthanized at approximately one year of age due to onset of seizures. Tumor- bearing females were imaged by FMISO-PET and FDG-PET.

Results: Unmated heterozygous *Nf1* R1276Q female KI rats did not display spontaneous tumors; however, mated females developed mammary tumors within two weeks of pregnancy. Metastases to the kidneys were detected by PET imaging. Heterozygous *Nf1* P1220fs*1223 KO females developed tumors spontaneously at sexual maturity, with acceleration of tumor formation post-mating. Male rats did not display significant cognitive deficits or behavioral differences when assessed at 6 months of age with any of the tasks performed; however, one KO and two KI males died of seizures (n=8 KO; n=6 KI) and brain lesions were apparent upon dissection. At 12-14 months of age, KI males displayed tumors in brain, adrenal gland, and colon.

Conclusions: Two novel mutant *Nf1* alleles, patient mutation c.3827G>A, p.R1276Q and deletion c.3661_3674del, p.P1220fs*1223, have been created in the rat, with sexually dimorphic and pleiotropic phenotypes. Females display mammary gland adenocarcinoma in sexually mature females and carcinoma in situ in young unmated females. Restriction of tumor development to pregnancy in *Nf1* R1276Q females suggests hormone induction plays a major role in tumor development. The divergence in phenotype between patient and null alleles may be due to residual function of R1276Q missense NF1 protein. Lack of full-term homozygous mutant pups indicates that these alleles are embryonic lethal, although rapid tumor onset post-mating may confound this result.

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Neurofibromin Loss Drives Tumorigenesis by Affecting SIRT3 Activation via Complex I Inhibition

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Neurofibromin loss drives neoplastic growth and a rewiring of mitochondrial metabolism. Here, we discover that neurofibromin ablation dampens expression and activity of NADH dehydrogenase, the respiratory chain complex I, in an ERK-dependent fashion. This provides cells with resistance to pro-oxidants targeting complex I and decreases both respiration and intracellular NAD+/NADH ratio. Expression of the alternative NADH dehydrogenase NDI1 raises NAD+ levels, enhances the activity of the mitochondrial NAD+-dependent deacetylase SIRT3 and interferes with tumorigenicity in neurofibromin-deficient cells. This anti-neoplastic effect is mimicked both *in vitro* and *in vivo* by administration of NAD+ precursors or by rising expression of the NAD+ deacetylase SIRT3, and is synergistic with ablation of the mitochondrial chaperone TRAP1, which augments succinate dehydrogenase activity further contributing to block pro-neoplastic metabolic changes of these cells. These findings shed light on chemotherapeutic resistance and on bioenergetic adaptations of tumors lacking neurofibromin, linking complex I inhibition to mitochondrial NAD+/NADH unbalance and SIRT3 inhibition, as well as to down-regulation of succinate dehydrogenase. This metabolic rewiring could unveil attractive therapeutic targets for neoplasms related to neurofibromin loss.

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Tumor Suppressor Status Alters MPNST Metabolic Profiles and Gene Expression

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Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are aggressive, highly metastatic sarcomas with a 20-35% 5-year survival rate, due to a high chemotherapy and radiation resistance. Metabolic reprogramming is a hallmark of cancer, allowing tumors to adapt to cellular stress and driving drug resistance. Cancer-specific metabolic vulnerabilities represent unique therapeutic targets, and several metabolic inhibitors are entering clinical trials. However, MPNST metabolism is not well-studied, and the impact of different genetic mutations on MPNST metabolism is unknown. MPNSTs activate the Ras pathway through mutations in neurofibromin (*Nf1*) and loss-of-function in a tumor suppressor, either *P53* or *CDKN2A.* The majority of MPNSTs have co-occurring disruptions in *NF1* and *CDKN2A,* while ~30% have mutations in *P53* instead of *CDKN2A*. Both *P53* and *CDKN2A* play critical roles in cell cycle progression, differentiation, senescence and apoptosis. We have used somatic CRISPR/Cas9 tumorigenesis approaches to generate MPNSTs in wild-type, littermate paired mice with disruptions in Nf1/Trp53 or Nf1/Cdkn2a. We investigated the impact of tumor suppressor mutation on tumor growth, immune infiltration, metabolic profile and gene expression. Mass spectrometry metabolomic profiling on tumor-derived cell lines identified tumor suppressor-dependent differences across multiple metabolic pathways, including glycolysis, pentose phosphate pathway, TCA cycle and amino acid biogenesis. Analysis of gene expression data is ongoing. Taken together, these data suggest that *P53* and *CDKN2A* differentially impact MPNST biology and cellular metabolism, and demonstrate how CRISPR/Cas9 approaches can facilitate for direct comparison of different genetic combinations.

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Utilizing Single-Cell Transcriptomics for Deeper Surfaceomic Profiling of Malignant Peripheral Nerve Sheath Tumors

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Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas, and 50% of these malignancies arise in patients with neurofibromatosis 1 (NF1). The lifetime incidence of MPNST in NF1 patients is 8% to 13%, and such tumors are often associated with poor prognoses. Chemotherapies and signaling pathway-targeting drugs are limited in efficacy, and complete surgical resection, which is the most effective treatment, is often infeasible for these tumors. With the advent of immune-based therapies, unique and highly expressed surface proteins on tumor cells are prime candidates for targetability. Identifying such targets in solid tumors poses a challenge due to intratumoral heterogeneity. Characterizing the repertoire of surface markers (surfaceome) for various cancers has been done using a multi-omics approach, many of which combine transcriptomics (RNAseq) and proteomics to filter and identify surface targets. Single-cell transcriptomics (scRNAseq) may provide an additional layer of information to disentangle cellular heterogeneity and more accurately identify markers on cancer cells within the bulk tumor. Here, to identify potential candidates for immunotherapeutic targeting, we take a systematic approach to characterize the MPNST surfaceome.

To minimize potential off-target tissue toxicity, a list of highly expressed genes was generated by comparing bulk RNAseq data obtained from 16 MPNST tumor samples and 5 MPNST cell lines to a panel of 42 normal tissues controls ($p < 0.01$, $\log_2$FPKM > 5). This gene list was then filtered using a surface protein predicting tool comprising of 11 annotated databases and was mapped on our annotated scRNAseq data obtained from a patient MPNST sample and 10 unique MPNST patient-derived xenografts (PDXs) to identify potential cancer cell-specific genes. We identified and ranked 30 candidate genes that have promising potential to be surface targets. Delta homolog 1 (DLK1), a non-canonical Notch ligand, was found to be highly expressed in our PDXs and in 99.5% of MPNST tumor cells and minimally in other cell types (average $\log_2FC = 3.06$) in our patient sample. Validation of targets of interest is ongoing along with a global surface proteomic analysis of MPNST cell surface protein isolates via biotinylation.

We demonstrate here that the MPNST surfaceome can be surveyed for the discovery of new immunotherapeutic targets, and the addition of single-cell transcriptomic data can provide a deeper look into various cell populations within the tumor. A proteogenomic integration of RNA and mass spectrometry datasets will also allow us to gain further insight into the MPNST cell surface landscape.

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Characterization of "Mild" Neurofibromatosis Type 1 Patient Mutation p.M992del in Knock-In Mouse Model

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Background: Animal models are critical for molecular understanding of pathophysiology of disease and preclinical studies testing new therapeutics. Patient-specific NF1 models are now more readily created with gene editing tools such as CRISPR/Cas9 technologies. We have created a three-base pair (bp) knock-in deletion (p.M992del) mutation in mice. Patients present with a mild clinical phenotype associated with café-au-lait spots but no neurofibromas. Therefore, we hypothesize that the mutation is hypomorphic and maintains partial neurofibromin activity.

Methods: Heterozygous mice were created using CRISPR/Cas9 and a repair template to introduce a 3 bp deletion in the corresponding region of the mouse Nf1 gene. We then crossed heterozygous mice (*Nf1*$^{M992del/+}$) to assess viability of homozygous mutants, as most Nf1 mutant alleles induce early embryonic lethality due to NF1 deficiency when homozygous. Of the nine *Nf1*$^{M992del/M992del}$ mice surviving post-weaning, one animal was euthanized (along with heterozygous and wild-type (WT) female sibling controls) to obtain tissues for Western blot analysis of NF1 in brain and assessment of downstream signals pERK and pAKT. An additional cohort is being monitored for phenotype.

Results: Analysis of the genotype ratios revealed a significantly lower number of *Nf1*$^{M992del/M992del}$ mice than the expected 25%, with only 9 capable of surviving to weaning of 18 born ($n=186$ total pups; $p<0.0001$). Surviving *Nf1*$^{M992del/M992del}$ mice are ~50% of the size by weight of littermate controls throughout life. Preliminary Western blot analyses of neurofibromin, pERK, and pAKT protein levels in brain show no significant differences between wild type, heterozygous, or homozygous mutant mice.

Conclusions: Heterozygous *Nf1*$^{M992del/+}$ mice appear to be healthy with no overt phenotype, whereas homozygous *Nf1*$^{M992del/M992del}$ animals are severely runted. The Western blots confirm that mutant neurofibromin protein is present, and at levels consistent with that found in control animals. Western blots also indicate no changes in pERK or pAKT in homozygous *Nf1*$^{M992del/M992del}$ animals, suggesting that mutant neurofibromin protein functionally regulates these pathways in brain. Further molecular characterization of downstream signals pERK and pAKT are ongoing.

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RUNX Represses Pmp22 to Drive Neurofibromagenesis

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Neurofibromatosis type 1 (NF1) patients are predisposed to develop plexiform neurofibromas but the underlying molecular mechanism(s) of neurofibromagenesis are not fully understood. We showed that dual genetic deletion of RUNT-related transcription factor 1 (Runx1) and Runx3 in Schwann cells (SCs) and Schwann cell precursors (SCPs) significantly delayed neurofibromagenesis and prolonged mouse survival in the *Nf1fl/fl;DhhCre* neurofibroma mouse model. We identified peripheral myelin protein 22 (Pmp22/Gas3) related to neurofibroma initiation. Knockdown of Pmp22 with shRNAs increased *Runx1fl/fl;Runx3fl/fl;Nf1fl/fl;DhhCre* tumor derived sphere numbers and enabled significantly more neurofibroma like micro-lesions on transplantation. Conversely, overexpression of Pmp22 in mouse neurofibroma SCs decreased cell proliferation. Mechanistically, RUNX1/3 regulated alterative promoter usage and induced levels of protein expression of Pmp22 to regulate SC growth. Finally, pharmacological inhibition of RUNX/core binding factor beta (CBFB) activity using a RUNX/CBFB interaction inhibitor, R05-3335, significantly reduced plexiform neurofibroma volume in vivo. Thus, we identified a novel signaling pathway involving RUNX1/3 suppression of Pmp22 in plexiform neurofibroma initiation and/or maintenance. Targeting disruption of RUNX/CBFB interaction might provide a novel therapy for neurofibroma patients.

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Identifying a Direct Role for Fibroblasts in Cutaneous Neurofibroma Development

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Cutaneous neurofibromas (cNF) are present in nearly all NF1 patients. While benign, these tumors are a major source of morbidity for NF1 patients, and elective surgery is currently the only approved therapy. Neurofibromas are complex peripheral nerve sheath tumors composed of a heterogeneous population of neoplastic *NF1*$^{-/-}$ Schwann cells surrounded by fibroblasts, mast cells, and other nerve components within a dense extracellular matrix. *NF1*$^{-/-}$ Schwann cells are required to initiate tumor formation, but the haploinsufficient microenvironment also plays a crucial role in neurofibromagenesis. Fibroblasts are a major cellular constituent of cNFs and have been shown to play an indirect role in neurofibroma growth by depositing excessive collagen and interacting with other stromal cells. Cancer-associated fibroblasts have been shown to interact directly with neoplastic cells in other tumor types, but a direct functional relationship between haploinsufficient *NF1*$^{+/-}$ fibroblasts and tumor-initiating *NF1*$^{-/-}$ Schwann cells in cNFs has not been thoroughly studied. We developed a genetically engineered *NF1*$^{+/-}$ minipig that develops hallmarks of NF1 syndrome, including tumors that resemble NF1-associated cNFs. Similar to human cNFs, primary Schwann cells isolated from minipig neurofibromas exhibit spontaneous loss of heterozygosity of the *NF1* gene and subsequent Ras hyperactivation. Notably, fibroblasts isolated from these tumors display altered morphology, resistance to fibroblast growth-suppressing drugs, and unhindered proliferation in serum-free media. We hypothesized that fibroblasts are reprogrammed by the neurofibroma environment and interact directly with *NF1*$^{-/-}$ Schwann cells to promote neurofibroma growth and maintenance. To determine whether fibroblasts are altered by the neurofibroma environment, we performed RNA sequencing of fibroblasts isolated from cutaneous neurofibromas, skin, and sciatic nerve from three *NF1*$^{+/-}$ animals. To determine whether neoplastic *NF1*$^{-/-}$ Schwann cells activate fibroblasts in the neurofibroma microenvironment through direct or paracrine interactions, we performed *in vitro* co-culture assays. The NF1 minipig neurofibroma model provides a unique opportunity to investigate the role of the microenvironment on neurofibroma growth and maintenance. The results of these studies will enable researchers to better understand the complex mechanisms of neurofibroma maintenance and reveal new targets for therapeutic intervention.

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Establishment of a Comprehensive Clinically-Annotated Nerve Sheath Tumor Bank from Patients with Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is a common genetic disorder characterized by a predisposition to the development of nerve sheath tumors, including cutaneous neurofibromas (cNF), plexiform neurofibromas (pNF), and malignant peripheral nerve sheath tumors (MPNST). The development of nonsurgical therapy for PN has been limited by: 1) lack of cell culture-based models, 2) limited number of animal models, and 3) limited access to primary tissue from patients with NF1. Although there is progress in the development of animal and cell culture models, the limited availability of primary patient tissue remains unaddressed. Comprehensively characterized banked tissue allows for the most efficient and targeted use of specimens in collaborative NF1 research efforts.

Methods: We have created and expanded a Johns Hopkins University biospecimen repository for the purpose of banking blood fractions and tumor tissue from patients with NF1, and generating cell culture and xenograft models to propagate primary human tissue. Under an IRB-approved research authorization, NF-associated tumor tissue is collected and processed according to a Standard Operating Procedure. H&E slides of each banked sample are reviewed by the study neuropathologist for quality control and accuracy of diagnosis. Banked specimens undergo comprehensive genomic characterization using RNAseq and whole exome sequencing (WES), and data are available through Synapse (https://www.synapse.org/#!Synapse:syn4939902/wiki/235907). A clinically-annotated database accompanies the bank, and includes NF1-associated symptoms and findings, tumor characteristics, and outcomes data. We have implemented an internal review process for researchers outside our institution to request access to specimens and accompanying de-identified clinical information.

Results: We have established a Johns Hopkins NF1 biospecimen repository of high quality, well-annotated, and genomically characterized nerve sheath tumor tissues and blood fractions. Since inception in January 2016, over 183 unique samples have been banked, and include neurofibroma (n=104), MPNST (n=23), blood fractions, and xenograft (n=4) specimens, from 92 unique patients. Ten researchers from outside institutions have requested access to our specimens. RNAseq data and WES data are available on the NF Data Portal.

Conclusions: Our biospecimen repository represents a high-quality, clinically and genomically characterized and valuable resource for ongoing scientific efforts in the NF1 research community.

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Mechanical Characterization of Healthy and Plexiform Schwann Cell Spheroids

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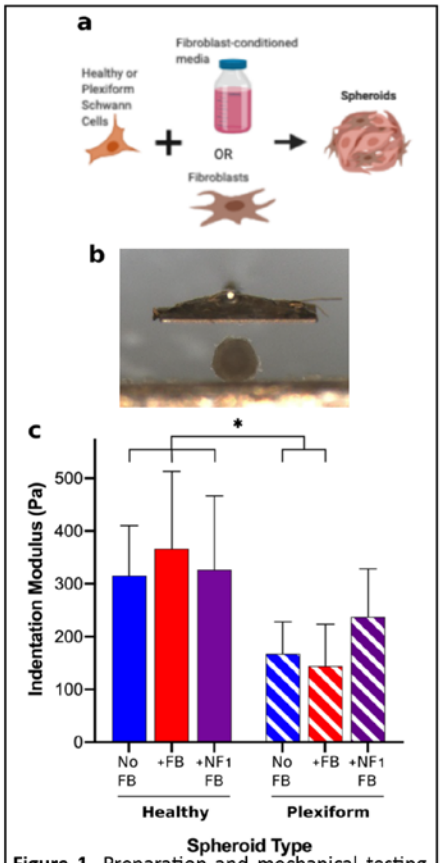

Figure 1. Preparation and mechanical testing of Schwann cell spheroids. (a) experimental design (b) A 550 micron diameter spheroid is tested in parallel-plate compression. (c) Healthy SC spheroids are stiffer than the plexiform SC spheroids regardless of FB inclusion.

Introduction: A hallmark of NF1 is the growth of benign and, sometimes, malignant tumors.(*1*) Biomechanics have been shown to play a role in cancerous tumor formation and cell invasion, which for these tumors has not yet been investigated.(*2*) For example, solid tumors, in general, are stiffer than healthy tissue and have stiffness profiles correlated with their malignancy.(*3*) NF1 fibroblasts cells have altered mechanosensitivity, linked to the mechanosensitive protein signaling pathways in which neurofibromin is involved.(*4*) To begin to understand the biomechanical microenvironment of NF1 tumors, systematic measurements of the mechanical properties of NF1 tumors need to be made. Here, we begin with a benchmark study of the mechanical properties of spheroids of healthy and NF1 Schwann cells (SCs).

Materials and Methods: Spheroids were produced with healthy SCs (97.4) or NF1-/- plexiform SCs (04.4). The contribution of other PNF components were investigated by adding either i) healthy or NF1 fibroblast (FB)-conditioned media (70% 5-day FB-conditioned media:30% fresh media) or ii) healthy FBs or NF1+/- FBs (10% of cells/spheroid) (Fig 1a). Stiffness was measured on day 5 using parallel plate compression (Fig 1b). The resulting force-displacement data was fit with the Hertz contact model for the elastic indentation modulus.

Results: Plexiform SC spheroids were more compliant (168 ± 61 Pa) than healthy SC spheroids (315 ± 95 Pa) (Fig 1c). The addition of healthy or NF1 FB-conditioned media (not shown) or healthy FBs (Fig 1c) had no effect. However, the addition of NF1 FBs resulted in stiffening of plexiform SC spheroids (237 ± 91 Pa) (Fig 1c).

Discussion: This work establishes the mechanical properties and influences of various neurofibroma components both individually and combined. This helps to build an understanding of what role each component plays in building the microenvironment of PNFs. These mechanics are also critical to consider when creating models for these tumors. Neither the softer environment created by the plexiform SCs or the effect of NF1 FBs has been explored and both may be relevant to the overall formation of plexiform neurofibroma.

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NF1 Somatic Mutation Triggering Cellular Apoptosis to Prevent Certain NF1 Syndrome Features

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Consider that the NF1 syndrome could be reversed or stopped early on through genetic manipulation – before elements of the disorder compromised the person. We could 1) modify the zygote's *NF1* mutation (*CRISPR*/*Cas9*); 2) amplify expression of wildtype allele; 3) use the occurrence of an *NF1* somatic mutation to trigger removal of the affected cell through induced apoptosis. Persons with ordinary, prototypical NF1 are born with a germinal/zygotic mutation at the *NF1* locus in all of their cells. Subsequently an *NF1* somatic mutation occurs in a variety of non-germinal cells, including, and especially the Schwann cell (SC). *NF1* haploinsufficiency seems to provide the conditions for the SC (and other cells) to develop *NF1* diploinsufficiency: recurrent *NF1* somatic mutation occurs repeatedly in multiple types of tissue, resulting in progression of the NF1 syndrome. What if we could prevent *NF1* diploinsufficiency or prevent persistence of *NF1* diploinsufficient cells? Imagine a viral-like construct with an apoptosis trigger held in abeyance unless an *NF1* somatic mutation occurs in the presence of an *NF1* germinal mutation or whole gene deletion. Destroying a cell immediately consequent to its developing *NF1* diploinsufficiency in a person with the NF1 syndrome seems reasonable, since we expect *NF1* diploinsufficiency to have only adverse results. That is, we could interfere with the intracellular and tissue progression of the disorder by removing the altered the cell using the viral homologue or paralogue. In summary, this molecular construct would occupy the nucleus in each of the NF1 person's cells and have two critical abilities: 1) monitoring of the long arm of Chromosome 17 to determine if the *NF1* wildtype allele is continuously present; 2) a trigger to initiate apoptosis in the event of loss of the *NF1* wildtype allele. In this way, any cell that converts from *NF1* haploinsufficiency to *NF1* diploinsufficiency will be shed immediately from the person through apoptosis. Thus, none of the NF1 person's cells would be able to survive the change from *NF1* haploinsufficiency to *NF1* diploinsufficiency. Only *NF1* haploinsufficient cells are allowed to survive. The risks are nil and the benefits substantial.

Identification of Inhibitors Selectively Targeting the Proneoplastic Mitochondrial Chaperone TRAP1 in NF1-Associated Tumors

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Dysregulation of the Ras/ERK transduction axis impacts on the metabolic rewiring of tumor cells through phosphorylation and thus activation of the mitochondrial chaperone TRAP1 (1), a conserved member of the Hsp90 family that downregulates oxidative phosphorylation by inhibiting succinate dehydrogenase (SDH), leading to a succinate-dependent stabilization of the pro-tumorigenic transcription factor HIF1alpha (2). These findings have suggested that TRAP1 could be a feasible target to develop novel therapeutic approaches for Neurofibromatosis type 1 (NF1)-associated tumors, where hyperactivation of the Ras/ERK pathway is crucial. In this context, we have exploited computational approaches based on the characterization of TRAP1 internal dynamics in order to identify TRAP1 regions that can be potentially targeted by selective TRAP1 inhibitors in an allosteric way. Based on this information, we have fished out and tested a set of putative TRAP1 allosteric inhibitors, finding that all these compounds are able to decrease TRAP1 ATPase activity in a highly selective way and with a degree of inhibition very similar to that displayed by the classical Hsp90 family inhibitors. At a cellular level, all tested compounds were able to reverse the inhibition of SDH activity exerted by TRAP1 in both mouse and human MPNST (Malignant Peripheral Nerve Sheath Tumors), while TRAP1 knock-out cells were completely insensitive to the effects of these small molecules. Notably, TRAP1 ligands are not toxic *per se* on cultured neoplastic cells but abolish the capability of MPNST cells to overcome contact inhibition and form foci. These findings provide evidence that novel computational approaches can be used to design highly selective allosteric inhibitors of TRAP1, which might be useful tools for the comprehension of TRAP1-mediated metabolic changes and their possible involvement in the onset and growth of MPNST. These molecules could constitute lead compounds for the development of novel anti-neoplastic strategies for NF1-associated tumors.

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Comprehensive Genomic and Epigenomic Characterization of the Spectrum of Peripheral Nerve Sheath Tumors Associated with NF1 Identifies Two Distinct MPNST Subtypes

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Introduction: Neurofibromatosis type 1 (NF-1) is a tumor predisposition syndrome that results in the development of innumerable peripheral nerve sheath tumors. These tumors fall within a spectrum of benign, premalignant and malignant tumors. Most benign neurofibromas have a very small risk of malignant transformation, but plexiform neurofibromas harbor a 5-15% lifetime risk of malignant transformation into a sarcoma called malignant peripheral nerve sheath tumor (MPNST). The molecular mechanism that drives malignant transformation has yet to be fully understood. Here, we provide an integrated molecular characterization of the spectrum of peripheral nerve sheath tumors and identify two novel molecular subtypes of MPNSTs that are driven by SHH or WNT pathway activation.

Methods: We investigated the genome-wide DNA methylation patterns of a spectrum of peripheral nerve sheath tumors (N=108, 19 MPNSTs, 22 premalignant NFs, 34 plexiform NFs and 33 cutaneous NFs). The methylation classes were further characterized by DNA copy number analysis, mutational profiling and RNA sequencing on a subset of tumors. In addition, functional validation of identified pathways was performed using CRISPR/cas9 knockout of relevant sonic hedgehog (SHH) and WNT pathway genes in immortalized neurofibroma cell lines.

Results: Unsupervised consensus hierarchical clustering of the top 20K most variable methylated probes yielded seven stable and robust subgroups that are clinically relevant. The high-grade MPNSTs formed two distinct methylation-based clusters (MPNST-G1 and MPNST-G2). *PTCH1* loss was prevalent in MPNST-G1 compared to MPNST-G2 (75% vs 12.5%, $p < 0.05$). In addition, MPNST-G1 harbored *PTCH1* CpG island promoter hypermethylation in (87.5% vs 0%, $p < 0.001$). Transcriptome profiling recapitulated the two distinct MPNST subgroups. Gene set enrichment analysis (GSEA) demonstrated that RB1 and PRC2 signaling pathways are aberrant in both MPNST-G1 and MPNST-G2. However, SHH pathway activation is observed in MPNST-G1, while WNT and CCND1 pathway activation is observed in MPNST-G2. To determine if SHH pathway activation is sufficient for malignant transformation, we knocked out *PTCH1* in immortalized neurofibroma cells lines. In 3 different cell lines with *PTCH1* knockout, we observed induction of a malignant phenotype, with increased cellular proliferation and invasion.

Conclusions: Our integrative genomics approach to a large cohort of the spectrum of peripheral nerve sheath tumors identified two novel MPNST subgroups. The MPNST subgroups can be reliably assigned to subgroups through methylome and transcriptome signatures. Future research on MPNSTs and the development of clinical trials should take into consideration these two distinct types of MPNSTs to target these pathways as a novel therapeutic approach.

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Antisense Oligos, Human Cell Lines, and Mouse Models for Evaluation of Exon Skipping as a Therapeutic for Neurofibromatosis Type I

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Previously, we investigated the feasibility of utilizing an exon skipping approach as a genotype-dependent therapeutic for neurofibromatosis type 1 (NF1) by determining which *NF1* exons might be skipped while maintaining neurofibromin function. After *in silico* analysis and utilizing a novel *Nf1* cDNA system, *NF1* exons 17, 47 and 52 were selected as the best candidates for the antisense PMO exon skipping approach. The Human Splice Finder was used to identify exonic splicing enhancer (ESE) motifs in each of the proposed exons and PMOs were designed to mask ESE motifs to aid exon skipping. Each of the exons and intronic flanking regions secondary structures were modeled using Visual OMP software in order to assess the biophysical binding properties of the PMOs to its targets. 25-mer PMOs were designed and tested in cell culture at 2 μM. A nested RT-PCR read-out was developed and optimized for detection of the exons skipping in HEK293T cell line. Semi-quantitative analysis was performed using ImageJ software. Following dose response examinations, the most efficacious PMOs were then further optimized by increasing their size to 28-mers and performing micro walk across their respective targets. The 28-mer PMOs show significantly increased skipping compared to their 25-mer counterparts. The detailed dose-response assessment of each PMO for exon skipping is in its final stages, as is determination of the optimal PMO for each target proposed for future cellular and in vivo studies. To this end, we have developed multiple cell lines and animal models. We have several cell lines with a patient-specific mutation in exon 17 (c.1885G>A; p.G629R; which creates a cryptic splice that results in Gln616Glyfs*4). We have generated an exon 47 mutation cell line with a homozygous c.6948insT that generates a frameshift. We have also generated exon 52 mutations containing the patient specific mutation R2550X. Homozygous CRISPR deletion of exon 17 in a novel mouse model is compatible with viable, fertile, and grossly healthy animals, providing proof-of-concept that exon 17 may not be essential for murine neurofibromin function. Creation of mice with the patient specific p.G629R mutation has begun. We have humanized all of mouse exon 17, including an extra 50 bp flanking of each intron. Finally, assessment of NF1 patient databases indicates that mutations resulting in deletion or skipping of exons 17 and 52 have not been reported; truncating pathogenic variants in each exon account for ~0.9 and 0.25% of unrelated NF1 cases respectively. Hence, exon skipping might be used for intragenic exon 17 mutations affecting a significant portion of individuals.

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NF1-Associated Cognitive Impairment in a Porcine Model of Neurofibromatosis Type 1

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Our team has generated and characterized a highly innovative NF1 miniswine model bearing a heterozygotic mutation in exon 42 orthologous to a mutation found in NF1 patients. The *NF1*$^{+/ex42del}$ miniswine phenocopies a range of manifestations seen in patients with NF1 disease (and often not seen in mouse models), including dermal lesions and neurological deficits. Here we further examine how mutations in *NF1* influence structural and cellular changes within the CNS and explore how these changes may contribute to cognitive impairment in patients. We have performed an extensive longitudinal study to examine changes in: a) behavior and motor functionsby performing assays for social interactions, sleep and home pen locomotion, learning and memory tests, and gait; b) neurotransmitter levels within discrete brain regions, including an imbalance in the ratio of GABA:glutamate within the hippocampus; c) regional brain pathology associated with NF1 dysfunction, including prefrontal lobe, anterior cingulate, and hippocampus, using histological and cell type-specific read-outs. In both sexes of pigs, we have observed an increase in mature oligodendrocytes within the prefrontal tracts and the anterior cingulate. Moreover, we have observed an increase in quiescent microglia in the prefrontal lobe and hippocampus of male *NF1*$^{+/ex42del}$ miniswine. Interestingly, male *NF1*$^{+/ex42del}$ miniswine present with cutaneous tumors at a greater frequency than female animals, and the prevalence of their tumor burden appears to correlate with the level of microglia within the CNS. Similar to what has been reported in NF1 mouse models, astrocytic activation within the CNS of our NF1 miniswine models appears to be variable across time and biological sex. However, there does appear to be a consistent increase in astrocytic activation within the CA1 region of the hippocampus in male *NF1*$^{+/ex42del}$ miniswine. Taken together, we report on the comprehensive characterization of neurocognitive abnormalities in NF1 miniswine, and this late breaking abstract will highlight how specific CNS related perturbations correlate to changes seen in NF1 patients.

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Genome Engineering of Disease Specific Cell Types Reveals Hidden Vulnerabilities and New Therapeutic Approaches for Treatment of Neurofibromatosis Type-1 Associated Malignancies

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Genome engineering technologies, such as CRISPR/Cas9, offer exciting opportunities to build more relevant cell line based models to advance cancer research. These techniques allow introduction of clinically relevant mutations into cells of the correct tissue type for the disease being studied. Using these principles, we built models of tumors that arise in Neurofibromatosis Type 1 (NF1) and used these models for therapeutics discovery with synthetic lethal pharmacogenomic and genetic screens.

Given plexiform neurofibromas and MPNSTs arise within the Schwann cell linage, we developed a drug discovery pipeline to identify targeted therapeutics for treating NF1-related neoplasia. Using CRISPR/Cas9, we created immortalized human Schwann cell lines that are deficient for the *NF1* gene or *NF1* and components of Polycomb Repressive Complex 2 (PRC2), such as *SUZ12*. ~80% of all MPNST harbor loss of function mutations in PRC2 genes, which is highly suggestive that perturbation of epigenetic homeostasis plays a role in malignant transformation of neurofibromas.

We previously reported results from our drug screening efforts against *NF1* deficient human Schwann cells, as well as preliminary work with *NF1*/*SUZ12* double mutants. Here we build upon this work through extensive *in vivo* testing of these therapeutic candidates in models of MPNST, mechanistic studies, and complete genome-wide CRISPR based genetic screens.

Our therapeutic screening efforts identified compounds showing selective lethality towards *NF1*/*SUZ12* double mutants, closely mimicking the genetics of an MPNST cell. These include classes of drugs affecting epigenetic homeostasis, such as HDAC inhibitors and DNA methyltransferase inhibitors. Moreover, many of these drugs showed strong synergy *in vitro* when tested in combination against *NF1*/*SUZ12* deficient human Schwann and MPNST cell lines. This was particularly true when MEKi was combined with HDACi. We have explored the mechanism of this synergy and confirmed our results by engineering mutations in other components of the PRC2 machinery (such as *EED*) and through pharmacologic inhibition of EZH2.

Clinically interesting drug candidates were advanced and tested as single agents and in combination in *in vivo* models of neurofibroma and MPNST. Some have shown dramatic efficacy and top combinations exhibit strong synergy *in vivo.* To date, we have attained long term durable responses in an MPNST xenograft model (S462TY) and dramatically shrank large MPNST xenografts established in immune deficient mice. We are now testing these combinations and new compounds coming through our testing pipeline in multiple PDX models of MPNST to assess the generalizability of the responses.

These pharmacogenomic screens leveraged synthetic lethal interactions with *NF1* and *SUZ12* to identify therapeutics for the treatment of NF1-related neoplasia. The discovery of agents effective against models of MPNST is exciting, as to date there are no approved targeted therapies for MPNST. Results from our studies are forming the basis for clinical trials we hope to propose for the treatment of MPNSTs and aggressive plexiform neurofibromas.

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Single-cell RNA Sequencing Identified Increased Schwann Cell Population as a Marker of Atypical Neurofibromatous Neoplasms with Uncertain Biologic Potential

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Patients with Neurofibromatosis 1 (NF1) present a number of clinical manifestations, one of which is the development of Plexiform Neurofibroma (PN). Although PN are considered benign tumors, they can represent precursor lesions as 10-15% of PN transform to Malignant Peripheral Nerve Sheath Tumors. Mechanistic understanding of steps in the malignant transformation is challenging but clinically meaningful. After a consensus meeting in October 2016, the term of "Atypical Neurofibromatous Neoplasms with Uncertain Biologic Potential (ANNUBP)" has been used for lesions with at least two of the following features: nuclear atypia, loss of neurofibroma architecture, high cellularity, and increased mitotic features. Genetically, ANNUBP frequently demonstrate loss of *CDKN2A* and exhibit increased risk of malignant transformation. While previous efforts have used a candidate gene approach to dissect this complicated transformative process, in this work, we applied single cell RNA sequencing (scRNAseq) to human primary ANNUBP and the adjacent PN to comprehensively evaluate differences between these two types of NF1 tumors. Six fresh core needle biopsies taken from different areas of one enlarging PN were dissociated into single cell suspensions in the presence of enzymatic digestive medium. The number of cells captured from each of these biopsies ranged from 2261-4378 and 6 unique capture libraries were sequenced independently. Histologic review of these tumors demonstrated that three out of the six biopsies were classified as ANNUBP. Data generated using the 10X Chromium platform were analyzed as 6 separate tumors and then integrated as PN and ANNUBP for comparisons using Seurat3.0. A total of 20,252 cells, including 11,261 cells from 3 ANNUBP biopsies and 8991 cells from 3 PN biopsies, were included in the downstream analysis. A total of 12 different cell types that belong to 18 distinct clusters were detected in this dataset and both PN and ANNUBP contributed similarly to each cell cluster. Interestingly, more than 35% of all cells were identified as Schwann cells (SC, 28.9% of PN and 46.6% of ANNUBP), in comparison to less than 5% of SC detected in a previous PN single cell dataset. Additionally, *CDKN2A* was identified as a marker of SC, along with other classical SC markers. When comparing expressions of marker genes of each cluster, we found that expression of *CDKN2A* was significantly lower in SC of ANNUBP (43.1-70.4%) than in SC of PN (64.3-84.8%). In summary, we report our findings of using scRNAseq to compare ANNUBP and adjacent PN that increased number of SC with partial loss of *CDKN2A* expression is a biomarker of this premalignancy.

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NF1: Clinical Science

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NF1: Clinical Science

Potential Predictors of Neuropsychiatric Symptoms Associated with NF1

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Introduction: Chronic history of spinal neurofibromas (sNF) and plexiform neurofibromas (PNF) can lead to significant pain, depression and other psychiatric complications depending on the burden of the disease. Previous studies of neuropsychiatric manifestations in patients with Neurofibromatosis type 1 (NF1) have typically involved cohorts at individual clinical or research centers. In this study, we evaluated for neuropsychiatric manifestations as a function of disease burden among 6850 patients with NF1 from the Children's Tumor Foundation (CTF) Registry. We hypothesized that neuropsychiatric manifestations such as depression and anxiety would be higher among NF1 patients with sNF and PNF compared to those without.

Methods: We obtained approval to access the CTF Registry (CTF) after submitting a letter of intent to CTF. The dataset contained information on 6850 patients diagnosed with NF1, including associated symptoms. We calculated odds ratios (OR) for selfreported neuropsychiatric manifestations among patients with NF1 and sNF or PNF and compared the results to those without sNF and PNF.

Results: 2828 patients (41%) reported having had genetic testing confirming an NF1 diagnosis. The majority were diagnosed before age 5 years (66%). Neurofibromas near the spine (sNF) diagnosed by MRI were reported in 1471 of NF1 patients (21.4%), 420 (28.5%) of whom had surgical intervention. Plexiform neurofibromas (PNF) were reported in 35% of patients (n=2451). The OR for anxiety if the person had more than 1 PNF compared to no PNF, and 1 PNF compared to no PNF, was 2.2 (95% CI 1.77- 2.79, $p<0.0001$) and 1.4 (95% CI 1.04-1.88, $p=0.026$), respectively. The OR of having depression if the person had more than 1 PNF compared to no PNF, and 1 PNF compared to no PNF, was 2.8 (95% CI 2.19-3.62, $p<0.0001$ and 1.55 (95% CI 1.11- 2.16, $p=0.010$), respectively. The OR of having difficulties with social interactions if the person had more than 1 PNF compared to no PNF, and 1 PNF compared to no PNF, was 1.75 (95% CI 1.32-2.31, $p=0.0001$) and 1.16 (95% CI 0.80-1.68, $p=0.43$), respectively. The OR of having fatigue if the person had more than 1 PNF compared to no PNF, and 1 PNF compared to no PNF, was 3.76 (95% CI 2.83-5.01, $p<0.0001$) and 1.55 (95% CI 1.04-2.31, $p=0.0316$), respectively. For sNF, the OR of having anxiety, depression, difficulties with social interactions, and fatigue was 2.0 (95% CI 1.64-2.53, $p<0.0001$); 2.35 (95% CI 1.85-2.98, $p<0.0001$); 1.7 (95% CI 1.27-2.2, $p=0.0002$), and 2.8 (95% CI 2.2-3.65, $p<0.0001$) respectively compared to those with no sNF.

Conclusions: We conclude that NF1 patients with more than 1 PNF (or sNF) have significantly higher OR of having depression, anxiety, difficulties with social interactions, and fatigue compared to those with only 1 PNF or no PNF (or no sNF). We propose to have standard recommendations for screening NF1 patients with high burden of sNF and PNF in all NF1 Clinic Network for psychiatric symptoms, and make referrals and treatment as seems necessary.

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An International Multi-Center Natural History Study of Newly Diagnosed Neurofibromatosis Type 1 Associated Optic Pathway Glioma (NF1-OPG): Preliminary Enrollment and Clinical Characteristics

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Introduction: Treatment and clinical management decisions for children with NF1-OPG remain challenging. In order to establish evidence-based guidelines, we prospectively enrolled children with newly-diagnosed NF1-OPGs, and gathered standardized neuro-oncology/NF1 clinicians' assessments and ophthalmology examinations.

Methods: Children with NF1 and newly diagnosed OPGs, confirmed by central review, were eligible. Indications for obtaining the initial MRI, as well as factors associated with the decision to treat with chemotherapy or observe without treatment, were obtained. Quantitative visual acuity (VA) was evaluated at enrollment and months 3, 6 and 12 after enrollment, using Teller acuity cards (TAC) and, when old enough to cooperate, the computerized amblyopia treatment study HOTV protocol (ATS-HOTV). Other ophthalmic features and MR images were collected at the same interval. Goal enrollment is 250 subjects.

Results: One-hundred thirty-three children (52% female) from 20 institutions met inclusion criteria, and were included in this preliminary analysis. The most common reasons for the diagnostic MRI included screening related to NF1 diagnosis (36.8%), ophthalmologic concerns (29.3%), and non-ophthalmologic concerns (24.8%), such as headache. Eighty-six percent of subjects were able to perform quantitative VA testing using TAC at enrollment. To date, twenty subjects have initiated treatment with chemotherapy (the majority with carboplatin-based regimens), only twelve (9%) at the time of the initial OPG diagnosis. Median age at OPG diagnosis was 3.1 years. Age and sex distribution were similar in subjects immediately entering the observation and treatment arms (median age 3.0 versus 3.5 years, respectively).

Conclusion: Most children with NF1-OPGs are observed at time of their initial OPG diagnosis, rather than treated. Importantly, a large proportion of children are able to complete quantitative VA testing at enrollment. Once enrollment is complete, these data will help to establish evidence-based guidelines for clinical management of NF1-OPGs. Furthermore, this robust and exceptionally well genotyped-phenotyped longitudinal cohort with accompanying brain imaging could be utilized to investigate the evolution of vascular, neurocognitive, social functioning and other neuro-oncologic manifestations of NF1.

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Impact of Chronic Pain on Psychosocial and Cognitive Functioning in Individuals with Neurofibromatosis Type 1

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Background: Individuals with NF1 may experience a range of conditions (e.g., plexiform neurofibromas (PN), scoliosis, joint pain, headache), many of which can lead to chronic pain. These pain-inducing manifestations of NF1 may inhibit daily functioning and quality of life amongst the NF1 population. They may also be associated with increased risk for psychological comorbidities, though the association between the experience of pain and mental health diagnosis is not well-understood.

Objective: We aimed to explore the association between chronic pain and neuropsychological functioning in individuals with NF1.

Method: This study included 229 individuals diagnosed with NF1 (50.6% female) between the ages of 5 and 67-years (M=14.87) who contributed data to a clinical registry of NF1 patients followed for outpatient care. When further broken down by age, the sample consisted of 65 adults (ages 18 and up), and 164 children (ages 5 to 17). Information regarding presence of chronic pain, psychological disorders, and cognitive functioning was extracted from patient medical records, neuropsychological reports, and parent questionnaires.

Results: Over a quarter of the sample (n=66; 27.8%) reported chronic pain, with adults (ages 18 and up, n=65), more likely to experience chronic pain than children (55.5% of adults vs. 18.3% of children, $p<.001$). Sources of pain included PNs (n=78; 32.9%), and headaches (n=94, 39.7%). 38.4% (n=91) of patients had at least one psychiatric diagnosis. Those with chronic pain were more likely to be diagnosed with depression than those without pain (64.3% vs. 35.7%, respectively, $p=.002$), but not with anxiety ($p=.925$) or ADHD ($p=.794$). We also examined a subset of pediatric patients who had completed neuropsychological testing (n=62). Chronic pain was not associated with differences in cognitive functioning, or parent-and teacher-reported executive function and attention. However, parent- ($p=.01$) and teacher-reported hyperactivity ($p=.01$) was reduced in children with pain compared to those without pain.

Conclusion: Because chronic pain is common amongst individuals with NF1, it is important to continue exploring the ways pain may interfere with daily functioning, including how it may impact academic, cognitive, and psychosocial domains. Given the high risk for depression amongst individuals with NF1 who experience chronic pain, future research should focus on the interaction of chronic pain and mental health.

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Whole Body MRI to Identify Atypical Neurofibromas in Patients with NF1 and High Plexiform Neurofibroma Tumor Burden

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Background: Recent studies have identified atypical neurofibromas (ANF) as a precursor lesion for malignant peripheral nerve sheath tumors (MPNST) in patients with Neurofibromatosis Type 1 (NF1). In a recent analysis, patients who developed MPNST had a history of ANF either at the site of interest or a different location. Importantly, none of the patients who had complete resection of the ANF had recurrence. This suggests that early identification and resection of ANF can potentially be preventative to malignant transformation in a subset of patients. Preliminary studies show that patients with increased plexiform neurofibroma (PN) burden have higher risk for developing MPNST. However, we currently do not have a screening method to effectively detect pre-malignant ANF, characterized as distinct nodular lesions (DNL) on magnetic resonance imaging (MRI). The primary goal of this study was to determine if whole body MRI (WBMRI) can effectively be used to screen for DNL in patients with NF1 and high PN burden.

Methods: We prospectively obtained two WBMRI scans, 4-6 months apart, on patients with high plexiform tumor burden (defined as >=1 PN that is >3cm in diameter on MRI) at the same time as their clinically scheduled regional MRI to allow for radiographic comparison of each identified target PN. MRI scans were reviewed for the presence of DNL and target PN volume. Plasma for cell free DNA (cfDNA) isolation was obtained with each MRI. Clinical characteristics including age, gender and symptoms were also collected.

Results: A total of 10 patients between 13-24 years of age were enrolled (2 Female: 8 Male), and 38 MR images were obtained (19 WBMRI, 19 regional). Baseline median target PN volume was 141cm^3 (range 6.1-3404cm3). Target PN volume was stable for all patients within the 4-6 month evaluation period (n= 9 subjects, range -16 to 11% volume change). Target tumor volume was not significantly different (n=11 MR pairs, p=0.9021) when comparing WBMRI (6 or 10mm slices) and regional MRIs (3-5mm slices). One patient had a DNL noted on WBMRI, which was confirmed on regional MRI.

Conclusion: WBMRI can be used to identify DNL in patients with high PN burden, although a larger study will be required to determine its utility as a screening modality for detecting DNL/ANF this patient population. Studies focusing on the combined utility of WBMRI plus cfDNA as biomarkers for the detection of ANF in patients with high PN volume are pending.

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Innovation in the Treatment of Persistent Pain in Adults with NF1: Implementation of the iCanCope Mobile Application

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Introduction: Over 50% of individuals with NF1 report significant pain and discomfort (1,2) which can be associated with tumors, but is often not localized to a structural lesion, thus presenting treatment challenges for patients and their medical caregivers. Moreover, the increased pain in this population can negatively impact an individual's perceived quality of life (3,4). Due to the complexity of the disorder (e.g., location, severity, number, and type of tumors), there are limited effective therapeutic options for treating pain. In this regard, tumor-related pain is primarily managed by either surgery or medication (5,6). Mobile technologies can be used to enhance the accessibility of pain self-management therapies (7). In addition to improving access, these technologies can empower individuals to take an active role in managing their condition by providing "in the moment" access to pain coping strategies. The purpose of this study was to understand the burden of pain in individuals with NF1 through qualitative interviews of and adults with NF1 to inform the refinement of an app to help them cope with pain.

Methodology: A purposive sample of adults with NF1 (N=32) engaged in one of six one-hour web-based, audio-recorded, semi-structured qualitative focus groups, conducted with Zoom, to explore four key themes: 1) daily impact of pain, 2) barriers of pain care, 3) strategies to self-manage pain, and 4) acceptability of using technology-based solutions to help assess and manage pain. Participants were predominantly female (n=25) with an average age of 31.2.

Results: Data saturation was reached after the 6th session (8). The results centered around four main themes: community need, pain tracking, functionality, and barriers of use. Interestingly, the community need was more frequently coded than any other theme, indicating the need for commonality and social support with others who are in pain because of their NF1 symptoms. Pain tracking content focused on including narrative descriptions along with quantitative measures. Lastly, individuals suffering from NF1 were successful resilient in managing pain symptoms with limited resources; however, they felt they would benefit from additional alternative strategies to better facilitate pain management.

Discussion: The current study provided the content needed to adapt the iCanCope mobile application and determine the acceptability of the identified features and self-management needs in adults with NF1. These preliminary findings provided clear indication that a pilot randomized control trial is needed to assess the applicability of the iCanCope mobile application for NF individuals suffering from pain symptoms.

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The Impact of Anxiety and Depression on Work Readiness within Neurofibromatosis Type 1 (NF1)

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Introduction: Neurofibromatosis Type 1 (NF1) is an autosomal dominant genetic disorder affecting an estimated 1 in 3500 individuals (1). Manifestations of NF1 commonly present as plexiform neurofibroma tumors (PNs), cafe au lait macules, dermal neurofibromas, as well as learning disabilities, attention problems, and social emotional problems (2). Subjective well-being (quality of life) has been investigated for several decades and employment has been shown to be a strong predictor of subjective well-being (3). However, quality of life improves even greater when barriers to employment are eliminated or reduced, and career goals and work motivations are met (4). The current study evaluated the perceived impact of work barriers onto individuals with suffering from NF1. First, what impact does perceived barriers to successful employment, anxiety, and depression have on hope for employment? Second, how does duration of NF1 influence employment hope and work readiness?

Methods: A total of 96 individuals (n=63 female) with a diagnosed case of NF1 completed an online survey hosted through Qualtrics. Each participant was asked to self-report on the following: Generalized Anxiety (Generalized Anxiety Disorder; GAD-7); Depression (Patient Health Questionnaire; PHQ-9); Work Readiness (Work Readiness Scale), Employment Hope Scale and Barriers to Employment.

Results: Two stepwise linear regressions found significant differences between work readiness, employment hope and barriers to work when compared to generalized anxiety ($p < .001$) and depression ($p < .001$). Specifically, the subscales of workplace readiness (communication, self-view, and skills) and employment hope (psychology empowerment, utilization of skills, and decision making) were highly significant when parsed out. Interestingly, no significance was found between the length of time of having the disease and employment hope and work readiness.

Discussion: Previous research has shown individuals with NF1 have lower quality of life than normalized populations (5). The current study is the first study, to the author's knowledge, to evaluate the impact of generalized anxiety and depression on workplace barriers, one of the factors within quality of life. The indication of how individuals with NF1 are perceived and their self-view have a direct effect on their anxious and depressive feelings, which can limit potential employment opportunities for those with the disease. Future research needs to directly evaluate the impact of stigma within work related activities.

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Clinical and Neuroradiological Characterisation of Spinal Lesions in Adults with Neurofibromatosis Type 1

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Objectives: Neurofibromatosis type 1 (NF1) can manifest itself in many ways in the spine. This study aims to report the types of spinal lesions, clinical and demographic data in a large cohort of NF1 patients from a dedicated complex NF1 centre. In particular, the characteristics of those with spinal neurofibromatosis, where neurofibromas are present on every spinal nerve root were sought for comparison with the wider group of patients with NF1.

Methods: This is a retrospective review of MDT minutes of 303 patients from a UK NF1 centre and the largest reported series of NF1 patients based on radiological data. Prevalence of each symptom and lesion was calculated and statistically significant associations were established.

Results: The most reported findings and symptoms were cutaneous lesions (44.9%) and neurological deficit. (27.4%). 28.4% had dural ectasia, 52.5% had some form of spinal deformity, mostly thoracic scoliosis. 57.8% had spinal nerve root tumours, the most common of which were at C2. The lesions that progressed the most were spinal nerve root tumours (29.1%). The only statistically significant association between the types of lesions was dural ectasia and spinal deformity ($P < 0.003$), where dural ectasia is associated with a 32.6% increase in spinal deformity incidence.

Conclusion: This is the largest reported descriptive study to date of spinal lesions in NF1. Spinal tumours and spinal deformity are prevalent in NF1. The predilection of spinal tumours for flexible regions of the spine suggests that repetitive movement might be an important factor in pathogenesis. The lack of significant associations between various lesions suggests differing pathways in their pathogenesis. Physicians and patients should be alert to the observation that although many spinal neurofibromatosis patients display no neurological deficit, they often have significant lesions which require monitoring and sometimes surgery.

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Psychological, Social, and Behavioral Functions in the RASopathies

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Objective: This research reviews and summarizes studies that have focused on psychological, social, and behavioral functions and outcomes across the RASopathies with the aim of identifying similarities and differences across disorders.

Methods: Empirical studies published in peer-reviewed, scientific journals over the past 32 years were identified through searches in PubMed, PsychInfo, and Google Scholar through July 23, 2019. Searches included the names of each of the RASopathies individually with one psychological search. The authors reviewed all titles and abstracts for relevancy. Full-text articles of abstracts deemed relevant were then fully reviewed by the authors. Case studies were excluded. 796 publications were identified through the initial search.

Results: 80 of 796 publications (10%) were deemed relevant after initial screening and full review by the authors. Research on Neurofibromatosis Type 1 (NF1) was most prolific, followed by Noonan syndrome (NS), Costello syndrome (CS), Cardiofaciocutaneous syndrome (CFCS), and Noonan syndrome with multiple lentigines (NSML). Very little research has been published in this area on Legius syndrome (LS) and Noonan syndrome-like disorder with loose anagen hair (NSLAH). Social dysfunction is one of the most researched deficits across all RASopathies. Additional similarities in these domains include increased behavioral problems, with a predominance of internalizing symptoms (e.g., anxiety, depression), and decreased adaptive functioning and quality of life.

Conclusions: There is a paucity of research on the psychological, social, and behavioral manifestations in children and adults with RASopathies, although an increase in research has emerged over the past seven years. Dysfunction in these areas can negatively impact functioning with regard to relationships, education, employment, and quality of life. Researchers should consider these difficulties within a biopsychosocial model to better understand the presence of psychological and social impairments within and across the RASopathies. This review highlights the need for prospective research in psychological, social, and behavioral functioning in the RASopathies, with the ultimate goal of developing interventions and therapies targeting psychosocial impairments.

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Factors Contributing to the Response of Children with NF1 and Plexiform Neurofibromas to Selumetinib

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Background: Plexiform neurofibromas (PN) in NF1 can cause substantial morbidity. The phase 1 and 2 studies of selumetinib (NCT01362803) showed 71 and 74% partial response (PR) rates, respectively, and the phase 2 study demonstrated improvement in PN-related morbidities. Here we analyze 1) factors contributing to the responsiveness to selumetinib, 2) response dynamics, and 3) the effect of treatment discontinuation on PN growth.

Methods: All participants of the phase 1/2 selumetinib study (patients 2-18 years old with inoperable +/- symptomatic PN) are included in this analysis. Selumetinib was administered in continuous 28-day cycles. Tumor response was evaluated with volumetric MRI analysis (PR = target PN volume decrease ≥20%). We collected age, PN growth rate prior to treatment, response and progression status, PN volume change at best response, time to PR, time to best response, selumetinib dose reductions and PN re-growth off treatment. We performed two-group comparisons using a Wilcoxon rank sum test or Spearman correlation analyses. Fractions of patients in two groups were compared using Fisher's exact test.

Results: The median age of the 99 participants was 10.6 years (range, 3.0 to 18.5). Best response was PR in 73 patients (74%), stable disease (SD) in 25, and 1 patient had no response data. Patients who achieved a PR were slightly younger compared to those who did not (median age at enrollment 9.5 vs. 13.3 years; P=0.01). Younger age was weakly correlated with more PN shrinkage at best response in the entire cohort (R=0.28, P=0.01), however age did not have a significant effect on the degree of maximal PN shrinkage in the subset with PR (R=0.14; P=0.23). Responses were seen in previously growing and stable PN and no correlation was found between the pre-treatment growth rate and maximum response to study drug (R=0.05; P=0.65). Among responders, the PR was initially observed after a median of 8 cycles of treatment (range, 4 to 28 cycles), or a median of 6.9 months (range, 3.2 to 25.9 months). Further shrinkage was observed for a median of 14.1 months (range 0 to 57.9 months) until reaching best response. Progressive disease after achieving a PR (≥20% volume increase above best response) was more likely in patients who required dose reduction compared to those who did not (55.6% [10/18] vs. 21.8% [12/55]; P=0.0156). The median duration of treatment was 38.9 months (range, 1.0 to 85.1 months) with 56 patients still receiving treatment as of March 2020. Of the 43 patients removed from treatment, follow-up PN volumetric information is available for 25. Younger age at treatment discontinuation was moderately correlated with more tumor growth on the next consecutive MRI (R=-0.46, P=0.02).

Conclusions: Response rate to selumetinib in the combined cohort of phase 1 and phase 2 patients was 74%. Responses were observed in previously growing and stable PN. Responses were typically detected within the first year of starting therapy, with some additional tumor shrinkage within the following year. Younger age at enrollment was associated with higher likelihood of tumor response and younger age at treatment discontinuation was correlated with more tumor regrowth. Patients who required dose reduction were more likely to develop disease progression. These observations may assist in guiding the selumetinib treatment of children with NF1 PN.

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Comparing Social Functioning Measures in Early Childhood for Children with Neurofibromatosis Type 1

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Background: Social functioning patterns vary across measures in children with neurofibromatosis type 1 (NF1), with a social functioning measure indicating poorer social skills (Barton & North, 2004; Huijbregts & de Sonneville, 2011) and broad screening measures indicating no impairment (Martin et al., 2012; Sangster et al., 2011). The current aim was to further characterize social skills using three different measures and determine which measure is best able to identify difficulties for young children with NF1 with comparison to typically developing siblings. The frequency of difficulty on each measure will also be explored.

Participants and Methods: 49 children with NF1 (M=4.37, SD=1.11) and 24 siblings (M=4.70, SD=1.20) were assessed in early childhood (ages 3-6). Parent report on three measures was used to assess social functioning: Social Skills Rating System (SSRS) Social Skills scale, Behavior Assessment System for Children – Second Edition (BASC-2) Social Skills scale and Scales of Independent Behavior – Revised (SIB-R) Social Interaction and Communication scale.

Results: SSRS social skills for children with NF1 were significantly lower than the normative mean (t=-4.24, p<.001, d=.65) while no differences were found for the BASC-2 and SIB-R and BASC-2 (t=-.89, p=.38, d=.11; t=-.51, p=.61, d=.005). Social scores were not significantly different from the normative mean for siblings (SSRS: t=-1.23, p=.23, d=.25; BASC-2: t=1.23, p=.23, d=.19; SIB-R: t=.14, p=.89, d=.13). There was no significant difference in social scores between children with NF1 and their typically developing siblings on the three measures (SSRS: t=.1.68, p=.098, d=.43; BASC-2: adjusted t=1.41, p=.16, d=.33; SIB-R: t=.52, p=.608, d=.13).

Conclusions: The SSRS appears to better identify social skills difficulties for children with NF1 as poorer performance was evident in comparison to the normative mean. While there were no significant differences in social skills between children with NF1 and their typically developing siblings on any of the measures, the SSRS showed the strongest effect size. It is likely that a measure specifically targeting social functioning is able to better capture social difficulties and aid in early identification. Implications of these findings will be discussed.

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Incidence of Malignant Peripheral Nerve Sheath Tumor (MPNST) Development in Patients with NF1 Receiving and Not Receiving Medical Therapies Directed at Plexiform Neurorifbromas (PN)

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Background: Neurofibromatosis type 1 (NF1) is a genetic tumor predisposition syndrome. Many patients with NF1 develop histologically benign plexiform neurofibromas (PN), a subset of which transform to malignant peripheral nerve sheath tumors (lifetime risk 10-15%). Three patients with NF1 < 18 yo receiving the MEK ½ inhibitor (MEKi) selumetinib as part of a pediatric phase 2 trial (NCT01362803) developed MPNST while on study. We performed a retrospective review to evaluate the association of MEKi treatment with MPNST formation in a high-risk study population.

Objective: To determine the proportion of MPNST in NF1 patients enrolled on clinical trials at the NIH overall and also by the type of PN-directed medical treatment received.

Method: We conducted a retrospective review of all patients with NF1 enrolled on trials at the NCI Pediatric Oncology Branch from 1/2001 to 1/2020, excluding patients referred for treatment of MPNST. Patients were categorized into 3 groups: 1) History of MEKi therapy, 2) treatment with medical therapy other than MEKi, and 3) no PN directed medical therapy. Proportions of MPNST rates and median age to MPNST development were calculated for each group. Kaplan Meier life tables were generated to compare risk of MPNST between groups starting from birth to age of event/last follow up.

Results: 302 patients (54% male) with a diagnosis of NF1 (median age of event/last follow up 20.1 yo, range 2.2-62.2) were identified, of which 34 (11.3%; 18 male and 16 female) developed an MPNST. MPNST rates were lower in the MEKi group (4.2%,n=3/72) compared to the non-MEKi (11.4%, n=10/88) or no history of PN-directed therapy group (14.8%, n=21/142). The median age at MPNST development was earlier in the MEKi group (10.4 yo) compared to non-MEKi and no treatment group (17.3yo and 21.1yo, respectively). Patients in the MEKi group had an average of 1.1 (range 0-6) prior lifetime medical therapies while the non-MEKi group had an average of 1.5 (range 1-4).

Conclusion: In this high-risk study population, more events of MPNST were seen in patients who had not received PN-directed medical therapy or non-MEKi PN-directed therapy compared to those with a history MEKi therapy. Patients treated with MEKi developed MPNST at a younger age than those in the other groups. We are currently conducting a time dependent co-variate analysis of other factors that may contribute to the development of MPNST including number and type of medical therapies used throughout the lifetime and *NF1* microdeletion status.

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NOTE: Elements of this abstract were incorporated into author's platform presentation.

Treating Cognitive Deficits in Neurofibromatosis Type 1: Efficacy of Computerized Cognitive Training

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Background: Cognitive deficits are common and impairing neurological manifestations of Neurofibromatosis Type 1 (NF1). However, evidence-based and efficacious interventions for improving cognitive functioning in this population remain lacking. Cognitive training (CT) programs have increasingly been utilized to improve functioning. CogmedRM is the most widely-used CT program for working memory (WM) deficit remediation.

Objective: This was a randomized, multi-site clinical trial of a home-based, computerized CT program, CogmedRM, to improve WM difficulties in children with NF1.

Method: Ninety-three children with NF1, age 8-16 years (mean 11.23 years) at four participating sites (51.6% Male, 79.6% Not Hispanic/Latino, 71.4% White, 41.1% with ADHD, 14.8% on stimulant medication), underwent a screening assessment using a battery of neurocognitive tests and parent-completed questionnaires. Following baseline testing, 74 (80%) children showed evidence of WM difficulties and comprised the study population. These participants were stratified by stimulant medication use and randomized equally between two interventions: CogmedRM ($n = 37$), and the active control, MobyMax, an online reading program ($n= 37$). Participants had up to 11 weeks to complete the intervention and returned for follow-up assessment within 2 weeks of finishing the CT program.

Results: There were significant differences in cognitive functioning at the screening evaluation, such that participants who qualified for the study exhibited lower full scale IQ scores ($p=.034$), perceptual reasoning skills ($p=.002$), and WM skills ($p<.05$) than participants who did not qualify. On average, participants randomized to CogmedRM completed 22/25 training blocks, with 81% of participants meeting the standard for treatment adherence (i.e., completing at least 20 training blocks). Few adverse events (AE) were associated with either training program; children "never" or "rarely" experienced physical pain or discomfort (93%). The most common reported AEs associated with CogmedRM were the experience of frustration (48%) or boredom (43%) at least some of the time. The majority of children (62%) "often" or "always" enjoyed completing the exercises and most parents (88%) were "somewhat" or "very" satisfied with participation in the study. Preliminary results indicate that participants randomized to CogmedRM demonstrated significantly higher scores on Digit Span Backwards ($p=.016$) than participants randomized to MobyMax.

Conclusion: Computerized, home-based WM training programs may positively impact aspects of WM in children and adolescents with NF1. Given support for the acceptability and efficacy of CogmedRM, translation of this intervention into clinical practice may be warranted, with the potential to improve quality of life.

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Granting Agency: U.S. Department of Defense

Examining a More Comprehensive Profile and Identifying Longitudinal Predictors of Verbal Memory Functioning in Children and Young Adults with NF1

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Background: Youth with Neurofibromatosis type 1 (NF1) are at increased risk for cognitive and academic impairment (Yang et al., 2020). While some domains of cognitive functioning have been well explored, the impact of NF1 on various aspects of memory is less clearly understood. Most studies evaluating memory in NF1 have focused on broad domains of functioning, rather than specific subdomains of memory. The present study aimed to provide a more in-depth characterization of verbal memory in NF1, and to examine factors associated with poorer memory functioning over time.

Methods: Children through young adults with NF1, most with plexiform neurofibromas (PN), were followed longitudinally as part of a natural history protocol that involved neuropsychological testing at baseline (T1) and 36 months (T2). Testing assessed verbal learning and memory (California Verbal Learning Test; CVLT-II or CVLT-C) and working memory (Wechsler; WISC or WAIS) Digits Backward subtest. NF1 symptom severity was rated by medical providers at T1 and dichotomized to distinguish mild from moderate/severe symptoms. Patients rated their pain during testing sessions on a continuous visual analogue scale, which was controlled in all analyses.

Results: Participants included 99 children and young adults (ages 6-33 years) with NF1 (age $M=13.85\pm4.76$; 61% male; 97% with PN). At T1, individuals with moderate/severe NF1 symptom severity had significantly decreased performance on CVLT tasks assessing memory interference ($p=.034$), free recall after a 20-minute delay ($p=.033$), and long-delay recall with contextual cues provided ($p=.033$) compared to those with mild symptoms. Also at T1, presence of a learning disability (LD) predicted decreased performance on memory interference ($p=.019$) and working memory ($p<.001$). At trending levels, NF1 symptom severity at T1 predicted decreased overall recall ($p=.054$) and use of semantic clustering strategies ($p=.058$) at T2 (N=74). Presence of a LD at T1 predicted decreased overall recall ($p=.048$) at T2. Further, while no differences existed at T1, children with moderate/severe NF1 symptoms were significantly more likely to have below average ($T<40$) scores on overall recall at T2 than those with mild symptoms ($p=.017$). Other relationships were significant prior to controlling for pain.

Conclusions: This study suggests that overall memory scores may not fully represent specific aspects of memory functioning in children and young adults with NF1. Results suggest that academic (LD) and potentially symptom severity relate to lesser-explored memory domains and predictive of poorer memory over time. These longitudinal patterns more fully characterize memory in NF1 and suggest that those with diagnosed learning difficulties and possibly those with more severe NF1 symptoms should be monitored more closely for memory challenges and targeted for interventions.

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Choroidal Abnormalities Behavior in Families with Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant, progressive and multisystemic genetic condition with intra and interfamily clinical variability. Recently described, the choroidal nodules are lesions with high frequency in this condition and are indicated as a new diagnostic criterion for NF1. The aim of this study was to evaluate the intrafamilial variability of this lesion. The sample consists of patients with NF1 treated at the Clinical Hospital of the Medical School of Ribeirão Preto (HCFMRP). The images were obtained by Confocal Ophthalmoscopic Laser with infrared monochromatic light (815nm) and analyzed by two ophthalmologists that evaluate presence (≥2 nodules in at least one eye) and pattern of involvement (delimited and / or confluent) of the choroidal lesions. We studied 23 families, composed of 57 patients, each one formed by at least one affected person. In 84.8% of the families, all the members had choroidal alterations. The nodules affect different numbers of quadrants among the members in 78.3% of the families. Regarding the types of lesions, in 65.2% of the cases, all of the family had both confluent and isolated lesions. The divergence between types and the extent of the lesions corroborate the inter- and intra-familial variation for the choroidal nodule, analogously to other NF1 changes. When evaluating families with at least two affected members in order to understand the intrafamilial behavior of the manifestations, we are aware that more severe forms of the disease can be excluded.

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Evolution of Bone Density Over Time in Children and Young Adults with Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) is a genetic tumor predisposition syndrome associated with bony abnormalities (e.g. scoliosis, tibial dysplasia, sphenoid wing dysplasia). Prior analysis in the NCI NF1 natural history study (NCT00924196) showed high plexiform neurofibroma (PN) burden was correlated with decreased lumbar spine (LS) height-adjusted bone mineral density Z-scores (HAZ)[1]. Here, we investigated whether local bone mineral density (BMD) differed with significant PN involvement and whether HAZ worsened over time. We also evaluated the impact of PN-directed medical therapy on HAZ.

BMD was evaluated with serial dual-energy X-ray absorptiometry (DEXA) scans. Subtotal body (SB, excluding head), LS, and femoral neck (FN) HAZ were calculated using data from the BMD in Childhood study[2]. Abnormal HAZ was defined as $\leq$-2. Independently, a clinical expert defined degree of PN involvement (none, minimal, or severe) by body quadrant. Raw BMD was compared between contralateral limbs with any difference in PN involvement. HAZ at baseline and most recent scan were compared for each subject. Change in HAZ was compared between patients who initiated PN-directed medical therapy between scans and those who did not.

A total 120 participants (44% female, median age 12.5 years at baseline scan) were included, of which 44 (37%) had asymmetrical limb PN involvement. BMD was slightly lower in limbs with more significant PN involvement compared to contralateral limbs (n=113 scans, median difference 0.02 g/cm^3, $p<0.0001$).

Multiple scans were available for 78 (65%) over a median of 3.9 years. Most had normal HAZ at baseline (90/120, 75%) and most recent scan (59/78, 76%). Of those with normal HAZ at baseline, nine (10%) later developed an abnormal HAZ. From baseline to most recent scan, SB HAZ increased (n=61, median -0.94 vs. -0.44, p=0.0073), LS decreased (n=68, -0.35 vs. -0.67, p=0.0029) and FN did not change (n=75, -0.94 vs. -0.98, p=0.8293). Between scans, 43 (55%) started any PN-directed medical therapy, including 20 (26%) who started a mitogen-activated kinase (MEK) inhibitor. No impact of PN-directed therapy initiated between scans was detected on HAZ.

This is the first longitudinal analysis of BMD in this population. Most subjects had normal HAZ at baseline and were unlikely to develop abnormal HAZ later, though HAZ may progress differently by body region. Despite therapeutic response of PN to medical treatments, we were unable to detect a difference in HAZ evolution by PN-directed therapy. Significant PN involvement may slightly decrease local BMD and could affect local fracture risk.

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Phase II Trial of the MEK 1/2 Inhibitor Selumetinib (AZD6244, ARRY-142886 Hydrogen Sulfate) in Adults with Neurofibromatosis Type 1 (NF1) and Inoperable Plexiform Neurofibromas (PN)

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Background: NF1-related PN are locally invasive tumors characterized by increased activation of the RAS pathway causing significant morbidity including disfigurement, pain and functional limitations. Selumetinib has received breakthrough designation for NF1 PN based on phase I/II trials in children. We present results for the ongoing phase II study of selumetinib in adults with NF1 PN, which includes pharmacodynamic (PD) evaluation of serial tumor biopsies as well as functional/patient-reported outcomes (PROs).

Methods: Open-label Simon 2-stage trial design. Eligibility: NF1 patients (pts) ≥18 years old with inoperable/symptomatic/progressive PN. First 2 pts received selumetinib 75 mg BID; subsequent pts received selumetinib 50 mg BID. Primary objective: response rate by volumetric MRI analysis (partial response [PR]; ≥20% volume decrease). Secondary objectives: PD studies on pre/on-treatment biopsies of PN and cutaneous neurofibromas, assessment of clinical benefit using PROs (Numeric Rating Scale-11, Pain Interference Index), and PN-specific functional assessments. Validated, fit-for-purpose, isozyme-specific measurements of pERK/ERK/pMEK/MEK performed using SOPs designed for labile phosphoproteins (PMID 27001313).

Results: As of February 2020, 27 pts have enrolled. Outcomes are reported for 23 pts (74% male; median age 33 years, range 18-60). Most common PN-related morbidities: pain (19 pts), motor dysfunction (17 pts), disfigurement (13 pts). Sixteen pts achieved PR, with 13 confirmed (57%, [.34-.77] 95% CI); no disease progression. Median time to confirmed PR response: 14 months (range 8-30); median change in PN volume at best response: -22% (range -41% to +5.5%); median duration of treatment: 28 months (range 2-50). Pt-reported target tumor pain intensity and pain interference scores significantly improved ($p=0.025$, $p=0.028$ respectively). Selumetinib suppressed tumor pERK1,2/ERK1,2 but not pMEK1,2/MEK1,2 ratios from 1-10 hours following oral dosing (one $t_{1/2}$). Pts 1 and 2 were dose-reduced due to grade 3 intolerable rash ($n = 2$) and pain ($n = 1$). Grade ≥3 drug-related toxicities on 50 mg (21 pts) include transaminitis (5 pts), rash (1 pt) and pancreatic enzyme elevation (1 pt). Two pts were dose reduced (rash = 1 pt, transaminitis = 1 pt). Two pts discontinued by choice, 2 pts withdrawn by PI (best interest of patient), and 1 pt each removed for transaminitis, surgical resection, serious concurrent medical illness, and non-compliance.

Conclusions: Selumetinib shrinks the majority of adult PN and results in molecular target suppression and clinical benefit.

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Composite Pheochromocytoma in Neurofibromatosis Type 1

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Learning objective: to review the incidence, biology and surveillance and management of neuroendocrine tumors in Neurofibromatosis type 1

Pheochromocytoma/paraganglioma (PCC/PGL), catecholamine-secreting neural crest-derived tumors arising out of the adrenal medulla or paravertebral tissues, are known to affect ~2-8% of individuals with Neurofibromatosis type 1 (NF1). Nevertheless, recommendations for routine screening for these tumors, which are often at least initially asymptomatic in affected individuals, are not currently widely employed and are controversial. Some groups have suggested routine screening in adults over the age of 18, (PMID 29977594), however, to our knowledge there are no proponents of screening in children as the age of onset for these tumors is thought to be later in life (PMID 27787920). As characteristically NF1-associated PCC/PGL are asymptomatic at the time of diagnosis, true age of onset is difficult to robustly characterize at this time and warrants further study. Interestingly, neuroblastoma, the neural crest tumor that predominates in children, is not significantly associated with NF1; while pathogenic variants in the *NF1* gene are found in ~6% of primary neuroblastoma tumors (PMID 27130733), the literature does not support an increased incidence of these tumors in patients with germline *NF1* variants/clinical features of NF1. Pathogenic variants found in tumor tissue are distinct from those in the germline (PMID 8490657). Composite pheochromocytomas, in which tissue contains predominantly PCC but also other neuroendocrine patterns, are individually very rare, but have been reported in NF1 patients with suspected PCC. There has been suggestion that composite PCC/neuroblastoma are in fact biologically distinct from either tumor individually. Crucially, while loss of *NF1* expression and *MYCN* over-expression are seen in neuroblastoma, it does not appear that this poor prognostic pattern is seen in composite tumor types (PMID19864235). Similarly, prognosis appears relatively favorable. Here we present a case of a ten-year-old male with an incidentally discovered composite pheochromocytoma/neuroblastoma tumor. This case argues for reconsideration of asymptomatic screening for NF1 patients for hormone secreting tumors both on therapeutic and data gathering grounds.

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Trametinib for Neurofibromatosis Type 1 Associated Plexiform Neurofibroma and Recurrent Low-Grade Glioma

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Background: Based on early clinical efficacy data, Seattle Children's Hospital established a standard clinical practice for MEK inhibitor therapy for children with Neurofibromatosis Type 1 (NF1) associated plexiform neurofibroma (PN) or recurrent low-grade glioma (LGG).

Methods: Data were collected under an IRB-approved retrospective chart review.
Trametinib was prescribed off-label at 0.025 mg/kg daily for up to two years. Physical exam and laboratory monitoring were monthly for 3 months, then every 3 months. Retinal examination, echocardiogram and electrocardiogram were every 3 months. Tumor response was evaluated by MRI every 3 months for LGG; imaging for PN was dependent on tumor location.

Results: 22 patients received trametinib; 9 LGG, 16 PN (3 both); 13 female/9 male; median age 11 years (range 4.1-22.6). Primary PN locations included face/neck (n = 10), extremity (n=4), paraspinal (n= 2); most patients had more than one PN (n=10). Most LGGs were located in the optic pathway (n=7), also in the cortex (n=1) and brainstem (n=1). Most common adverse events (AE) were dermatologic and gastrointestinal. Eight had dose interruption/reduction, only one discontinued therapy for AE. Four received dermatology specialty care for AE. With median follow-up of 12 month, only 1 patient had progression. One-year EFS was 100% for PN and PN/LGG groups, and 83% for LGG. Central radiology review of response will be presented.

Conclusions: This real-world pediatric cohort supports efficacy and tolerability of MEK inhibitor therapy for short-term control of plexiform neurofibroma and low-grade glioma with NF1. Further studies are warranted to evaluate comparative efficacy, combination therapy and duration of therapy.

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Evolution of the Circulating DNA Landscape During Progression from Neurofibroma to Malignant Peripheral Nerve Sheath Tumor (MPNST)

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Background: Patients with the cancer predisposition syndrome neurofibromatosis type 1 (NF1) have a ~15% lifetime risk of developing malignant peripheral nerve sheath tumors (MPNST). MPNSTs are aggressive, soft tissue tumors with as low as a 20% 5-year survival rate due to rapid development of metastases and resistance to radiation/chemotherapy. Early detection and resection of MPNST is associated with improved outcomes, however, this is challenging in the setting of multiple, large but benign NF1 associated plexiform neurofibromas (PN). Studies have described the molecular pathogenesis, characterized by increasing aneuploidy and recurrent, defined accumulating genetic lesions associated with transformation from PN to pre-malignant atypical neurofibromas (ANF) and histologically higher grade atypical neurofibromatosis neoplasms of uncertain biologic potential (ANNUBP) to malignant MPNST. We hypothesize that presence of these changes in cell free DNA (cfDNA) can detect early transformation of PN to ANF/ANNUBP and MPNST.

Methods: 620 serial plasma samples from patients with NF1 and PN (n=117), ANF (n=36) and MPNST (n=22) were banked through the NCI, Johns Hopkins NF1 Biospecimen Repository and SARC031 Clinical Trial. Isolated cfDNA (n=98) was sequenced using ultra low pass whole genome sequencing (ULP-WGS) for detection of copy number variations (CNV) and estimations of tumor fraction using iChorCNA.

Results: Mean ploidy showed an overall increase with histological grade from controls (2.005, n=14), PN (2.009, n=52), ANF (2.002, n=12), ANNUBP (2.018, n=8) to MPNST (2.019, n=12). Mean tumor fraction estimates overall increased with histological grade from controls (0.4%), PN (0.4%), ANF (0.4%), ANNUBP (0.9%) and MPNST (0.6%). All sequenced samples were pre-treatment.

Dampened signals may result from inconsistent processing of samples; rapid plasma isolation is required to prevent genomic DNA contamination from lysed white blood cells (WBC). A subset of samples along with data from collaborators were reanalyzed using in silico size fragment selection for reads consistent with circulating tumor DNA (ctDNA, 90- 150bp). Pilot data show improved differentiation in estimated tumor fraction between groups: normal 0.3% (n=6), PN 2.0% (n=9), ANF 2.2% (n=1) and MPNST 5.9% (n=14).

Conclusions: ULP-WGS of plasma from patients with NF1 detect increasing ploidy and tumor fraction with malignant transformation. Ongoing efforts, including in silico enrichment for ctDNA and targeted deep sequencing (CAPP-Seq), may improve sensitivity. Together, these technologies hold promise for cancer surveillance in a high- risk population where early detection is critical for improved outcomes.

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Social-Emotional Functioning in Children and Adolescents with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PNs) Over Time: The Impact of Inhibitory Control

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Background: Cross-sectional studies have indicated that youth with NF1 exhibit difficulties in social-emotional functioning, with neurocognitive impairments in inhibitory control (an aspect of executive functioning) as a contributing factor (Huijbregts and de Sonneville, 2010; Toledo- Tamula et al., 2012). Further, a recent longitudinal study showed a declining trajectory in inhibitory control (Yang et al., 2020). Given these findings, this study sought to explore patterns of social-emotional and behavioral functioning over 6 years and the contribution of declining inhibitory-control in children with NF1.

Methods: NF1 children and adolescents (3-18 years) with PNs on a NCI Natural History study (NCT00924196) were administered a psychological evaluation that assessed inhibitory control (CPT-II: Commissions) and social-emotional functioning (BASC-2 Parent). One-way repeated measures ANOVAs were performed to investigate changes in within-subject BASC variables across the baseline, 3-year, and 6-year evaluations. Linear regressions examined change in inhibitory control over time in relation to social-emotional functioning at 6 years.

Results: Seventy four participants with NF1 and PNs (M age=9.97 years, range 3-18; 55% males) and their parents provided baseline data. A substantial percentage of parents (range 25 to 45% depending on the sub-domain) reported their child to be at-risk or experiencing clinically significant problems with anxiety, depression, internalizing problems, somatization, and/or social withdrawal across evaluations. No significant change over time was seen in parental reports of child anxiety, atypicality, depression, internalizing problems, social skills, or somatization (n=39). However, social withdrawal significantly increased from baseline to 6 years (ps<.001). Worsening inhibitory control was associated with higher parent-reported atypicality (ps<.05) and withdrawal at 6 years (ps<.05). Improvements in inhibitory control over time was related to better social skills (ps<.01) at 6 years.

Conclusions: Most parents of youth with NF1 and PNs perceive their child/adolescent's emotional functioning to be within normal limits. Still, a substantial proportion of these parents see their child as being at-risk or experiencing significant problems with anxiety, depression, internalizing problems, somatization, and/or social withdrawal over time. Importantly, inhibitory control is associated with aspects of social-emotional functioning over six years. This longitudinal study adds to the cross-sectional findings on this relationship, indicating that inhibitory control may be a target for interventional studies designed to improve social- emotional functioning in NF1 youth. Both psychosocial and medical treatments are needed to help children with NF1 and PNs better manage their social-emotional and cognitive difficulties.

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Beat NF: A Jazz Motor Therapy Program for Toddlers with Neurofibromatosis Type 1 (NF1)

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Young children with NF1 frequently struggle with learning, attention, social, and motor skills, which are most often addressed with traditional outpatient therapy interventions. Unfortunately, access to these services is highly variable, and can be limited by geographical location and financial resources. In attempt to increase accessibility to effective early intervention therapy for children with NF1, The Washington University Neurofibromatosis (NF) Center, in collaboration with Jazz St. Louis and St. Louis Children's Hospital Foundation, created Beat NF, a jazz motor therapy program specifically designed to address frequently delayed developmental skills in toddlers with NF1.

This jazz music motor therapy curriculum uses jazz music and physical therapy to promote social, attention, and motor skills in toddlers with NF1, while also fostering healthy parent-child interactions, peer relationships, and jazz appreciation. During each of the five consecutive weekly sessions, professional jazz musicians play live music, while children learn social rules as a group, explore "mystery instruments", and engage in gross motor activities. The Beat NF Team members carefully design each weekly program to target specific social and motor delays commonly encountered in young children with NF1, and provide families with a corresponding home program to complete prior to the next session.

Sixty percent of parents surveyed found that their child made measurable developmental improvements as a result of participating in the Beat NF program, and all parents appreciated benefits in motor and social development. Objective assessment of participants revealed advancement in developmental progress in all children, where 27% exhibited improvements in gross and fine motor skills by the end of the program. These included increased vocabulary, emergence of walking and jumping skills, and a greater interest in music and musical activities in the home setting.

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Implications of Corpus Callosum Splenial Lesions in NF1

Grace Vassallo, MD, *Manchester University Hospitals NHS Foundation Trust*

Introduction: Splenium lesions of the Corpus Callosum on Brain MR scans of patients with Neurofibromatosis type 1 patients are rare and their significance unclear.

Patients & Methods: The NHS nationally commissioned Highly Specialised Service (HSS) for NF1 patients in Manchester has served 797 adult complex NF1 patients (females n=439-55%) over 10 years. A retrospective review of their neuroimaging was conducted.

Results: 148 patients (18%) had lesions in the corpus callosum and 37 (4%) had a lesion in the splenium. Analysis of these lesions by our neuro-radiologist and correlation with data collected from case records classified these lesions into (1) demyelinating, (2) neoplastic and (3) non-specific lesion groups. Data on neurological symptoms, genotype/phenotype information and other cranial lesions was included.

Demyelination lesions. 5 patients (13%) identified. Female (60%). Median age 41 years. 80% (n=4) of these patients had more than one corpus callosum lesion. 60% (3) of the patients had other demyelination lesions located outside the corpus callosum. Two have a diagnosis of multiple sclerosis.

Neoplastic lesions. Corpus callosum splenial tumours were present in 6 patients (16%), female (50%).
Median age 28 years. Follow up ascertained that in 67% (n=4) patients the most likely tumour was pilocytic astrocytoma.

Non-specific (NS) lesions. 26 patients had non-specific lesions of the splenium of the corpus callosum. These lesions are T1W-iso- to minimally hypointense, T2W-hyperintense and do not show any contrast enhancement. Follow up neuroimaging classified the behaviour of these lesions into **(1) Static (2) Regressive (3) Progressive.**

Static lesions: 13 patients (50%) had static lesions over prolonged follow up. The majority were female (62%). Median age 39 years.

Regressive lesions: 8 (31%) patients had regressive non-specific lesions-female (87%). Median age 30 years.

Progressive: 5 patients (19%) with progressive non-specific corpus callosum lesions were identified. F=3. Median age 23 years. First noted at 20 years.

Conclusion: Splenial lesions of the corpus callosum in NF1 have been characterized. These lesions are more common in females with an onset before 30 years of age. We found no genotype/phenotype association nor any specific associated clinical symptoms. Further analysis of our results suggest that in an NF1 female patient under the age of 30 years, with at least one other cerebral tumour that is not an OPG the presence of a new splenial lesion on MRI shows T1W-isointensity and T2W-hyperintensity, with or without contrast enhancement, ongoing surveillance imaging is recommended in view of the likelihood of progression into a splenial tumour.

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Preventing Stroke in Paediatric NF1

Grace Vassallo, MD, *Manchester University Hospitals NHS Foundation Trust*

Introduction: Moyamoya is well recognised in NF1. We present our centre's experience and novel neuroradiological studies that help timely interventions to prevent stroke in this group of children.

Patients & Methods: The NHS Nationally Commissioned Highly Specialised Service (HSS) for NF1 patients in Manchester serves NF1 patients in the North of England. At present the service looks after 351 children. (F=159 M=192) A retrospective review of their neuroimaging, clinical phenotype and management including neovascularization (where indicated) was conducted.

Results: 10 patients (3%) 5 M=F, Median age = 9 1/2 years had Moyamoya with 7 having unilateral and 3 bilateral disease. 9 children had the "IVY" sign on their scans on the affected side. 4 patients were symptomatic at presentation and had neuroimaging as a result of this. (2M/ 2F). 2 children had a history of transient ischemic attacks. One child had a history of intermittent choreoathetosis and already had a small and clinically undetectable ischemic infarct on their MR scan at presentation. One child presented acutely with a haemorrhagic stroke after weeks of severe headaches. One child presented with deteriorating speech and cognition (All these children have had successful revascularization surgery and are now asymptomatic on Aspirin.)

One asymptomatic child had been operated in another centre. She did not have the "IVY" sign in her initial scans and no perfusions studies had been carried out pre-operatively.

One asymptomatic child has had surgery as his perfusion studies and "Ivy sign" were deteriorating suggestive of increase stroke risk. He remains well, on aspirin, with improvement in his IVY sign and ASL. The other 3 children are being monitored. One is not on aspirin as she is asymptomatic and her perfusion studies are reassuring. Two children are asymptomatic but on Aspirin due to concerning perfusion studies; one has already had a small asymptomatic infarct. They are under close clinical and neuroradiological follow up and the decision to offer revascularization surgery will be based on changes in either.

Conclusion: MoyaMoya in paediatric NF1 may be asymptomatic. While there are no recommendations for screening for vasculopathy in this population of children it is important that scans carried out of other reasons are examined for signs of ischemia and management tailored accordingly if these are present. This group of child may be protected from future stroke if this is carried out appropriately.

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Brain Volume Alterations in Adults with NF1 Characterized Using MRI

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Although multiple studies have explored the differences in brain volume of children with neurofibromatosis type 1 (NF1), there are few studies of adults with NF1. We present the largest study to date characterizing brain volume alterations in adults with NF1.

3D MPRAGE volumetric measurements were obtained for 351 adults with NF1 and 43 adults with NF2 or Schwannomatosis (control group), neither of which affects brain volume. Volumes for total brain matter, total grey matter, total white matter, and white matter of specific regions: frontal lobe, parietal lobe, inferior frontal gyrus, corpus callosum, brainstem, and pons were measured. Brain volumetric measurements were compared between adults with NF1 and controls using Student T test or Mann-Whitney U test, as appropriate. Total brain volume, grey matter volume, and white matter volume were compared between NF1 adults and control as a function of age and sex using multivariate linear regression in R 3.6.0. All p-values were adjusted for multiple comparisons using false discovery rate.

Total brain volume and total and all regional white matter volumes were significantly larger in adults with NF1 compared to the control group. Total gray matter volume was not significantly different between the two groups. As well, total brain volume ($\beta = 144.22$; 95%CI 99.97 to 188.47; $p = 4.27x10^{-10}$) and total white matter volume NF1 ($\beta = 94.11$; 95%CI 72.40 to 115.81; $p = 6.08x10^{-16}$) of the control group increased significantly faster with age compared to adults with NF1. In contrast, total gray matter volume decreased with age, with the adults with NF1 ($\beta = 24.81$; 95%CI 2.49 to 47.12; $p = 0.029$) declining significantly faster than the control group. This is especially apparent in males with NF1. Younger adult males with NF1 have larger total grey matter volumes than controls but deteriorate rapidly with age so that adult males over 45 with NF1 have smaller total grey matter volume than controls.

Our findings indicate that there is an enlargement, especially of white matter, in brain volume of adults with NF1. This is consistent with the hypothesis that dysregulation of brain myelin production is an important manifestation of NF1.

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Correlation Between Brain Volume Alterations and Neurological Changes in Adults with NF1

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Multiple studies have explored the differences in brain volume in people with NF1, but the relationship of those morphological changes to neurological function has rarely been studied in adults. We correlated brain volume alterations in adults with NF1 with neurological function and neuropsychometric measurements.

Nineteen adults with NF1 received 3D MPRAGE scans for brain volumetric measurements and were assessed for clinical severity of neurological symptoms and psychometric scores. Volumes for total brain, total grey matter, total white matter, corpus callosum white matter and brainstem were measured. Clinical severity was determined by NF1 features and neurological problems. Psychometric tests included tests for attention deficiencies and IQ. Correlation analysis was conducted between brain volumes and clinical and psychometric features using Pearson's r or Spearman's rho in R 3.6.0. All p-values were adjusted for multiple comparisons using false discovery rate.

Larger total brain volume correlated with higher IQ ($r = 0.691$, $p = 0.018$). Larger total white matter volume correlated with higher IQ ($r = 0.656$, $p = 0.026$) and lower attention deficit score ($r = 0.783$, $p = 0.001$). Larger corpus callosum white matter volume correlated with lower attention deficit score ($r = 0.722$, $p = 0.005$). Larger brainstem white matter volume correlates with higher IQ ($r = 0.686$, $p = 0.018$) and lower attention deficit score ($r = 0.546$, $p = 0.049$). Total gray matter volume was not correlated with any clinical severity or psychometric measurement.

Our findings indicate that enlargement, especially of white matter, in the brain volume of adults with NF1 is correlated with less severe psychometric deficiencies, particularly IQ and attention deficiency.

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We are grateful to the Bundesverband Neurofibromatose organization for funding this project.

Panel Germline Cancer Genetic Testing on Individuals with Neurofibromatosis Type 1

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The diagnosis of Neurofibromatosis type 1 (NF1) has relied on the clinical diagnostic criteria for decades. Germline *NF1* gene testing has played a supplementing role in the diagnosis and care of individuals with NF1. With the advancing technology and decreasing cost of next generation sequencing (NGS), untargeted germline cancer genetic testing panel has become increasingly available.

We conducted a review on the pattern of germline *NF1* genetic testing in the multidisciplinary NF clinic in Moffitt Cancer Center from 2017 to 2020. There are 88 consecutive cases of NF1 meeting the clinical diagnostic criteria. All individuals were offered genetic testing. Standard genetic counseling was given and the individuals were made aware of the following: 1) The personal health management plan is not likely to change whether an *NF1* mutation is or is not identified. 2) If an *NF1* mutation is identified, it may help to facilitate the diagnosis of a child or other blood relative whose clinical signs and symptoms are too ambiguous to establish a diagnosis. 3) Using the exon NGS technology in a commercial lab, an *NF1* mutation may not be found in up to 5% of the individuals affected with NF1. 4) The cost is the same to be tested for one gene (*NF1*) or a wide panel of cancer predisposition genes. 5) Genetic testing may or may not be covered by the health insurance. Self-pay option is available. 6) Personalized cancer surveillance and prevention may or may not change based on the finding of panel genetic testing.

Twenty seven out of 88 patients elected to proceed with genetic testing. Of these patients, untargeted panel genetic testing is preferred. Twenty six individuals opted for panel testing including *NF1,* only one opted for *NF1* single gene testing. Among them, no *NF1* PV was found in 3 individuals, 3 individuals were found to carry other genetic PV in addition to *NF1* PV. One carries an *NF1* splice PV and an *APC* PV, one carries an *NF1* truncation PV and a *PMS2* PV, one carries an *NF1* truncation PV and a *MITF* PV. One additional individual carries an *NF1* missense variant of uncertain significance (VUS) and a *RAD51D* PV. This *NF1* VUS is highly suspicious for being pathogenic.

Untargeted panel genetic testing for cancer and tumor predisposition is preferred by individuals affected with NF1. There is a noticeable number of cancer predisposition PVs coexisting with an *NF1* PV in this cohort, which have impact on the management of the patients and/or their relatives.

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Test-Retest Reliability of Goldmann Perimetry in Children with NF-1 Associated Optic Pathway Gliomas

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Introduction: Neurofibromatosis type 1 (NF-1) associated optic pathway gliomas (OPGs) may cause profound loss of vision and visual field (VF). Many children undergo formal VF testing to monitor for signs of tumor progression. The purpose of this study is to determine the test-retest reliability and variability of testing using Goldmann perimetry in children with NF-1-associated OPGs in an effort to provide evidence-based guidelines for tumor progression.

Methods: Children between 4 and 18 years with NF-1 and an OPG were enrolled in this prospective, cross-sectional study. A single perimetrist performed Goldmann perimetry using targets III4e and I4e which was repeated within the visit. Mean visual field areas were digitized and calculated using Adobe Photoshop CS3 for analysis.

Results: Fourteen (71% male) patients participated. Mean age at enrollment was 12 years (range 6.6 to 17.3) and 3 (21%) participants had a VF defect. Mean total planimetric area was 166.37cm^2 for III4e targets and 127.66 cm^2 for I4e targets. There was significant correlation between tests for each isopter and eye. The variability of testing was found to have a maximum range of 8.13% decrease in area to 10.25% increase in area for the III4e target and 10.37% decrease in area to 13.01% increase in area for the I4e isopter and was not correlated with the participant's age.

Discussion: Goldmann perimetry testing in children with NF-1 associated OPGs is feasible and shows good test-retest reliability.

Conclusion: Goldmann perimetry is important for evaluating VF dysfunction in children with NF-1 associated OPGs, and quantitative studies defining the variability of testing are relevant for monitoring tumor progression.

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Constitutional Mismatch Repair Deficiency is the Differential Diagnosis in 0.41% of NF1/SPRED1-Mutation Negative Children Suspected of Sporadic Neurofibromatosis Type 1

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Purpose: Biallelic germline mismatch repair (MMR) gene mutations cause constitutional MMR deficiency (CMMRD), a highly penetrant childhood cancer syndrome with a broad tumor spectrum. CMMRD phenotypically overlaps with neurofibromatosis type 1 (NF1) and is a legitimate differential diagnosis in suspected NF1 children without *NF1/SPRED1* mutations. Testing these children for CMMRD enables inclusion of CMMRD positives into monitoring programs prior to tumor onset. However, testing is associated with potential harms. The prevalence of CMMRD among these children, although unknown, is estimated to be as low as 0.39%.

Methods: We have previously developed a simple and scalable microsatellite instability (MSI) assay of non-neoplastic leukocyte DNA to reliably detect CMMRD. Here we used this assay to retrospectively screen an anonymized cohort of >700 children suspected of sporadic NF1, but lacking *NF1/SPRED1* germline mutations after highly sensitive mutation analysis.

Results: For three MSI-positive patients, identification of MMR gene germline mutations confirmed the diagnosis of CMMRD. A *PMS2* founder mutation, prevalent in Europe and North America, and an *MSH6* founder mutation, affecting 1:400 French Canadians, represented two of the five MMR gene mutations identified in these patients. CMMRD prevalence was 3/735 (0.41%, 95% CI: 0.08-1.19%).

Conclusion: Our empirical data provide urgently needed reliable numbers for genetic counseling, and confirm previous prevalence estimations on which the *Care for CMMRD* (C4CMMRD) consortium based its guidelines for CMMRD testing as a differential diagnosis of NF1/Legius syndrome in a child without malignancy. These guidelines advocate CMMRD testing of preselected patients rather than offering reflex testing to *all* suspected sporadic NF1 children lacking *NF1/SPRED1* mutations. The MMR mutations in the CMMRD patients identified here suggest that the possibility of founder effects should be considered, in addition to C4CMMRD guidelines, when counseling *NF1/SPRED1* mutation negative children.

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NF2: Basic Science

LAST	FIRST	TITLE
Hehman	Charles	Targeting of Focal Adhesion Kinase (FAK) Decreases Cell Proliferation in Nf2/Merlin Deficient Murine Schwann Cells
Laraba	Liyam	TEAD Inhibition Reduces Tumour Proliferation in Merlin Null Meningioma and Schwannoma
Leek	Miranda	The Potential of Targeting Nitrated Hsp90 as a Therapeutic Approach for Neurofibromatosis Type 2
Petrilli	Alejandra	Synergistic Combinatorial Phosphatidylinositol-3-Kinase (PI3K) Targeting for NF2 Schwannoma

NF2: Basic Science

Targeting of Focal Adhesion Kinase (FAK) Decreases Cell Proliferation in Nf2/Merlin Deficient Murine Schwann Cells

Charles Hehman, MD, *Indiana University School of Medicine*

Neurofibromatosis type 2 (NF2) is a devastating syndrome of central and peripheral nervous system tumors. Current treatments are limited and are mostly focused around surgical resection or radiation treatment. Brigatinib (Alunbrig®) is an Anaplastic Lymphoma Kinase (ALK) inhibitor FDA approved for use in crizotinib- resistant, ALK-positive non-small cell lung cancer. Mice lack the ALK phosphorylation site that is targeted in humans, and ALK is not highly expressed in murine or human schwannoma cell lines. Even so, previous work in our lab utilizing a genetically engineered mouse model (GEMM) of NF2 showed that 12 weeks of brigatinib treatment effectively reduced tumor burden and decreased the progression of hearing loss. These data suggest that brigatinib's effectiveness is due to off-target kinase inhibition.

In addition to ALK, brigatinib has strong activity against Focal Adhesion Kinase (FAK/PTK2). It is demonstrated that when FAK is knocked down in murine Schwann cell lines the Akt and Erk1/2 pathways show less activation by Western blot. It is also shown that mouse Schwann cells treated with brigatinib have less growth *in vitro* than cells not treated with the tyrosine kinase inhibitor. Some human schwannomas show increased expression and upregulation of FAK supporting the theory that this may be a suitable target for therapy. Defactinib, a selective FAK inhibitor currently in phase I clinical trials, is shown to have a stronger effect on cell growth *in vitro* than the less selective brigatinib. A novel target for therapy would be very beneficial to understanding the biology of schwannomas and the treatment of disease.

Further research is needed to fully establish whether FAK inhibition by brigatinib is the primary target of interest in the context of NF2 *in vivo,* however these data suggest that a selective FAK inhibitor, defactinib, may have a more robust effect on schwannoma progression. A therapeutic study of defactinib in the NF2 GEMM would provide critical data to determine the effectiveness of targeted therapy *in vivo.*

Additionally, the genetic proof-of-concept is currently in development to determine if additional conditional deletion of FAK in the NF2 GEMM is sufficient to ameliorate tumor burden and hearing loss.

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TEAD Inhibition Reduces Tumour Proliferation in Merlin Null Meningioma and Schwannoma

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Introduction: Meningiomas are the most common intracranial tumour and arise from the arachnoidal cell layer of the meninges. Schwannomas develop from Schwann cells which provide the myelin sheath and trophic support within the peripheral nerves. The tumor suppressor Merlin is deleted in approximately 50-60% of meningiomas and 70% of schwannomas. Loss of Merlin results in dysregulation of the Hippo pathway and consequent aberrant activation of the co-transcriptional activators YAP and TAZ. Nuclear localisation of YAP and TAZ has been shown to drive tumor phenotypes in many cancers. The TEAD family of transcription factors are major targets of YAP- and TAZ-dependent signaling. TEAD family members undergo autopalmitoylation and this modification is required for transcriptional activation by YAP or TAZ. Vivace Therapeutics have identified small molecule inhibitors of autopalmitoylation that block the interaction of TEAD family members with YAP and TAZ. This study seeks to establish the efficacy of small molecule inhibitors of TEAD activity in meningioma and schwannoma tumor cells.

Experimental Procedures: Meningioma cell lines, primary human meningioma and schwannoma cells, as well as the schwannoma mouse model (PeriostinCRE-NF2$^{fl/fl}$), which spontaneously develop tumors within Schwann cells of the dorsal root ganglia (DRGs) and vestibulocochlear nerves, were treated in vitro and in vivo with Vivace inhibitors to assess their effects upon tumor cell proliferation and TEAD-driven transcription.

Results: TEAD inhibition significantly reduced the proliferation of the BenMen-1 meningioma cell line, as well as in primary human meningioma cells. In schwannoma, these inhibitors blocked cell proliferation and also reduced the expression of CTGF, a target of TEAD activation in primary human meningioma and schwannoma cells. These novel inhibitors are also currently being tested in the Periostin CRE-NF2$^{fl/fl}$ schwannoma mouse model to evaluate their efficacy in vivo. At 3-months of age, proliferation in the DRGs of Periostin CRE-NF2$^{fl/fl}$ animals was significantly reduced by 14 days oral gavage of 30 mg/kg of Vivace inhibitor. The drug also significantly blocked CTGF protein expression when compared to vehicle controls.

Conclusions: The novel Vivace inhibitors have been shown to significantly reduce proliferation in primary meningioma and schwannoma cells, as well as in the Periostin CRE-NF2$^{fl/fl}$ schwannoma mouse model. The use of these novel compounds to inhibit TEAD function in these two Merlin-null tumor types shows great potential as an effective treatment for reducing the rate of cell proliferation in meningioma and schwannoma, two clinically important tumor types.

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The Potential of Targeting Nitrated Hsp90 as a Therapeutic Approach for Neurofibromatosis Type 2

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Heat Shock Protein 90 (Hsp90) is an evolutionarily conserved, pro-survival chaperone protein. The functionality of Hsp90 is linked to a cycle of conformational changes, regulated in part by ATP hydrolysis, a host of co-chaperones, and many post-translational modifications (PTMs). Of these PTMs, we showed that the biologically relevant peroxynitrite-induced tyrosine (Tyr) nitration on residues 33 and 56 in human Hsp90 leads to a pathological gain of function that regulates mitochondrial metabolism in tumor cells. Further, we uncovered that nitrated Hsp90 supports survival of merlin-deficient Schwann cells. We found that while Hsp90 is endogenously nitrated in vestibular schwannomas from neurofibromatosis type 2 (NF2) patients and in merlin-deficient human and mouse Schwann cells, nitrated Hsp90 is absent in normal Schwann cells. Together, these findings position nitrated Hsp90 as a novel category of tumor-directed targets for the development of safe, long-term therapeutic strategies for NF2 treatment. We hypothesize that structural changes induced by nitration on Tyr33 and 56 in Hsp90 are responsible for the pro-tumorigenic gain-of-function. We can then take advantage of the structural differences between the nitrated and normal Hsp90 to design or modify drugs that specifically target and inhibit the nitrated form, sparing the activity of the normal protein. Here, we used biochemical and biophysical techniques to study the structural changes induced by nitration on Tyr33 and 56 in human Hsp90. While Hsp90 functions as a dimer, our results showed that treatment of recombinant Hsp90 with peroxynitrite dramatically increased the presence of higher-order oligomers commensurate with a higher percentage of monomers compared to dimers. Peroxynitrite-treated Hsp90 dimer also showed an electrophoretic shift on Native-PAGE, consistent with a change in conformation when compared to WT Hsp90. Further, site-specific incorporation of nitrotyrosine at Tyr33 and 56 using genetic code expansion increased the formation of monomer vs. dimer and resulted in a differential distribution of oligomeric species dependent upon which site was probed. Together, these results suggest that nitration significantly alters the structure of Hsp90 which may account for the pathological gain-of-function. Uncovering these nitration-dependent structural changes may lead to the development of safe, long-term therapeutic strategies for NF2 that selectively target nitrated Hsp90 function while leaving the unmodified, pro-survival heat shock chaperone unaffected.

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Synergistic Combinatorial Phosphatidylinositol-3-Kinase (PI3K) Targeting for NF2 Schwannoma

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Mutations in the *NF2* gene cause Neurofibromatosis Type 2 (NF2), a tumor predisposition disorder of the nervous system. The *NF2* gene encodes merlin, a tumor suppressor that modulates activity of numerous signaling pathways controlling Schwann cell proliferation and survival. Despite increasing knowledge of merlin function, there are no FDA approved drug therapies for NF2. A rich history of target identification studies performed by us and others using high-throughput and high-content assays revealed that PI3K inhibitors are strong candidates for development of NF2 therapeutics. However, it is well established that activation of compensatory signaling mechanisms frequently limits success of targeted therapies. To overcome drug resistance, combinations of inhibitors are becoming standard in oncology. Therefore, we conducted a small-scale combination screen of 3 PI3K inhibitors with 9 non-PI3K pathway inhibitors using a 10X10-matrix cell viability protocol in 384 well plates. The screens were performed using both human and mouse NF2 schwannoma model cells. The best-performing synergistic combination was pictilisib, a PI3K alpha/delta inhibitor with PF-3758309, an inhibitor of p21-activated kinase (PAK), a well-established merlin effector. This combination was advanced to in vivo assessment in an orthotopic allograft mouse model. Fifty engrafted NOD.*Cg-Prkdcscid Il2rgtmlWjl*/*SzJ* mice were enrolled into four treatment groups (vehicle, pictilisib, PF-3758309, and their combination), and received daily oral gavages for 14 consecutive days. Pharmacokinetic studies showed that pictilisib, administered as a single agent was rapidly absorbed and distributed in plasma and nerve, indicating penetration of the normal blood-nerve barrier (BNB). In contrast, PF-3758309 was undetected in the normal nerve. Drug efficacy was assessed by excised tumor weights. There was a 66% reduction in tumor weight in mice treated with pictilisib alone as well as in combination with PF3758309 compared to the vehicle group ($p=0.012$ and $p=0.022$; Kruskal-Wallis with post-hoc analysis). This indicates a lack of in vivo synergy for the combination. PF3758309 promoted a 42% reduction in tumor weight vs vehicle that did not reach significance ($p=0.37$). Mass spectrometry analyses of tissue from treated mice revealed that PF-3758309 was present in the intraneural tumor but not in the contralateral normal nerve. This indicates that tumors had compromised BNB. Relative to other PI3K inhibitors tested in the mouse schwannoma model in our lab, pictilisib was the most effective. In vivo and in vitro target modulation and mechanism of action studies are underway.

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NF2: Clinical Science

LAST	FIRST	TITLE
Cotter	Kristina	Development of a Real-World Evidence Platform in NF2 to Examine Treatment Patterns and Outcomes
Early Kan	Samuel Charlotte	Progression of Contralateral Hearing Loss in Patients with Sporadic Vestibular Schwannoma
Engelson	Celia	Spontaneous Regression of Multiple Meningiomas Following Meningoencephalitis in a Patient with Neurofibromatosis Type 2

NF2: Clinical Science

Development of a Real-World Evidence Platform in NF2 to Examine Treatment Patterns and Outcomes

Kristina Cotter, MS, PhD, CGC, *RDMD, San Francisco, CA*

Background: NF2 is a rare disease, and its low prevalence can make it challenging to conduct clinical research on long-term clinical outcomes of patients who use off-label therapies for management. Real-world data (RWD), or data collected outside a clinical trial, can be used to understand disease progression, treatment patterns, and outcomes. Here, we describe the development of a RWD platform that allows for abstraction of high-quality data from medical records of patients with NF2.

Methods: A RWD platform was designed to abstract data from medical records of patients with NF2. An umbrella research consent allowing for minimal-risk research on de-identified data was employed. With patient permission, complete records (including clinical notes, test reports, and radiology images) from patient-reported institutions were requested and digitized. Data relating to diagnosis, NF2 tumors, and management were abstracted to evaluate data depth. An electronic audit trail to source documents was maintained.

Results: Thirty-six patients with NF2 consented to research and had records available. The cohort was 58% female, with an average age of 35 and representation from all U.S. Census regions and Canada. On average, 12 years of data and 178 clinical documents were available per patient. The average age of diagnosis was 20. All patients had ≥1 NF2-related tumor and 86% had ≥2 NF2 tumor types. Ninety-four percent of patients had at least one vestibular schwannoma and all but two had bilateral vestibular schwannomas. Additional NF2-related tumors included meningiomas (69%), non-vestibular schwannomas (64%), and ependymomas (33%). None had astrocytomas. NF2-related medication data was available for 33% of patients; of these, 42% had tried two or more therapies. The most common were bevacizumab (83%), everolimus (25%), and lapatinib (17%). Ninety-two percent had documented procedures (average 6 / patient). Fifty-seven percent of procedures involved NF2 tumor resection; on average, patients had 4 NF2 tumor-related procedures. Thirty-nine percent of patients had emergency visits (average 3 / patient), while 53% had inpatient admissions (average 3 / patient).

Discussion: Medical records are a robust source of RWD. However, collection of high-quality RWD requires evaluation of complete medical records and maintenance of an audit trail to source documentation. We developed a RWD platform for NF2 and demonstrated that it may be used to understand clinical characteristics and treatment patterns, regardless of geographic location. This platform will be used for additional NF2 outcomes work in the future.

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Progression of Contralateral Hearing Loss in Patients with Sporadic Vestibular Schwannoma

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Charlotte Rinnooy Kan, *Massachusetts Eye and Ear; Harvard Medical School; Leiden University Medical Center, Leiden, The Netherlands*

Background & Introduction: Vestibular schwannomas (VSs) are the most common tumors of the cerebellopontine angle, typically presenting unilaterally with ipsilateral sensorineural hearing loss (SNHL). The mechanism of tumor-induced hearing loss has recently been shown to be related to secreted tumor factors, in addition to mechanical compression of the adjacent auditory nerve, and these factors may percolate through CSF or blood to affect contralateral hearing as well.

Methods: Retrospective study of medical records for patients treated for VS at Mass Eye and Ear from January 1994 through October 2018. Included patients had unilateral VS and sequential audiometry allowing for longitudinal assessment of hearing over time. Mass Eye and Ear's audiology database was used to select age- and sex-matched case controls, also with sequential audiometry, from the non-VS population. Subgroup analysis was performed by age, sex, baseline hearing, and tumor size at initial diagnosis. Hearing loss progression was performed using Kaplan-Meier analysis to account for variable follow-up times.

Results: A total of 661 patients were identified with VS and sequential audiometry. The population was predominantly female vs. male (368 vs. 293, $p=0.0035$), driven primarily by younger patients with Koos 4 tumors (76 female vs. 49 male, $p=0.016$). Patients with normal baseline hearing bilaterally ($N=241$) demonstrated no significant difference in hearing loss progression in VS-contralateral vs. control ears. Patients with abnormal baseline VS-ipsilateral hearing ($N=190$), however, demonstrated significantly higher likelihood of reaching moderate SNHL in VS-contralateral ears. Subgroup analysis by age, sex, and baseline tumor size did not yield any subgroup-specific trends for hearing loss progression.

Discussion & Conclusion: This is the largest study to date tracking long-term bilateral hearing outcomes in patients with VS, and demonstrates that, in patients with abnormal hearing in the VS-ipsilateral ear, there exists a long-term risk of progression to moderate hearing loss in the contralateral ear as well. Combined with the absence of significant changes in word understanding in the affected ears, these findings may provide clues to the nature of tumor- secreted factors involved in VS-associated hearing loss. Female predominance within the VS patient population is confirmed, driven mostly by younger female patients with Koos 4 tumors.

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Spontaneous Regression of Multiple Meningiomas Following Meningoencephalitis in a Patient with Neurofibromatosis Type 2

Celia Engelson, FNP-C, *NYU Langone Health*

Introduction: Herein, we present a woman with NF2 and synchronous involution of multiple meningiomas in the setting of meningoencephalitis. Spontaneous regression of meningiomas is extremely rare. Immunologic factors have previously been implicated in spontaneous regression of other tumor types. Though a causative relationship cannot be definitively established in this case, based upon the time course and imaging findings we hypothesize an immune mediated tumor response.

Case Report: A 40-year-old woman with NF2 and multiple intracranial and spinal meningiomas and schwannomas, bilateral deafness and epilepsy was admitted to the inpatient epilepsy unit for increased seizure frequency and medication adjustment. One week prior to admission she was diagnosed with influenza and completed a 5-day course of oseltamivir.

On the first day of hospitalization she was febrile to 39.4°C. She was treated empirically for suspected otitis media and was found to be Influenza A positive. Late in the day she continued to be febrile and became hypotensive and lethargic. WBC was elevated at 16. EEG showed no epileptic events. She was transferred to the intensive care unit for possible sepsis. She was started on vancomycin and aztreonam for presumed meningitis and peramivir for flu. Head CT showed possible ventricular enlargement and LP was deferred.

MRI of the brain and IACs the following day showed features consistent with meningoencephalitis including diffuse leptomeningeal enhancement and new foci of FLAIR hyperintensities in the right frontal and left parietal lobes. In comparison to an MRI 9 months prior, there was mild interval enlargement of several intracranial meningiomas with new areas of central hypoenhancement suggestive of necrosis.

During the hospitalization the patient had intermittent fever, persistent headache and neck stiffness and developed bilateral pleural effusions (thought to be iatrogenic). Additionally she was found to have Strep pneumococcus antigen in urine. An infectious disease consult was obtained and it was determined that pneumococcal meningitis was the most likely diagnosis.

Over the course of several days the patient returned to her baseline mental status and neurologic function. The patient was discharged on hospital day 9 to complete her antibiotic course at home.

MRI obtained four months after discharge demonstrated marked interval decrease in the size of multiple meningiomas with cystic degeneration, specifically the meningiomas that had previously shown new central hypoenhancement. The remaining meningiomas and schwannomas were stable. MRI obtained eight months after discharge demonstrated interval filling or resorption of the central cystic degenerative areas with stable regression.

Full List of Authors: Celia Engelson, FNP-C and Kaleb Yohay, MD

SPECIAL THANKS TO THE 2020 NF CONFERENCE CHAIRS

Matthias Karajannis, MD, MS, Memorial Sloan Kettering Cancer Center

Conxi Lázaro, PhD, Catalan Institute of Oncology

Nicole J Ullrich, MD, PhD, Boston Children's Hospital

OUR THANKS ALSO TO THE FOLLOWING INDIVIDUALS FOR THEIR EFFORTS:

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Berg	Dale		None		
Berger	Carly		Children's National Hospital		
Berman	Mimi		Royal North Shore Hospital		
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Branch	Nicola		Manchester Metropolitan University		
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Chistoprudova	Anna		Interregional non-government organization for patients with neurofibromatosis 22/17		
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Cho	Seongree		N/A		
Clapp	David		Indiana University School of Medicine		
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Conrady	Christopher		OHSU	USA	Oregon
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Cowsert	Amanda		University of Florida	USA	Florida
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Creus Bachiller	Edgar		Fundació Institut d'Investigació Biomedica Bellvitge- Idibell		
Crooks	Bruce		IWK Health Centre	Canada	Nova Scotia
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del Castillo	Allison	Student	Children's National Hospital	USA	District of Columbia
Derdak	Joanne		National Cancer Institute	USA	Maryland
Dhaenens	Britt		Erasmus MC Rotterdam		
Dhamija	Radhika		Mayo Clinic		
Dirksen	Sara		Novo Nordisk		
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Dodd	Rebecca		University of Iowa	USA	Iowa
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Fidler	Meredith		Vertex Pharmaceuticals	USA	California
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Haughton	Frank		FH Petroleum Corp		
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Heller	Jonathan		Ocean BioMedical		
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Hupton	Eileen		Manchester Foundation trust	United Kingdom	Lancashire
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Isibor	Steve		Exelixis Inc.	USA	California
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Kim	YooRi		Gilbert Family Foundation		
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Lewis	Courtney		Boston Childrens Hospital	USA	Massachusetts
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Lipkovskaya	Vera		Russian Patient Association for NF "22/17"		
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Lozada-Sipe	Evelyn		St. Joseph's Children's Hospital		
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MacDonald	Maria		London Regional Cancer Program	Canada	Ontario
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Mansouri	Sheila		University Health Network	Canada	Ontario
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Martin	Vanessa		Childhood Tumour Trust	United Kingdom	East Sussex
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Miller	Matthew		OHSU		
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Mo	George		National Institutes of Health		
Moertel	Christopher		University of Minnesota	USA	Minnesota
Mohamad	Teddy		Université de Sherbrooke	Canada	Québec
Mohler	Alexander		Miami Cancer Institute	USA	Florida
Moots	Paul		Vanderbilt University Medical Center	USA	Tennessee
Moran	Jon		Ashfield Healthcare Communications Group Ltd	United Kingdom	Cheshire
Morin	Caroline		Gilbert Family Foundation	USA	Michigan
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Morris	Jill	Ph.D.	NIH	USA	Maryland
Murawski	Alexis		University of Alabama at Birmingham	USA	Alabama
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Ortenberg	June		McGill University Health Centre	Canada	Québec
Ortiz	Kayla		University of Central Florida		
Ostrow	Kimberly	Ph.D.	Johns Hopkins University	USA	Maryland
Packer	Roger		Children's National Hospital		
Pancza	Patrice		Children's Tumor Foundation	USA	New York
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Parhomchuk	Elena		UF Health Jacksonville	USA	Florida
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Pasmant	Eric	Ph.D.	Inserm	France	
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Piotrowski	Arkadiusz		Medical University of Gdansk	Poland	
Plotkin	Scott		Massachusetts General Hospital	USA	Massachusetts
Pope	Kimberly		Congressionally Directed Medical Research Programs	USA	Maryland
Post	Leonard	Ph.D.	Vivace Therapeutics	USA	
Powlovich	Lauren		Focused Ultrasound Foundation		
Prada	Carlos		Cincinnati Children's Hospital Medical Center	USA	Ohio
Pratilas	Christine	M.D.	Johns Hopkins	USA	Maryland
Przypyszny	Michele		Children's Tumor Foundation		
Puccetti	Diane	M.D.	America Family Childrens Hospital-University of Wisconsin-Madison	USA	Wisconsin
Puri	Ratna		Sir Ganga Ram Hospital		
Quelle	Dawn		The University of Iowa	USA	Iowa
Radtke	Heather	M.S., CGC	Children's Tumor Foundation / Medical College of Wisconsin	USA	New York
Rambo	Micah		Rensselaer Polytechnic Institute	USA	New York
Ramesh	Vijaya	Ph.D.	Massacusetts General Hospital/Harvard Medical School	USA	Massachusetts
Randolph	Linda		Children's Hospital Los Angeles		
Rangan	Kasey		Children's Hospital Los Angeles	USA	California
Ratner	Nancy	Ph.D.	Cincinnati Children's Hospital	USA	Ohio
Rauen	Kate	M.D., Ph.D.	UC Davis	USA	California
Rednam	Surya		Baylor College of Medicine		
Reiser	Catherine		Univ of WI Hospital and Clinics		
Rennie	Andrea	R.N.	Children's Hospital of Philadelphia	USA	Pennsylvania
Renstrup	Jens		SpringWorks Therapeutics		
Rezende	Nilton	M.D.	Federal University of Minas Gerais	Brazil	Minas Gerais
Rhodes	Steven	M.D., Ph.D.	Indiana University School of Medicine/Riley Hospital for Children	USA	Indiana
Riccardi	Vincent	M.D.	The Neurofibromatosis Institute	USA	California
Ritchie	Karleen		Astrazeneca	USA	Illinois
Robinson	J. Elliott		Cincinnati Children's Hospital Medical Center	USA	Ohio
Robinson	Kim		Children's Tumor Foundation	USA	California
Rodrigues	Luiz Oswaldo		Federal University of Minas Gerais	Brazil	Minas Gerais
Röhl	Claas		NF Kinder / NF Patients United	Austria	Wien
Romo	Carlos	M.D.	Johns Hopkins University	USA	Maryland
Rose	Traceann		Children's Tumor Foundation	USA	New York
Rosenfeld	Adam		Parent		
Rosser	Tena		CHLA	USA	California
Rottenberg	Ian		N/A		
Rukin Gold	Deborah	M.D.	Rainbow Babies & Children's Hospital	USA	Ohio
Rustad	Cecilie Fremstad		Oslo University Hospital	Norway	
Ryan	Lori		Verastem Oncology		
Sadighi	Zsila	M.D.	The University of Texas MD Anderson Cancer Center	USA	Texas
Saji	Bibitha		NHS	United Kingdom	Manchester
Salsman	Scott		Simpson Healthcare		
Salvador	Hector		Hospital Sant Joan de Deu		
Sammons	Josh		University of Alabama at Birmingham	USA	Alabama
Sanchez	Pedro		Cedars-Sinai/UCLA	USA	California
Sanchez-Martin	Carlos		University of Padova	Italy	Padova
Sandaradura	Sarah		Children's Hospital at Westmead	Australia	New South Wales
Sankaran	Hari		NIH	USA	Maryland
Santoro	Claudia		Vanvitelli university if Campania	Italy	Campania
Sarin	Kavita	M.D., Ph.D.	Stanford School of Medicine	USA	California
Sarnoff	Herb		Infixion Bioscience, Inc	USA	
Sato	Aimee	M.D.	Seattle Children's	USA	Washington
Saydam	Okay		University of Minnesota	USA	Minnesota
Schenck	Annette		Radboud University Medical Center	Netherlands	Gelderland
Schmidt	Christa		St. Olav Hospital	Norway	
Schorry	Elizabeth (Betty)		Cincinnati Children's Hospital	USA	Ohio
Schutt	Samantha		University of Iowa Hospitals & Clinics	USA	Iowa
Sehested	Astrid		Rigshospitalet	Denmark	
Sellars	Elizabeth		Arkansas Children's Hospital	USA	Arkansas
Serbinski	Carolyn		Cincinnati Children's	USA	Ohio
Serra	Eduard	Ph.D.	The Institute for Health Science Research Germans Trias i Pujol (IGTP)	Spain	
Shabason	Emily		CHOP	USA	Pennsylvania
Shah	Nidhi	M.D.	Penn State Health	USA	Pennsylvania
Shallow	Natalie		UF Health - Jacksonville	USA	Florida
Shea	John		NF Northeast		
Sheerin	Una	Ph.D.	Guy's and St Thomas' NHS Foundation Trust	United Kingdom	London,City of
Sherman	Larry	Ph.D.	Oregon Health & Science University	USA	Oregon
Shuhaiber	Hans		UF Health		
Sidhu	Alpa		University of Iowa Hospitals and Clinics	USA	Iowa
Simpson	Brittany		Cincinnati Children's Hospital Medical Center	USA	Ohio
Sitton	Madeleine		Duke University		
Skelton	Tammi		UAB	USA	Alabama
Slobogean	Bronwyn		Johns Hopkins University	USA	Maryland
Smith	Miriam	Ph.D.	University of Manchester	United Kingdom	Greater Manchester
Smith	Abbi		Indiana University School of Medicine	USA	Indiana
Smith	Lisa		Indiana University/Riley Hospital	USA	Indiana
Smith	Katherine	R.N.	CHOP	USA	Pennsylvania

Attendees List – 2020 NF Virtual Conference

LAST NAME	FIRST NAME	SUFFIX	COMPANY	WORK COUNTRY	WORK STATE
So	Karen	M.D.	AstraZeneca	United Kingdom	Cambridgeshire
Soll	Richard		WuXi AppTec	USA	Massachusetts
Song, PA-C	Young-Bin		University of Washington	USA	Washington
Sorman	Connie		Children's Tumor Foundation	USA	New York
Souza	Juliana		Neurofibromatosis Outpatient Reference Center (CRNF)/ The Federal University of Minas Gerais (UFMG)		
Stavinoha	Pete	Ph.D.	UT MD Anderson Cancer Center	USA	Texas
Stemmer-Rachamimov	Anat	M.D.	Massachusetts General Hospital	USA	Massachusetts
Stern	Edward		Boston University	USA	Massachusetts
Sun	Daochun	Ph.D.	Memorial Sloan Kettering Cancer Center	USA	New York
Tamula	Mary Anne		Leidos Biomedical Research, Inc.	USA	Maryland
Taylor	Lynne		University of Washington	USA	Washington
Thain	Emily	M.S., CGC	University Health Network	Canada	Ontario
Therrien	Janet		KGS	USA	Maryland
Thomas	Mary		Guy's Hospital	United Kingdom	
Thompson	Heather		California State University, Sacramento	USA	California
Thomson	John		NF Northeast		
Tibery	Cecilia		Leidos/POB at NIH	USA	Maryland
Tonsgard	James	M.D.	University of Chicago	USA	Illinois
Topilko	Piotr		IMRB	France	Ile de France
Traber	Jennifer		Washington University School of Medicine, St. Louis	USA	Missouri
Trapane	Pamela		UF College of Medicine	USA	Florida
Trevisson	Eva		University of Padova	Italy	Veneto
Tschiffely	Anna		CDMRP - Ripple Effect		
Tumminello	Brad		SpringWorks Therapeutics		
Ullrich	Nicole	M.D., Ph.D.	Boston Children's Hospital, Boston, MA	USA	Massachusetts
Upadhyaya	Meena	Ph.D.	Cardiff University	United Kingdom	Cardiff
Urish	Ken		University of Pittsburgh		
Vallee	Beatrice		CBM, UPR4301 - CNRS	France	
Vassallo	Grace		NHS HSS Complex NF1 service	United Kingdom	Manchester
Vasudevan	Harish		UCSF	USA	California
Vidaud	Dominique		AP-HP	France	
Vishwanath	Vijay		Children's Hospital Los Angeles		
Viskochil	David	M.D., Ph.D.	University of Utah	USA	Utah
Voeller	Julie		Baylor College of Medicine / The Children's Hospital of San Antonio		
Vukelj	Simon		Children's Tumor Foundation	USA	New York
Walbert	Tobias	M.D., Ph.D.	Henry Ford Health System	USA	Michigan
Walker	James		Massachusetts General Hospital	USA	Massachusetts
Wallace	Margaret	Ph.D.	UF College of Medicine	USA	Florida
Wallis	Deeann	Ph.D.	UAB	USA	Alabama
Walsh	Karin	Ph.D.	Children's National Hospital	USA	District of Columbia
Wang	Xia	M.D., Ph.D.	Moffitt Cancer Center	United States Minor Outlying Islands	Florida
Wang	Zhihong	M.D., Ph.D.	Children's Hospital of Richmond at VCU	USA	Virginia
Wang	Su		University of British Columbia		
Warrier	Akshaya		University of Iowa	USA	Iowa
Watson	Adrienne	Ph.D.	Recombinetics	USA	Minnesota
Weber	Mike		SpringWorks Therapeutics	USA	North Carolina
Wee	Michaela		Manchester Metropolitan University	United Kingdom	
Wegscheid	Michelle	Student	Washington University School of Medicine	USA	Missouri
Weimer	Jill		Sanford Research	USA	South Dakota
Weintraub	Lauren		Albany Medical Center	USA	New York
Weisman	Hannah		Children's National Hospital	USA	District of Columbia
Weiss	Brian		CCHMC	USA	Ohio
Whipple	Emily		Focused Ultrasound Foundation	USA	Virginia
Whitcomb	Patricia		National Cancer Institute	USA	Maryland
White	Jessica		SpringWorks Therapeutics	United States Minor Outlying Islands	CT
Widemann	Brigitte		National Cancer Institute	USA	Maryland
Wiemer	Erik		Erasmus University Medical Center	Netherlands	Zuid-Holland
Wilging	Sally		NA		
Williams	Kyle	Ph.D.	University of Minnesota	USA	Minnesota
Williams	Danaia		The Children's Tumor Foundation	USA	New York
Wimmer	Katharina		Medical University Innsbruck		
Wirtanen	Tracy	M.S.	Littlest Tumor Foundation	USA	Wisconsin
Wojciechowska	Sonia		Dana-Farber Cancer Institute		
Wolf	David		Emory University/CHOA		
Wolkenstein	Pierre	M.D., Ph.D.	APHP, UPEC	France	Ile de France
Wolters	Pamela		National Cancer Institute	USA	Maryland
Woodall	Lesli		Medical University of South Carolina	USA	South Carolina
Woodall	Lesli	M.S.	Medical University of South Carolina, Division of Physician Assistant Studies	USA	South Carolina
Wootton	Michael		NF2 Therapeutics	USA	Massachusetts
Wright	Erin		Akron Childrens Hospital	USA	Ohio
Wu	Lai Man (Natalie)	Ph.D.	Cincinnati Children's Hospital Medical Center	USA	Ohio
Wu	Jianqiang		Cincinnati Children's Hospital	USA	Ohio
Yasko	Liudmila		Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology	Russian Federation	Moscow
Yates	Charles		Indiana University	USA	Indiana
Yoshinaga	Akiko	M.D.	Kyoto University Hospital	Japan	Kyoto
Young	Lucy	Ph.D.	University of California, San Francisco	USA	California
Younger	Vanessa		Children's Tumor Foundation	USA	
Zadeh	Gelareh		University Health Network, Princess Margaret Cancer Centre	Canada	Ontario
Zhang	Xiyuan		NCI		
Zhao	Fu	M.D., Ph.D.	Beijing Tian Tan Hosptial	China	Beijing
Zisson	Alex		H.I.G. Capital	USA	Connecticut

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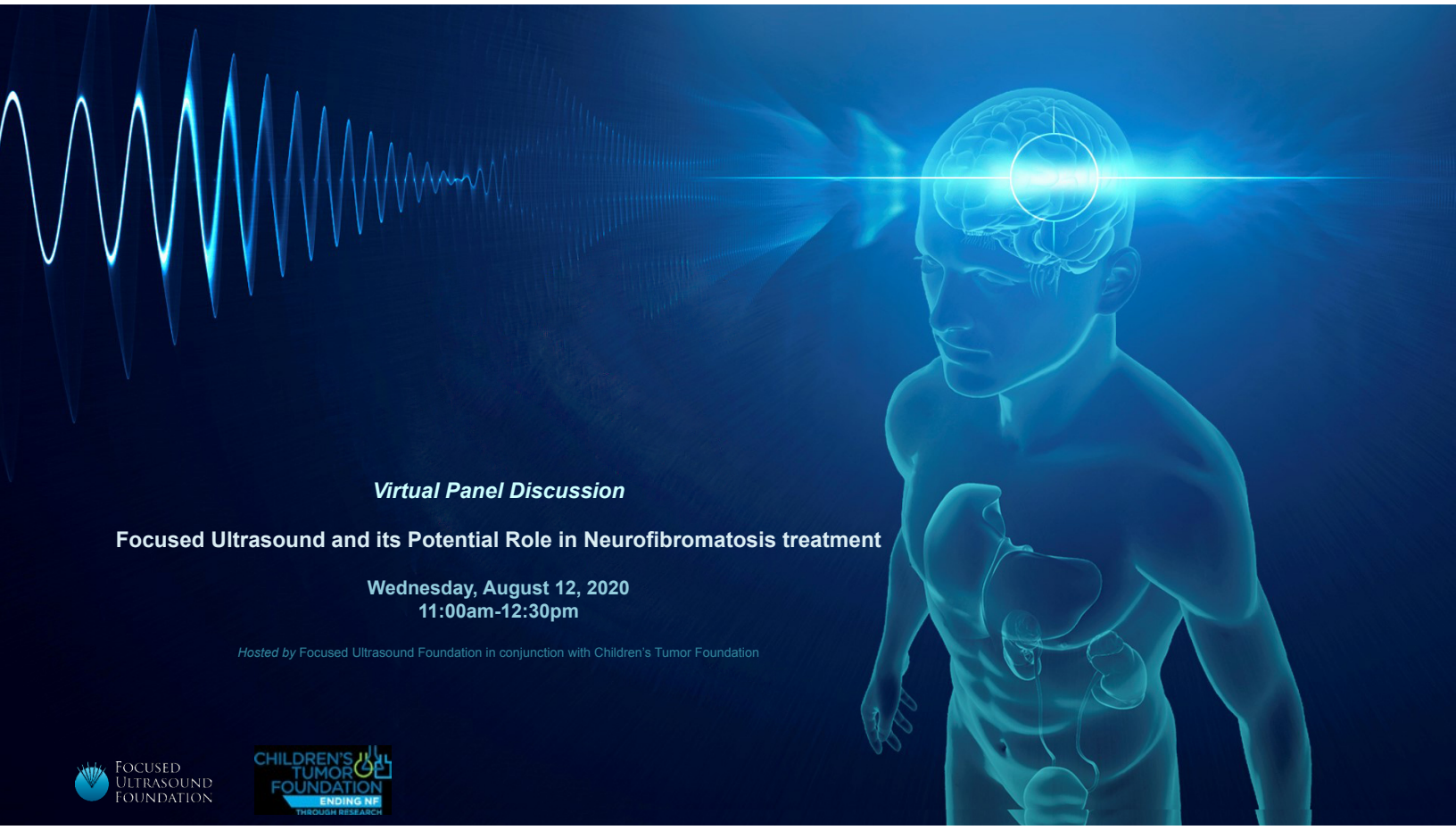
Virtual Panel Discussion
Focused Ultrasound and its Potential Role in Neurofibromatosis treatment
Wednesday, August 12, 2020
11:00am-12:30pm
Hosted by Focused Ultrasound Foundation in conjunction with Children's Tumor Foundation
Focused Ultrasound Foundation
Children's Tumor Foundation
Ending NF
Through Research