2019

NF Conference

**SEPTEMBER 21-24, 2019
HYATT REGENCY
SAN FRANCISCO, CA**

**CHILDREN'S
TUMOR
FOUNDATION
ENDING NF
THROUGH RESEARCH**

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MEDICAL COLLEGE OF WISCONSIN
CONTINUING EDUCATION PROGRAM

CME

ACCME Accreditation Statement:

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education through the joint providership of the Medical College of Wisconsin and Children's Tumor Foundation. The Medical College of Wisconsin is accredited by the ACCME to provide continuing medical education for physicians.

AMA Credit Designation Statement:

The Medical College of Wisconsin designates this for a maximum of 30.0 *AMA PRA Category 1 Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Hours of Participation for Allied Health Care Professionals:

The Medical College of Wisconsin designates this activity for up to 30.0 hours of participation for continuing education for allied health professionals.

Important Notes to Speakers, Chairs & Poster Presenters

Important Notes to Speakers, Chairs & Poster Presenters

NOTE TO SPEAKERS

- Bring your slides to the meeting on a flash drive. You may use your own laptop if you prefer. CTF staff will be available at registration to assist you uploading your presentation. Please make every effort to have your slides ready well in advance of your presentation.
- Please be available at the podium prior to the session in which you will be speaking to understand the a/v setup and make sure your slideshow is running smoothly.
- Verify the length of your talk and be prepared to complete it on time. There will be a CTF staff member seated in the front row to assist you with visual prompts.
- If you run over time, you may be "cut off". Briefly summarize what you see as the "take home" points of the session.

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NOTE TO SESSION CHAIRS

- Please stand by the podium 30 minutes before the start of the session you are chairing to ensure speakers have arrived, go through a/v setup, etc.
- It is your responsibility to convene and conclude your session PROMPTLY per the schedule.
- Introduce speakers by name and affiliation, and whether they invited or platform speakers.
- It is your responsibility to keep your speakers ON TIME. A CTF staff member will be seated in the front row to assist with visual prompts. You are also encouraged to give a 3-minute warning.
- When fielding questions from the audience, have the audience member identify him/herself, and ensure they speak into the microphone.
- At the close of the session, please briefly summarize what you see as the key 'take home' points of the session.

PREPARING A SUMMARY OF YOUR SESSION

- The meeting co-chairs will be assembling a report from the Conference that can translate into a publication after the meeting. Session co-chair(s) are requested to collaborate on providing a one to two page summary of your session. This should be succinct but sufficiently comprehensive to be meaningful. You are encouraged to liaise with your session speakers in putting this together. If there are critical references you want to mention please include the citation for reference.
- PLEASE SUBMIT YOUR SUMMARY TO PATRICE PANCZA BY THE END OF OCTOBER.

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NOTE TO POSTER PRESENTERS

- Posters will be on display throughout the Conference, SEPTEMBER 21 – 24TH in Pacific H-O.
- Posters can be set up Friday afternoon, Sept. 20th, starting at 6pm; your poster should be on display for the duration of the Conference.
- This year's poster sessions are combined for both basic and clinical science and is scheduled for Sunday afternoon from 5:05pm – 7:05pm. You must be present at your poster during this time.
- Preceeding the poster session will be the "Poster Advertisement" session, where the pre-selected top candidates for the poster contest will give a one minute/one-slide presentation promoting their poster.
- A jury will select the top three posters presented. The winners will be given the opportunity to present for 10 minutes including 3 minutes of Q&A and will receive an award during the poster review session scheduled for Tuesday, 9am –10am.
- All attendees will be invited to vote for their choice of the top basic and clinical science poster – the winners will receive a "People's Choice Poster Award".

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Questions?

Please contact a Foundation staff member!

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ABSTRACTS

Speaker Abstracts

CLINICAL CARE SYMPOSIUM: NF Diagnostic Criteria and Care Guidelines

Organized by the Clinical Care Advisory Board of the Children's Tumor Foundation. *Saturday, September 21, 8:00am – 12:00pm*

Planning Committee: Scott Plotkin, MD, PhD, Massachusetts General Hospital; Elizabeth Schorry, MD, Cincinnati Children's Hospital; Pamela Trapane, MD, University of Florida Health Jacksonville; Nicole Ullrich, MD, PhD, Boston Children's Hospital

Revised Diagnostic Criteria for NF1, NF2, and Schwannomatosis

Saturday, September 21, 8:05am – 8:30am

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

Saturday, September 21, 8:30am – 9:00am

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

When revising the diagnostic criteria we used the following guiding principles: represent best consensus among NF experts, address diagnostic (rather than clinical management) issues, be broadly representative of medical specialties and national groups, accessible to generalists as well as NF specialists, recognize advances in genetics over last 30 years without requiring genetic analysis for diagnosis, be acceptable in different countries and health care systems, now and in the coming years.

We used the anonymous Delphi procedure to ask the opinion of many specialists in the field about possible changes to the criteria. We met with a large group of experts in New York in June 2018 to discuss the Delphi results and proposed possible changes in the criteria and the science behind it. We subsequently performed a second round of Delphi in October 2018 and presented preliminary revised diagnostic criteria at the Global Neurofibromatosis Meeting in Paris in November 2018. Experts were asked to provide feedback after the Paris meeting. We asked non-expert specialists from different specialties to provide feedback on the use and clarity of the criteria. Finally the representatives from patient organizations were asked to provide feedback. We will present the latest version of the revised criteria and explain the process how we got there.

Full List of Authors: Gareth Evans[1], Susan Huson[2], Eric Legius[3], Ludwine Messiaen[4], Scott Plotkin[5], Pierre Wolkenstein[6]
[1]St. Mary's Hospital/University of Manchester, UK; [2]Consultant Geneticist, UK; [3]University of Leuven, Belgium; [4]University of Alabama, Birmingham, USA; [5]Massachusetts General Hospital, USA; [6]Hôpital Henri-Mondor, France

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Health Supervision for Children with NF1: AAP/ACMG Guidelines

Saturday, September 21, 9:00am – 9:30am

David T. Miller, MD, PhD and Nicole Ullrich, MD, PhD, *Boston Children's Hospital*

Clinical management guidelines for children with NF1 were revised and updated in 2019. These new American Academy of Pediatrics (AAP) and American College of Medical Genetics (ACMG) guidelines for the care of individuals with NF1 have an emphasis on decision-making for providers with regards to clinical management. Updates related to differential diagnosis and genetic testing is also addressed. Based on promising results of recent clinical trials, children with NF1 now have new treatment options available, some of which have become standard of care.

This educational session will help attendees to identify changes they could make in their practice related to guidelines for ongoing management of children with neurofibromatosis type 1, as well as identifying children who may be appropriate for intervention with novel therapeutics.

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Strategic Vision for the Neurofibromatosis Clinic Network

Saturday, September 21, 9:30am – 9:40am

Heather Radtke, MS, CGC, *Children's Tumor Foundation*

The NF Clinic Network (NFCN) was established in 2007 and has expanded to over 55 affiliate clinics throughout the US. The NFCN has oversight from the Clinical Care Advisory Board with the goal of improving access to quality NF care throughout the country.

Quality Metrics Project Description and Discussion

Saturday, September 21, 9:55am – 10:30am

Justin Jordan, MD, MPH, *Massachusetts General Hospital*

This presentation will provide background and evidence for the use of disease-specific guidelines to optimize healthcare delivery. Then, it will explore the state of current NF care guidelines, followed by an interactive discussion on future guideline development and clinical implementation thereof.

The European Reference Network (ERN) for Rare Disease Tumour Predisposition (GENTURIS) and its Clinical Management System

Saturday, September 21, 10:30am – 10:55am

D Gareth Evans, MD, *ERN GENTURIS*

Approximately 27-36 million patients in Europe have one of the ~5.000-8.000 known rare diseases. These patients often do not receive the care they need or they have a substantial delay from diagnosis to treatment. In March 2017, twenty-four European Reference Networks (ERNs) were launched with the aim to improve the care for these patients through cross border healthcare, in a way that the medical knowledge and expertise travels across the borders, rather than the patients. It is expected that through the ERNs, European patients with a rare disease get access to expert care more often and more quickly, and that research and guideline development will be accelerated resulting in improved diagnostics and therapies.

The ERN on Genetic Tumour Risk Syndromes (ERN GENTURIS) aims to improve the identification, genetic diagnostics, prevention of cancer, and treatment of European patients with a genetic predisposition for cancer. The ERN GENTURIS focuses on syndromes such as hereditary breast cancer, hereditary colorectal cancer and polyposis, neurofibromatosis and more rare syndromes e.g. PTEN Hamartoma Tumour Syndrome, Li Fraumeni Syndrome and hereditary diffuse gastric cancer. GENTURIS has already been involved in the revision of diagnostic criteria for NF and schwannomatosis as well as management guidelines for *TP53* carriers. NF and schwannomatosis is being used as the lead condition for the paradigm of healthcare management

Diagnostic Dilemmas and Case Studies

Saturday, September 21, 10:55am – 11:55am

Facilitator: Peter de Blank, MD, MSCE, *Cincinnati Children's Hospital Medical Center*

Discussion Panel: Matthias Karajannis, MD, MS, Memorial Sloan Kettering Cancer Center; Scott Plotkin, MD, PhD, Massachusetts General Hospital; Tena Rosser, MD, Children's Hospital Los Angeles; Elizabeth Schorry, MD, Cincinnati Children's Hospital; Pamela Trapane, MD, University of Florida Health, Jacksonville; Pierre Wolkenstein, MD, PhD, Henri-Mondor Hospital, Paris East University, France

The diagnostic and case studies session will present patient cases to demonstrate the clinical care session highlights including the new diagnostic criteria and current management recommendations. Several panel members will discuss approaches to medical care.

KEYNOTE LECTURE: From Patients to Patients: And a Whole Lot In-Between

Saturday, September 21, 1:00pm – 2:00pm

Stephen Groft, PharmD, *National Center for Advancing Translational Sciences, NIH USA*

Developing an orphan product for a rare disease frequently requires 10–15 years of research and development activities and costs approximately $2 Billion for a new compound to receive approval from regulatory agencies. A coordinated effort requires considerable planning to establish a research agenda, support research studies, identity research partners from patients, academic and biopharmaceutical industry investigators, when they will be needed, and how to obtain the needed resources. Patients with rare diseases have expressed a strong willingness to be research partners. Data collection can start with gathering information from appropriately designed patient registries and natural history studies, progress through clinical trials, and ends with post-approval studies with patients to confirm longer term safety and efficacy of treatments. Patient Advocacy Groups, in their expanded roles, are the main connection between the patient community, biomedical researchers, the biopharmaceutical industry, Federal research and regulatory agencies, clinical specialists, the public, the media, and reimbursement organizations. They often support research studies, training programs of new investigators, and research-based conferences. The leaders of PAGs are frequently viewed as the major stimulus leading to the successful development of diagnostics and interventions for rare diseases. They also respond to significant challenges to ensure the advances and research activities reach the community level at multidisciplinary specialty clinics with referral pathways to research and care. There is a need to expand awareness, advocacy, and outreach to everyone including those with low income, poor literacy, minority ethnic status, and living in underserved and marginalized populations in urban and rural areas How will we utilize advances in telehealth /telemedicine, mobile health devices, social media for application in all communities. Existing challenges to the research community for the investigation of rare diseases will be discussed including the value of patient registries, natural history studies, research consortia, and international networks. There is a need to address the psychological, sociological, and financial impact on patients, their families, and caregivers.

SESSION 1: Natural History of Neurofibromatosis

Chairs: Brigitte Widemann, MD, National Cancer Institute; Ana-Maria Vranceanu, PhD, Harvard University; Ethan Lester, PhD, Harvard University

Natural History of the NF1 Cognitive Phenotype in Early Childhood

Saturday, September 21, 2:10pm – 2:35pm

Belinda Barton, PhD, *Kids Neuroscience Centre and Children's Hospital Education Research Institute, The Children's Hospital at Westmead, Australia*

Background: The most common complication of neurofibromatosis type 1 (NF1) during childhood is cognitive deficits with up to 80% of school aged children demonstrating moderate-to-severe impairments in one or more areas of cognition. Literacy difficulties are also highly prevalent, with around two thirds of school aged children with NF1 having a reading disability.

Aims: To date the majority of research has focused on identifying the cognitive phenotype of school aged children with NF1 (8 to 16 years old). However it is critical to understand the natural history of the NF1 cognitive phenotype from infancy to early school years. This will allow us to identify early predictors of later cognitive difficulties and areas to target with early intervention.

Methods: Over a 15 year period we have prospectively followed the cognitive development of children with NF1 from 5 months to 7 years of age. Of the 91 children with NF1 enrolled, 67 of them were matched by age, sex and socioeconomic status to an unaffected control. Depending upon their age at enrolment (latest enrolment age was 40 months), children underwent scheduled neurodevelopmental assessments at 5 months, 9 months, 15 months, 21 months, 30 months, 40 months, 5 years and 7 years. Areas assessed depending upon their age included intelligence, behaviour, attention, executive functioning, early literacy and academic achievement.

Outcomes: All participants have now completed their final assessment at 7 years of age. In this presentation I will discuss the developmental trajectories of young children with NF1 and early predictors of later cognitive and reading difficulties.

Full List of Authors: Jennifer Lorenzo[1], Kathryn N North[2,3]
[1]Kids Neuroscience Centre, The Children's Hospital at Westmead, Australia; [2]Murdoch Children's Research Institute, Australia; [3]Department of Paediatrics, University of Melbourne, Australia.

Platform: Externalizing Symptoms Drive Social Functioning Difficulties in Children with Neurofibromatosis Type 1 (NF1)

Saturday, September 21, 2:35pm – 2:50pm

Allison del Castillo, BA, *Children's National Health System, Washington, D.C.*

Objective: Children with Neurofibromatosis Type 1 exhibit increased social dysfunction compared to typically developing children. Elevated internalizing and externalizing problems are also well-documented in children with NF1, with internalizing difficulties being more prevalent. Prior studies have found mixed results with regard to the relationship between internalizing and externalizing symptoms and social difficulties in children with NF1. We aim to further explore the relationship between internalizing and externalizing behaviors and social functioning in children with NF1.

Participants and Methods: We included 45 children with NF1 (age M=9.4, SD=4.86, 42% male) with a complete Child Behavior Checklist (CBCL) and Social Responsiveness Scale (SRS) collected as part of our NF clinic registry.

Results: Findings on the SRS demonstrate that 35.5% of children with NF1 demonstrate overall social functioning deficits. Clinically significant elevations were found in social awareness (26.7%), cognition (37.8%), communication (33.3%), motivation (26.7%) and restricted interests and repetitive behaviors (40%) subscales. Internalizing problems on the CBCL were found in 22% of children and 13% showed significant externalizing problems. Internalizing and externalizing symptoms were correlated with all domains of social functioning (r = .370-.607, p = .000-.002). Externalizing problems account for 21–42% of variance across SRS scales (R^2 = .213-.416, p = .000). Internalizing problems only accounted for an additional 1-6% of the variance across SRS domains (R^2 change = .008-.065, p = .056-.492).

Conclusions: Social functioning deficits and increased internalizing and externalizing behaviors are prevalent among children with NF1. Consistent with recent literature, children with NF1 demonstrate significant difficulties with social cognition and report greater internalizing than externalizing problems. Externalizing problems such as disruptive, aggressive, oppositional, and rule-breaking behaviors appear to be the predominant driver of social dysfunction across a range of skills in children with NF1. Internalizing symptoms had little effect on social functioning once externalizing symptoms were considered. In light of prior discrepant findings, these results suggest that externalizing symptom severity may explain deficits in social functioning in children with NF1. Future research should further examine the symptoms captured by measures of social and behavioral functioning to better understand the specific problems underlying the unique social functioning profiles of children with NF1 and inform interventions.

Platform: Long-Term Follow-Up of Adult Neurofibromatosis Type 1 Patients Using Whole-Body MRI Demonstrates Dynamic Changes in Internal Neurofibroma Size

Saturday, September 21, 2:50pm – 3:05pm

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

Background: Neurofibromas affect 40-50% of neurofibromatosis type 1 (NF1) patients and can cause significant morbidity and mortality. They appear to grow more rapidly during childhood and adolescence but studies in adults are limited by their retrospective nature and follow-up time < 3 years. The long-term natural history of neurofibromas remains unknown and there are no guidelines on the need and frequency of surveillance imaging for patients. Whole-body MRI (WBMRI) is a non-invasive technique that can detect whole-body tumor burden, including internal neurofibromas.

Methods: 17 adult NF1 patients who underwent a WBMRI between 2007-2010 (Scan 1) underwent a repeat WBMRI (Scan 2) between 2018-2019. Internal neurofibromas were segmented on short tau inversion recovery (STIR) sequences and tumor volume was calculated using a computerized volumetry and three-dimensional segmentation software (3DQI, Massachusetts General Hospital, Boston). Circumscribed tumors were defined as discrete; invasive tumors or those involving multiple nerves were defined as plexiform. Tumor growth and shrinkage were defined as a change in volume by ≥ 20% over the entire study period. Treatment history was obtained from medical record review and patient interview.

Results: Median patient age was 43 years (range 18-57) at the time of Scan 1 and 53 years (range 27-65) at the time of Scan 2. Median time between Scan 1 and 2 was 9 years. A total of 140 neurofibromas were assessed. 24% (33/140) of tumors grew by a median 63% (6.8% per year). 54% (76/140) of tumors spontaneously decreased in size by a median 60% (7% per year) without treatment. On a per-patient basis, 18% (3/17) of patients had overall tumor growth and 41% (7/17) overall tumor shrinkage. 8 new tumors developed in 7 patients. 16 tumors resolved entirely without medical or surgical intervention. Growth behavior did not correlate with discrete or plexiform morphology.

Conclusions: A subset of internal neurofibromas in adult NF1 patients grow significantly over a long-term period, suggesting that continued monitoring of these patients may be warranted. Surprisingly, more than half of neurofibromas shrink spontaneously without intervention. Continued patient enrollment and correlation of imaging findings with functional outcomes are underway.

Full List of Authors: K. Ina Ly[1], Raquel D. Thalheimer[1], Wenli Cai[2], Miriam A. Bredella[3], Vanessa L. Merker[4], Scott R. Plotkin[1], Justin T. Jordan[1]
[1]Pappas Center for Neuro-Oncology, Massachusetts General Hospital, Boston, MA, [2]3D Imaging Service, Massachusetts General Hospital, Boston, MA, [3]Department of Radiology, Massachusetts General Hospital, Boston, MA, [4]Center for Healthcare Organization and Implementation Research, Edith Nourse Rogers Memorial Veterans Hospital, Bedford, MA

Platform: Autism Spectrum Disorder and Social Functioning in Children with Neurofibromatosis Type 1: Preliminary Findings from the PANDA Study

Saturday, September 21, 3:05pm – 3:20pm

Jonathan M Payne, DPsych, *Murdoch Children's Research Institute*

Background: Children with the single-gene disorder neurofibromatosis type 1 (NF1) demonstrate social difficulties and appear to be at an increased risk for autism spectrum disorder (ASD). Despite the recent advances into understanding ASD in NF1 over recent years, critical questions remain. An explanatory framework is needed for understanding ASD in NF1 that delineates the effect of NF1 from gene expression and functional biology through to brain development, neurobehavioral abilities and functional outcomes. The primary aims of the Predictors of Autism in Neurofibromatosis type 1: Development to Adolescence (PANDA) study are to (1) establish the frequency of ASD in children with NF1, (2) examine the social cognitive phenotype, (3) investigate the neuropsychological processes contributing to ASD symptoms and poor social functioning in children with NF1, and (4) identify novel structural and functional neurobiological markers of ASD and social dysfunction in NF1.

Methods: This is an international, multisite, prospective, cross-sectional cohort study of children with NF1 and typically developing controls (3-15 years of age). Participants completed a detailed assessment of their cognitive abilities, behavior and adaptive functioning. Cognitive domains selected for assessment are based on a biopsychosocial model for social functioning that integrates abilities thought to underlie the development and expression of social behavior, including executive function, communication, and social cognitive skills. Children screening *at risk* of ASD (Social Responsiveness Scale-2; SRS-2 *T*-score $\geq$60) completed a comprehensive assessment which will be used to guide the formulation of an ASD diagnosis. Children aged $\geq$7 years were invited to complete structural, functional and spectroscopic neuroimaging.

Results: To date, preliminary data have been collected and analyzed in 109 children with NF1 (60 male, 49 female; mean full scale IQ=88.9, SD=12.9). The distribution of SRS-2 total scores was unimodal and elevated by 1.2 SD relative to population norms (mean SRS total score, 61.97; SD, 15.1). 52% of children with NF1 scored at or above the *at risk* range for SRS-2 total scores, with 19% scoring in the *severe* range (*T*-score $\geq$75). SRS-2 social communication and SRS-2 restricted interests and repetitive behaviors were highly correlated (r=0.85, p<.001), suggesting these two primary autism symptoms tend to be strongly related in children with NF1. SRS-2 total scores were highly related to ADHD symptoms (r=0.77, p<0.01), and executive functioning (r=0.78, p<0.01), but only weakly related to IQ (r=-0.35, p<0.01). Severity of ASD symptoms were comparable in males and females (p=0.20). Further analyses are underway.

Conclusions: Preliminary results from this study will improve current understanding of ASD in NF1, including neuroimaging markers, cognitive predictors, symptom profiles and functional impairments. Results will help guide the development of appropriate and effective interventions.

Full List of Authors: Jonathan M Payne[1,2], Natalie A Pride[3,4], Kristina M Haebich[1,2], Anita Chisholm[1,2], Karin S Walsh[5], Melissa Rouel[3], Belinda Barton[3,4], Alice Maier[1], Francesca Lami[1], Yael Granader[5], Allison del Castillo[5], Srishti Rau[5], Vicki Anderson[1,2], Kathryn N North[1,2]
[1]Murdoch Children's Research Institute, Australia; [2]Department of Paediatrics, University of Melbourne, Australia; [3]Kids Neuroscience Centre and Children's Hospital Education Research Institute, The Children's Hospital at Westmead, Australia; [4]Discipline of Paediatrics & Child Health, University of Sydney, Australia; [5]Center for Neuroscience and Behavioral Medicine, Children's National Health System, USA.

Platform: Longitudinal Examination of Attention Problems and Anxiety in Children with Neurofibromatosis Type 1

Saturday, September 21, 3:20pm – 3:35pm

Kristin Lee, MS, *University of Wisconsin–Milwaukee, Department of Psychology*

Background: Children with neurofibromatosis type 1 (NF1) are at increased risk for difficulties with psychosocial functioning including anxiety (Pasini et al., 2012) and attention difficulties (Hyman, Shores, & North, 2005). Research examining psychosocial functioning longitudinally is sparse for children with NF1. Thus, this study helps to further characterize the development of psychosocial functioning in children with NF1.

Methods: Children with NF1 (n=21; 12 boys/9 girls) were seen at two time points: ages 3-5 (T1) and ages 9-12 (T2). Parents completed questionnaires at both time points. Anxiety was assessed using the Preschool Anxiety Scale (T1) and the Spence Children's Anxiety Scale (T2). Attention was assessed using the Conners' Parent Rating Scale – Revised Short Form (T1) and Conners 3rd Edition Short Form (T2).

Results: Children with NF1 had significantly higher symptoms of inattention ($t(20) = 2.54$, $p = .020$, $d = .55$) and hyperactivity ($t(20) = 3.99$, $p = .001$, $d = .87$) at T2. Kendall's tau-b correlations between T1 and T2 were not significant for inattention and hyperactivity symptoms. The percentage of children in the elevated range significantly increased from 23.8% to 66.7% for inattention symptoms (exact McNemar's test $p = .022$) and from 4.8% to 47.6% for hyperactivity symptoms (exact McNemar's test $p = .012$). There was a significant median increase in generalized anxiety ($Mdn = 6$) at T2 ($Mdn = 53$) compared to T1 ($Mdn = 40$), $p < .001$. Significantly higher social anxiety ($t(20) = 2.70$, $p = .014$, $d = .59$) and overall anxiety scores ($t(20) = 2.50$, $p = .021$, $d = .55$) were observed at T2. The percentage of children with elevated anxiety scores ranged from 0-4.8% at T1 and 4.8-23.8% at T2. Correlations between T1 and T2 were significant for generalized anxiety scores ($Tau_b = .587$, $p = .002$). At T2, overall anxiety scores significantly increased with age ($Tau_b = .323$, $p = .047$).

Conclusions: Difficulties with attention and anxiety increased for children with NF1 over time from early childhood to the school-age years. Psychosocial functioning for children with NF1 needs to be regularly monitored as children develop, as these difficulties may not be apparent in early childhood. The implications of these results will be discussed.

References:
Pasini, A., Lo-Castro, A., Di Carlo, L., Mariabernarda, P., Martina, S., Rosa, C., & Galasso, C. (2012). Detecting anxiety symptoms in children and youths with neurofibromatosis type I. *American Journal of Medical Genetics Part B, 159B,* 869-873.
Hyman, S. L., Shores. A., & North, K. M. (2005). The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology, 65,* 1037-44.

Full List of Authors: Kristin Lee, Brianna D. Yund, Erin L. Corrigan, Bonita P. Klein-Tasman

Platform: Modeling of Long-Term NF1 Natural History Data to Predict Plexiform Neurofibroma Growth

Saturday, September 21, 3:35pm – 3:50pm

Cody J. Peer, MS, PhD, *National Cancer Institute, Clinical Pharmacology Program*

Background: Neurofibromatosis type 1 (NF1) is a multisystem disorder with variable tumor and non-tumor manifestations. The NCI NF1 natural history study (NCT00924196) was designed to collect long-term clinical and imaging data, including plexiform neurofibroma (PN) volumes. We previously described more rapid PN volume increase in young children and observed that patients with faster growing PN are more likely to develop tumor related morbidities. Accurately predicting future PN growth may help to identify patients who are most at risk of worsening symptoms and require medical or surgical intervention.

Objective: To use longitudinal volumetric MRI analyses of NF1 PN to develop a model of PN growth.

Methods: Participants of the NCI NF1 natural history study were included in the analysis if they had a PN measurable by volumetric MRI analysis with at least one year of follow up. Tumors with imaging characteristics or pathologic confirmation of atypical neurofibromas were excluded, as these tumors have a different biologic behavior. Concurrent PN directed medical therapy was allowed, with the exception of MEK-inhibitor treatment, which significantly alters the disease course. A linear mixed-effects model (LMEM) was built using volume data of one PN per patient. The independent variable was time and the dependent variable was tumor volume, with available predictor variables (covariates) of age, sex, ethnicity, body size, treatment type and tumor location. Model development started with a two-parameter LMEM consisting of an intercept (baseline tumor size; alpha) and a slope (linear growth rate; beta). Additional parameters were added until a base structural model was optimized, then a covariate analysis was performed until a final model was optimized based on statistical improvement.

Results: Median age of the 95 subjects was 8.6 years (range 0.7 – 40 years) and the median baseline tumor volume was 384 cm^3 (range 3.7 – 4895 cm^3). As most tumors increased proportionally in size, initially a two parameter LMEM was attempted, but this proved inadequate to capture some tumors that grew exponentially over time. A third parameter (gamma) was added and the model greatly improved the model fit ($p < 0.0001$), however it couldn't capture tumors that regressed in size over time. Therefore, a fourth parameter (delta) was added ($p < 0.0001$) that served as an optimized base model. There was significant inter-individual variability (IIV) on all four parameters (CV% > 140%) due to the wide range in patient characteristics. The covariates found to significantly improve the model were tumor location on the alpha and beta parameters, height on alpha, and age on beta that together explained 40% of the IIV.

Conclusions: Our model can adequately predict changes in PN size based on patient and tumor characteristics. Validation of this model, which may have utility in patient counseling and clinical decision making, with additional patient data is in progress.

Full List of Authors: William D. Figg, Andrea Baldwin, Andrea Gross, Brigitte C. Widemann, Eva Dombi, Srivandana Akshintala

SESSION 2: Psychosocial Issues of the NF Patient

Chairs: Ana-Maria Vranceanu, PhD, Harvard University; Ethan Lester, PhD, Harvard University

Virtual Mind-Body Groups Improve Resiliency Factors in Internationally Diverse Patients with NF1, NF2 and Schwannomatosis: Results of Three Randomized Controlled Trials (RCTs) in Adults, Adolescents and Patients Who Are Deaf

Saturday, September 21, 4:35pm – 4:55pm

Ethan Lester, PhD, *Harvard University*

Background: Patients with NF have lower quality of life (QoL) relative to the general population and cancer patients. Although biomedical treatments have greatly improved outcomes across NF patients an enormous need remains for interventions to increase resiliency – the ability to bounce back when faced with adversity - in patients with NF across the lifespan. Our team has adapted a mind body program – The Relaxation Response Resiliency Program (3RP) for the specific needs of: 1) adults with NF1, NF2 and schwannomatosis (3RP-NF); 2) adolescents with NF1 and NF2 (Resilient Youth with NF; RY-NF); 3) adults with NF2 who are deaf (d3RP-NF-CART). The three programs teach the same core resiliency skills (i.e., gratitude, mindfulness, coping, optimism and social support) but have been iteratively tailored to each population through direct qualitative feedback and prior research. The programs are highly feasible, accepted and efficacious in improving QoL when tested individually against an attention placebo control via three separate RCTs. Here we report data on the efficacy of the three programs in improving resiliency factors over and above the attention placebo control.

Methods: Patients (63 adults, 51 adolescents, 45 patients who are deaf) were recruited through an international NF registry. Screening and consent occurred via live video, with CART for patients who were deaf. Data was collected electronically. The intervention programs and control (8 sessions; 90 minutes for adults, 45 for adolescents, and 60 for deaf adults) were delivered by a clinical psychologist. Participants in the intervention received a patient manual and age/symptom tailored meditation recordings for home practice.

Results: Although specific outcomes varied by population, participants in all three interventions experienced significant improvements ($p < .05$) in one or more resiliency factors (mindfulness, gratitude, social support, coping and optimism) over and above their respective attention placebo controls, with many of these improvements maintained at six months.

Conclusions: A virtual mind-body program adapted for the needs of adults, adolescents and patients with NF2 who are deaf was associated with sustained improvement in resiliency factors. Promoting resiliency may be particularly important for individuals with NF who are living with a chronic incurable condition with an unpredictable trajectory. Details on program adaptations, results comparison by patient populations, future directions and implications for NF care will be discussed.

<u>Platform</u>: Acceptance and Commitment Training (ACT) in Individuals with NF1 and Plexiform Neurofibroma Tumors (PNs) and Chronic Pain: How Important is Treatment Engagement in Daily Life?

Saturday, September 21, 4:55pm – 5:10pm

Staci Martin, PhD, *Pediatric Oncology Branch, National Cancer Institute, NIH*

Background: Chronic pain is common in individuals with neurofibromatosis type 1 (NF1) and plexiform neurofibromas (PNs) and is associated with diminished quality of life (QOL) and interference with everyday activities. A small number of psychosocial interventions have been used effectively in patients with NF1 and chronic pain. While research strongly supports the efficacy of ACT across pain populations including NF1, little is known about how treatment engagement at home relates to pain outcomes. We hypothesized that more engagement in ACT techniques in daily life would relate to improved pain interference post-intervention among individuals with NF1 and chronic pain.

Methods: Adolescents and adults with NF1 and chronic pain ($n=55$; $M_{age}=29.9\pm11.32$ years; 41.8% male) enrolled in a randomized controlled trial of a multi-method ACT intervention. Participants completed questionnaires assessing pain intensity, pain interference, pain acceptance, and pain-related inflexibility. After a 2-day (4-hour) in-person intervention, participants engaged in an 8-week at-home treatment period involving videochats every other week and emails weekly. During this time, participants were instructed to practice newly-learned ACT skills (i.e., mindfulness, defusion [not letting thoughts control behavior], and values-consistent activity) in their daily lives. Post-intervention, they completed the same questionnaires and reported how often they had practiced these skills.

Results: Individuals who practiced defusion techniques on a daily or near-daily basis exhibited significantly more improvement in pain inflexibility ($t=3.38$; $p<0.01$) and pain acceptance ($t=-2.05$; $p<0.05$) at follow-up compared to participants who used these strategies once a week or less. In addition, participants who engaged in mindfulness exercises at least a few times per week had significantly greater reductions in pain intensity after treatment compared to those who practiced less frequently ($t=2.30$; $p<0.05$). The extent of engagement in values-based activity did not significantly impact pain-related outcomes. As hypothesized, engagement in home-based ACT exercises (collectively) significantly predicted improvements in pain interference at follow-up, when controlling for baseline levels of pain and pain acceptance [$F(3, 50)=4.44$; $p<0.01$]. Just over half (53%) of the participants spent time each week listening to audio meditations, while 64% reported reading ACT materials either on ACT websites or in their workbook at least once per week.

Conclusions: Most participants practiced ACT techniques on a weekly basis during the at-home phase of the intervention. Engagement in ACT skills in daily life had a direct and adaptive effect on pain interference at follow-up. Thus, the capacity to decrease pain interference via ACT skills practice at home has the potential to improve QOL in patients with NF1.

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Platform: Physical Functioning Outcomes in Children with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibroma (PN) Motor Morbidity on SPRINT: a Phase II Trial of the MEK 1/2 Inhibitor Selumetinib (AZD6244, ARRY-142886; NCT01362803)

Saturday, September 21, 5:10pm – 5:25pm

Pamela L. Wolters, PhD, *Pediatric Oncology Branch, National Cancer Institute*

Background: PNs may cause substantial morbidity, including pain and functional impairments, which affects quality of life (QOL). We prospectively assessed physical functioning using patient-reported (PROs) and functional outcomes in children with NF1 and PN motor morbidity in a phase II trial of selumetinib to assess clinical benefit.

Methods: Children 2-18 years with NF1, inoperable PNs, and ≥ 1 PN-related morbidity were enrolled. Children (≥ 8) and parents (of children ≥ 5) completed PROs to assess physical functioning (PROMIS-mobility/upper extremity [UE] function & PedsQL), tumor pain intensity (NRS-11), pain interference (PII), QOL (PedsQL), and global impression of change (GIC). Functional evaluations included the 6-minute walk (mobility) and grooved pegboard (fine motor dexterity) tests. MRI analysis assessed PN volume.

Results: Fifty children (60% male, *Mdn* age$=10.2$ years, 3-17) enrolled at four sites; 33 had a motor morbidity (61% upper body; 39% lower body). At baseline, child self-report (SR; n$=23$) PROMIS mobility ($M=46.6$; $p=0.02$) and UE ($M=36.8$; $p<.0001$) and parent report (PR; n$=32$) mobility ($M=37.4$; $p<0.0001$) and UE ($M=38.1$; $p<0.0001$) T-scores were significantly worse than 50 (normative mean) and pegboard z-scores (*Mdn*$=1.08$; dominant hand; *Mdn*$=2.22$ non-dominant; $ps<0.001$) were significantly poorer than 0 (normative mean). As expected, 6-min walk raw distance scores were associated with age (p$=0.0003$), limiting further analyses to only within-subjects change. Larger target PN volumes were associated with worse PROMIS UE function (PR/SR; $ps\leq 0.01$), mobility (PR; $p=0.03$), and PedsQL physical functioning (PR/SR; $ps\leq 0.03$). In contrast, PN volumes were not correlated with pegboard z-scores. When comparing pegboard and PROMIS UE function scores, only non-dominant hand z-scores were associated with PR PROMIS UE function ($p=0.02$). From baseline to cycle 12, the SR PROMIS mobility and UE mean scores did not change significantly; PR PROMIS mobility mean scores improved significantly ($p=0.0003$) but not UE. Both PR and SR PedsQL physical functioning mean scores improved significantly ($ps<0.001$). There was no significant change in 6-min walk distance or pegboard z-scores, but inadequate normative data and large, variable standard deviations limit the use of these data. Cycle 12 GIC qualitative responses noted improved mobility, range of motion, and strength by PR and increased mobility, range of motion, and physical activity by SR.

Conclusions: Children with NF1 and PN motor morbidity exhibited significant impairments in mobility and UE function on the PROMIS and poor fine motor dexterity on the pegboard test at baseline. PN volume was associated with the physical functioning PROs but not the functional tests. Further analysis of the pegboard data examining affected and unaffected hands is planned. On PROs, PR PROMIS mobility and PR/SR PedsQL physical functioning (mostly mobility items) improved significantly with selumetinib, indicating perceived clinical benefit in mobility. Functional evaluations of upper/lower extremity function did not show significant changes and have limited normative data. These results support the validity of the physical functioning PROs in NF1 while further evaluation of and normative data for the functional tests in children with NF1 and PNs are imperative for their use as clinical trial outcomes.

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KEYNOTE LECTURE: The Precision Health Future: Predicting, Preventing, and Curing Cancer

Sunday, September 22, 8:00am – 9:00am

Lloyd Minor, MD, Dean, *Stanford University School of Medicine*

Today, researchers and clinicians have the unprecedented opportunity to not just treat disease but to predict it, prevent it, and cure it—*precisely.* Stanford Medicine calls this proactive approach to patient care Precision Health, and it is revolutionizing all facets of biomedicine, including cancer diagnosis and treatment. Lloyd Minor, Dean of the Stanford University School of Medicine, will provide an overview of Precision Health, outline the critical role of fundamental research, and detail the factors that facilitate interdisciplinary collaboration—a vital component for ushering in this new era of proactive and personalized medicine. Additionally, he will highlight innovative Precision Health initiatives within Stanford Medicine that are disrupting traditional care strategies for cancer, share how emerging technologies will evolve approaches to research, and discuss the many factors accelerating the realization of Stanford Medicine's Precision Health vision.

SESSION 3: Cancer

Chairs: Rebecca Dodd, PhD, University of Iowa; Michael Fisher, MD, Children's Hospital of Philadelphia; Juha Peltonen, MD, PhD, University of Turku, Finland

MPNST: From Beginning to End

Sunday, September 22, 9:00am – 9:25am

Luis Parada, PhD, *Memorial Sloan Kettering Cancer Institute*

Malignant peripheral nerve sheath tumors are incurable sarcomas primarily associated with germline NF1 mutation. Progression from benign plexiform neurofibroma (PN) to malignancy is associated with secondary mutations at either the Ink4/arf or the P53 loci. Genetically engineered mouse models have provided the unique advantage of permitting study of the earliest events in both PN and MPNST origin. The collective work from several laboratories including our own, points to embryonic or early neonatal origin and to neural crest derived stem progenitor cells as the cells that give rise to these tumors. These sophisticated mouse models (GEM) and newly developed patient derived xenografts (PDX) from patient derived MPNST allow direct comparison and validation of the preceding mouse modelling data and advancement of translational preclinical studies aimed at blocking and reducing tumor burden. Studies on primary cultures from GEM and PDX tumors provide added consistency to tests of combinatorial drug therapies *in vitro* and *in vivo.*

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<u>Platform</u>: Somatic CRISPR/Cas9 Tumorigenesis Approaches to Study Myeloid Cell Function in MPNST

Sunday, September 22, 9:25am – 9:40am

Rebecca Dodd, PhD, *University of Iowa*

CRISPR/Cas9 technology provides cancer biologists with unprecedented opportunities to introduce multiple somatic mutations into adult, wildtype mice. We recently reported generation of CRISPR/Cas9-based malignant peripheral nerve sheath tumors (MPNSTs), aggressive sarcomas arising from the myelinating nerve sheath. Spatially-controlled MPNSTs were generated by localized injection of adenovirus expressing Cas9 and guide RNAs for *Nf1* and *p53.* By using *in vivo,* somatic delivery of Cas9 and guide RNAs into wild-type mice, this approach facilitates the use of true isogenic controls, allowing for studies in mice of identical backgrounds, simplifying gene expression studies, and reducing potentially confounding genetic events that occur in experiments with genetically-engineered mice. By exogenously delivering Cas9, this method is unique in allowing scientists a wide selection of background strains for experimental design. We have compared CRISPR/Cas9-induced MPNSTs across four common backgrounds of wild-type mice—C57BL/6, 129X1, Balb/c, and 129/SvJ. Our data shows that mouse background impacts tumor onset and immune cell profiles. While C57BL/6, 129X and 129/SvJ mice showed similar tumor initiation, Balb/c mice developed tumors more quickly than other strains, possibly due to Balb-specific mutations in the *p16* gene. Indel pattern analysis demonstrated that indel frequency and size were similar across all genetic strains. Gene expression and IHC analysis identified strong differences across mouse backgrounds in infiltration of T cell and myeloid cell populations, including macrophages and mast cells.

We have combined these somatic CRISPR/Cas9 approaches with Cre/loxP control of the tumor microenvironment to understand the role of myeloid cell populations in MPNST formation. We are using myeloid lineage-specific Mcpt5-Cre and LysM-Cre drivers to control mast cell and macrophage populations, respectively. This approach allows us to generate MPNSTs in mice with conditional or temporal depletion of myeloid cells using diphtheria toxin-based ablation models. Our data shows that constitutive depletion of mast cells slows tumor initiation and modifies tumor-infiltrating T cell populations. Current studies are examining the effects of temporal myeloid cell depletion at distinct points in tumor development. These experiments highlight useful applications of somatic CRISPR/Cas9 tumorigenesis approaches to study the tumor microenvironment in diverse backgrounds of mice.

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Platform: Pharmacogenomic and Synthetic Lethal Genetic Screens Reveal Hidden Vulnerabilities and New Therapeutic Approaches for Treatment of NF1-Associated Tumors and Malignancies

Sunday, September 22, 9:40am – 9:55am

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

Treatment options for plexiform neurofibromas and malignant peripheral nerve sheath tumors (MPNST) are limited, relying mostly on surgical resection and broad-spectrum chemotherapy. The genetic basis of NF1 syndrome makes well suited for using synthetic lethal genetic screens and related approaches to uncover unique variabilities in *NF1* deficient cells as well as cells closely mimicking the genetics of an MPNST.

Genome engineering technologies, such as CRISPR/Cas9, allow introduction of clinically relevant mutations into cells of the correct tissue type for the disease being studied. We built models of tumors that arise in Neurofibromatosis Type-1 (NF1) patients and used these models for therapeutics discovery with synthetic lethal pharmacogenomic screens as well as genome-wide synthetic lethal genetic screens.

Given plexiform neurofibromas and MPNSTs arise within the Schwann cell linage, we developed a drug discovery pipeline to identify 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 *SUZ12*. ~80% of all MPNST harbor loss of function mutations in Polycomb Repressive Complex 2 (PRC2) genes, such as *SUZ12,* which is highly suggestive that perturbation of epigenetic homeostasis plays a role in malignant transformation of neurofibromas. We have previously reported results from our drug screening efforts against *NF1* deficient human Schwann cells. Here we build upon this work by engineering additional mutations relevant to MPNST formation into these models and identify new classes of useful therapeutics, complete genome-wide CRISPR based genetic screens, and perform extensive *in vivo* drug testing of candidates in models of MPNST.

Our small molecule screening efforts identified compounds showing selective lethality towards *NF1*/*SUZ12* double mutants that more closely mimic 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. Informed by these studies we tested the most promising two and three drug combinations using *in vivo* models of MPNST. 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 also testing these combinations in human MPNST PDX models.

The discovery of agents effective against models of MPNST is exciting, as there are no approved targeted therapies for MPNST to date. 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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Platform: Defining the Mechanisms Underlying Pediatric Brain Tumor Penetrance

Sunday, September 22, 9:55am – 10:10am

Nicole M. Brossier, MD, PhD, *Washington University in St. Louis, Division of Pediatric Hematology-Oncology*

While most children develop cancer without a clear etiology, some pediatric cancers arise in the context of tumor predisposition syndromes, typically caused by germline mutations in genes that regulate cell growth. Interestingly, many of these mutations also occur as somatic mutations in sporadic (non-syndromic) childhood gliomas, making insights obtained from studying glioma predisposition syndromes applicable to the broader field of pediatric brain tumorigenesis. The most common of these syndromes, Neurofibromatosis type 1 (NF1), affects ~1:3,000 individuals worldwide, 15% of whom will develop low-grade tumors of the optic pathway (optic pathway gliomas; OPGs). However, it is currently unclear which children with NF1 will develop an OPG, making risk assessment for each individual child difficult.

While the risk factors that underlie glioma penetrance are currently unknown, emerging evidence from both human and animal studies has raised the intriguing possibility that the specific *NF1* germline mutation that each child is born with may be one such factor. To address the hypothesis that specific germline *NF1* gene mutations differentially increase the risk of optic glioma formation through cell-intrinsic effects on the tumor cell of origin, we designed a series of studies to examine the impact of different NF1 patient-derived germline *NF1* gene mutations on the neuroglial progenitor cell populations in the developing brain.

We first defined the putative cells of origin – neuroglial progenitor cells within the third ventricular zone (TVZ) – over normal mouse brain development using *Nestin-CFPnuc* reporter mice. Three cell populations were identified by immunochemical methods, which each displayed distinct spatial localizations and were dynamic over the course of late embryonic and early postnatal development. One of these three populations was more abundant during embryogenesis when TVZ neuroglial progenitors exhibited the highest levels of proliferation. This population also displayed increased clonogenic incidence *in vitro*, suggesting that these cells may be more capable of expansion following the acquisition of a somatic *NF1* genetic mutation. Transcriptional differences between these populations were then identified by RNA-sequencing, which are currently being validated and functionally explored. We then evaluated the spatiotemporal dynamics of these populations in mice genetically engineered to harbor different NF1 patient-derived germline *Nf1* gene mutations. Interestingly, some, but not all, *Nf1* gene mutations resulted in increased GFAP$^+$ progenitor content and progenitor proliferation. Together, these findings provide early experimental evidence for mutational specificity in specific putative brain tumor cells of origin relevant to glioma penetrance.

Full List of Authors: Nicole M. Brossier, MD, PhD, Oliva Cobb, BS, and David H. Gutmann, MD, PhD

Preclinical and Clinical Studies in NF1-Associated Blood Cancers

Sunday, September 22, 10:45am – 11:10am

Kevin Shannon, PhD, *University of California San Francisco*

Children with NF1 are predisposed to juvenile myelomonocytic leukemia (JMML), an aggressive myeloproliferative neoplasm (MPN) that progresses to acute myeloid leukemia (AML) in 15- 30% of patients. Our studies of JMML specimens showed that *NF1* functions as a tumor suppressor gene in hematopoietic cells, and that *NF1* inactivation results in deregulated Ras/Raf/MEK/ERK signaling. This work suggested a central the role of hyperactive Ras signaling in JMML pathogenesis and our group and other investigators subsequently discovered mutations in other Ras pathway genes including *NRAS, KRAS, PTPN11,* and *CBL.* Ras pathway mutations are invariably present at high allelic frequency at both diagnosis and relapse. Overall, >85% of JMML patients have founder mutations in one of these genes, including 15-20% with clinical NF1 or mutations in the *NF1* gene. Consistent with the molecular genetics of JMML, using the *Mx1-Cre* transgene to inactivate the conditional mutant *Nf1^{flox}* allele generated in the Parada lab (Project 2) or to express oncogenic *Kras^{G12D}* or *Nras^{G12D}* in the hematopoietic compartment of mice induces a JMML-like MPN. We utilized these genetically engineered mouse models to perform preclinical trials and observed remarkable efficacy of MEK inhibitors in *Kras* and *Nf1* mutant mice with MPN. These data informed a national phase 2 clinical trial of the MEK inhibitor trametinib as monotherapy for patients with relapsed/refractory JMML (ADVL1521). We also developed sensitive and reproducible assays for monitoring molecular responses in patients enrolled on ADVL1521 and have used these to address additional translational research questions. Finally, we have conducted preclinical trials of novel agents – alone and in combination with MEK inhibitors – in our genetically accurate models of JMML and AML. We anticipate that these studies will inform "next generation" clinical trials in children with JMML and AML.

Development and Progression of NF1-Associated Peripheral Nerve Sheath Tumors

Sunday, September 22, 11:10am – 11:35am

Eduard Serra, PhD, *The Institute for Health Science Research Germans Trias I Pujol (IGTP)*

Two key factors to understand tumor development and progression are the identity of the cell of origin and the somatic genetic alterations that this cell acquires. In our lab, we combine the genomic characterization of plexiform neurofibromas (pNFs), atypical neurofibromas (aNFs) and malignant peripheral nerve sheath tumors (MPNSTs), with the generation of models based on derived human cells and tissues to investigate their development and malignization.

pNFs originate during development from a precursor cell of the neural crest (NC) - Schwann cell (SC) differentiation lineage that losses *NF1* function. We have generated *NF1*(-/-) pNF-derived induced pluripotent stem cells (iPSCs) and set up conditions to differentiate iPSCs towards NC and further to SCs. This model is helping us to understand the role of *NF1* loss in SC biology, tumor formation and cellular composition. We took advantage of the higher proliferation capacity of *NF1*(-/-) iPSC differentiating SCs and their tendency to form spheres, to further develop a multiplexed 3D model which will allow us to investigate tumor formation, progression and therapy, when combined with DNA editing techniques.

In fact, the analysis of the somatic *NF1* mutation shared by pNFs or aNFs and their progressed adjacent MPNST, link the cells of origin of the benign and the malignant tumor counterparts. Additional genomic analysis revealed that an aNF is formed when in addition to the *NF1* inactivation, the *CDKN2A/B* locus is also lost, generating cells with a higher predisposition to progress towards an MPNST. These soft tissue sarcomas are characterized by a low mutation burden and a highly rearranged hyperploid genome, probably due to a catastrophic chromosomal event that somehow is afterwords stabilized. Compared to aNFs, MPNSTs bear few additional highly recurrent mutations, like those altering the correct functioning of the polycomb repressive complex 2 (PRC2). In addition, recurrent alterations in large chromosomal regions have an important impact on the overall gene expression of MPNST cells. This clustered regional expression can help us in identifying genes important for MPNST development and maintenance. The combination of experimental work using developed models together with a fine integrative genomic analysis of both tumors and models is helping us in understanding *NF1*-associated tumors with the final goal of identifying effective therapeutic strategies.

<u>Platform</u>: 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)

Sunday, September 22, 11:35am – 11:50am

Geraldine O'Sullivan Coyne, MD, PhD, *Developmental Therapeutics Clinic, Division of Cancer Treatment and Diagnosis, National Cancer Institute (NCI)*

Background: PN in NF1 are locally invasive tumors characterized by debilitating complications including pain, disfigurement and functional limitations. Selumetinib, an oral MEK inhibitor, has recently received breakthrough designation for NF1-related PN based on two early phase studies for children with NF1 and inoperable PN showing an unprecedented 71% and 74% response rate (RR) respectively. We report the initial results of the currently ongoing phase II single site study of selumetinib in adults with NF1 and inoperable PN (NCT02407405), which includes a prospective evaluation of serial PN and cutaneous neurofibroma (cNF) biopsies, together with standardized patient-reported outcomes (PROs).

Methods: Open-label Simon 2-stage phase II study in patients ≥18 years old with NF1, inoperable PN and ≥1 PN-related morbidity; overall target RR of 45%. Selumetinib 50 mg is administered twice daily orally on a continuous dosing schedule (28-day cycles). Primary study objective: determine the partial response (PR; ≥20% volume decrease) and complete response rate of PN using volumetric MRI analysis; secondary objectives include pharmacodynamic (PD) studies on mandatory pre- and on-treatment biopsies of PN and cNF, and assessment of clinical benefit using PROs (Numeric Rating Scale-11 and Pain Interference Index).

Results: As of May 20, 2019, a total of 23 patients (pts) have been enrolled (66% male; median age 33 years, range 18-60). Outcomes reported for the first 21 pts, with a 1-year minimum time on study. Eighteen pts had a typical PN target lesion on imaging; most common baseline PN-related morbidities were motor weakness (13 pts) and disfigurement (11 pts). Nineteen pts underwent serial photography. The median change in PN volume at the time of best response was -22% (range -41% to +5.5%); 14 pts (67%) achieved a PR, with 11 pts confirmed on ≥2 consecutive restaging scans. Three of these pts had a biopsy-confirmed atypical neurofibroma (aNF) as target lesion, of which 2 had a PR and one had continued growth, which was resected after coming off treatment. Four pts were dose-reduced (rash=3 pts, transaminitis=1 pt); two of these pts required a second reduction. Grade ≥3 drug-related toxicities include transaminitis (5 pts, 23%), rash (4 pts, 19%) and pancreatic enzyme elevation (4 pts, 19%). Nineteen pts had percutaneous neurofibroma and/or cNF biopsy with confirmed neurofibroma pathology. PD target inhibition assessment using phospho-AKT/phospho-ERK levels is ongoing; baseline PN core biopsies and cNF shave biopsies produced sufficient protein yield and phospho-ERK/phospho-MEK levels from total cell lysates for PD assessment. Seventeen pts (80%) remain on study; 4 pts discontinued treatment (rash, surgical resection, patient choice and non-compliance). Between baseline and end of 1-year evaluation, patient-reported target tumor pain intensity and pain interference scores significantly improved (p<0.002).

Conclusions: Selumetinib shows activity and clinical benefit in adult patients with symptomatic NF1-associated PN. PD marker analysis is continuing. Enrollment on stage 2 is underway.

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SESSION 3B (CONCURRENT): Mechanisms of NF2 Loss-Driven Tumorigenesis

Chair: Chunling Yi, PhD, Georgetown University

Mechanistic Underpinnings of TORC1 Activation in NF2 Mutant Tumors

Sunday, September 22, 10:45am – 11:05am

Filippo G. Giancotti, MD, PhD, *MD Anderson Cancer Center*

Inactivation of NF2 promotes tumor development through activation of YAP and TOR. While NF2 inhibits YAP by activating LATS through divergent mechanisms at the cell cortex and in the nucleus, the mechanisms through which NF2 suppresses TOR are not known. We are investigating these mechanisms by using a combination of somatic cell genetics and biochemistry and we are developing therapeutic strategies that build on this knowledge.

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MD Anderson Cancer Center

The Hippo-YAP Pathway in Schwannoma – Novel Functions and Therapeutic Implications

Sunday, September 22, 11:05am – 11:25am

Joseph Kissil, PhD, *The Scripps Institute*

Neurofibromatosis type 2 (NF2) is a dominantly inherited autosomal disease with most common manifestation being the development of bilateral schwannomas of the 8th cranial nerve. Although our understanding of the molecular mechanisms underlying NF2 has significantly improved over the past 2 decades, effective therapies remain lacking for this disease and there is an urgent need to develop therapeutic options for patients. Recent data implicates The Hippo-YAP pathway as a therapeutic target in NF2.

The Hippo pathway regulates cell proliferation and organ size 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 associated with enhancers regions and gene promoters. YAP has been mainly described as a transcriptional activator of genes involved in cell proliferation and survival. Using a genome-wide approach, we show that in addition to its known function as a transcriptional activator, YAP functions as a transcriptional repressor in Schwann cells. Validation of these findings in cell-based and in vivo models will be presented, as well as the mechanisms underlying YAP's transcriptional repressive functions. The therapeutic implications of these findings for NF2 will be discussed.

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NF2 and Cell Contact Regulation of TEAD Stability and Function

Sunday, September 22, 11:25am – 11:45am

Barry Gumbiner, PhD, *University of Washington/Seattle Children's Hospital*

NF2 mediates contact inhibition of proliferation and an upstream event in the Hippo signaling pathway in response to various physical and biochemical stimuli. The Hippo pathway is a kinase cascade that negatively regulates the activity of co-transcription factors YAP and TAZ, which interact with TEAD transcription factors and activate the expression of target genes. We find that the palmitoylation of TEAD, which controls the activity and stability of TEAD proteins, is actively regulated by cell contact and NF2, independent of Lats, the key kinase of the Hippo pathway. The expression of fatty acid synthase (FASN) and acetyl-CoA carboxylase involved in de novo biosynthesis of palmitate is reduced by cell density in an Nf2/ Merlin-dependent manner. Depalmitoylation of TEAD is mediated by depalmitoylases including APT2 and ABHD17A. A palmitoylation deficient TEAD4 mutant is unstable and degraded by proteasome through the activity of the E3 ubiquitin ligase CHIP. These findings show that TEAD activity is tightly controlled through the regulation of palmitoylation and stability via the orchestration of FASN, depalmitoylases, and E3 ubiquitin ligase in response to NF2 signaling and cell contact.

Full List of Authors: Nam-Gyun Kim and Barry M. Gumbiner

Lipid Signaling in Hippo Pathway Regulation and Cancer

Sunday, September 22, 11:45am – 12:00pm

Wenqi Wang, PhD, *University of California, Irvine*

The Hippo pathway plays a crucial role in organ size control and tumor suppression, and its downstream target YAP is highly activated in multiple human cancers including NF2-deficient schwannomas, meningiomas and mesotheliomas. However, the precise regulation of the Hippo pathway has not been fully understood. Here, we reported phosphatidic acid (PA)-related lipid signaling as a key regulator of the Hippo pathway. Supplementing PA in various Hippo-activating conditions activates YAP. Mechanistically, PA directly interacts with Hippo components LATS and NF2 to respectively disrupt the LATS-MOB1 complex formation and NF2-mediated LATS membrane translocation and activation. Inhibition of phospholipase D (PLD)-dependent PA production suppresses YAP oncogenic activities. PLD1 is highly expressed in breast cancer and positively correlates with YAP activation, suggesting their pathological relevance in breast cancer development. Taken together, our study not only reveals a role of PLD-PA lipid signaling in regulation of the Hippo pathway, but also indicates the PLD-PA-YAP axis as a potential therapeutic target for cancer treatment.

KEYNOTE LECTURE: Whole Body Imaging of Children with Cancer

Sunday, September 22, 12:45pm – 1:45pm

Heike E. Daldrup-Link, MD, PhD, *Stanford University*

Molecular imaging enables *in vivo* detection, characterization and quantification of molecular and metabolic processes that cause tumors. While current diagnostic imaging technologies depict anatomical and pathological processes predominantly at the macroscopic-anatomical level, molecular imaging provides more detailed information about the underlying physiology and function. The extent of neurofibromas in children is typically evaluated with magnetic resonance imaging (MRI). The metabolic activity of suspicious lesions on ^{18}F fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) scan can provide additional information about the risk of malignant degeneration. Therefore, integrated ^{18}F-FDG PET/MR imaging techniques can be a time-efficient and convenient approach for whole body evaluations of patients with neurofibromatosis by providing information about the extent of neurofibromas throughout the entire body and the presence of focal lesions that are suspicious for malignancy. Recently, whole body diffusion weighted scans have evolved as a radiation-free alternative to PET/MR scans. This presentation will provide an overview of advantages and limitations of these new molecular imaging technologies, discuss the additive value of artificial intelligence algorithms and provide an outlook on possible future developments. The continued development, testing and refinement of these novel imaging techniques will significantly impact the future of pediatric cancer diagnoses and long-term outcomes.

SESSION 4: Diagnostics and Imaging

Chairs: Shivani Ahlawat, MD, Johns Hopkins University; Rosalie Ferner, MD, Guy's and St. Thomas' NHS Trust; Michel Kalamarides, MD, PhD, Hôpital Pitié-Salpêtrière, Paris

Quantitative Imaging Analysis in Assessment and Prediction of Tumor Response

Sunday, September 22, 1:45pm – 2:10pm

Wenli Cai, PhD, *Director, 3D Quantitative Imaging Laboratory, MGH*

The objective, accurate, and standardized evaluation of tumor response to treatment is an indispensable procedure in clinical oncology. Compared to manual measurement, computer-assisted linear measurement can significantly improve the accuracy and reproducibility of tumor burden quantification. For irregular-shaped and infiltrating or diffuse tumors, which are difficult to quantify by linear measurement, computer-assisted volumetric measurement may provide a more objective and sensitive quantification to evaluate tumor response to treatment than linear measurement does. In the evaluation of tumor response to novel oncologic treatments such as targeted therapy, changes in overall tumor size do not necessarily reflect tumor response to therapy due to the presence of internal necrosis or hemorrhages. This leads to a new generation of imaging biomarkers to evaluate tumor response by using texture analysis methods, also called radiomics. Computer-assisted texture analysis technology offers a more comprehensive and in-depth imaging biomarker to evaluate tumor response. The application of computer-assisted quantitative imaging analysis techniques not only reduces the inaccuracy and improves the reliability in tumor burden quantification, but facilitates the development of more comprehensive and intelligent approaches to evaluate treatment response, and hence promotes precision imaging in the evaluation of tumor response in clinical oncology. This article summarizes the state-of-the-art technical developments and clinical applications of quantitative imaging analysis in evaluation of tumor response in clinical oncology.

Platform: Feasibility of Magnetic Resonance-Guided High Intensity Focused Ultrasound for Treatment of Atypical Neurofibromas/Distinct Nodular Lesions

Sunday, September 22, 2:10pm – 2:25pm

Caitlin Tydings, MD, *Children's National Medical Center*

Background: Malignant peripheral nerve sheath tumors (MPNST), an aggressive sar-coma, is the leading cause of death in patients with Neurofibromatosis Type 1 (NF1). MPNST often arise from plexiform neurofibromas (PN), benign nerve sheath tumors; and in particular, atypical neurofibromas (ANF) have been described as precursor le-sions to MPNST. ANF typically present as distinct nodular lesions (DNL), identified radiographically as round, well-demarcated, >3 cm lesions. Pain, increased growth, and greater PET avidity than PN raise concern for ANF. Removal of ANF, even with suboptimal margins, has been shown to prevent recurrence and transformation to MPNST. However, removal of lesions may carry significant surgical morbidity and may not always be feasible.

Magnetic resonance-guided high intensity focused ultrasound (MR-HIFU) is a novel therapy which provides controlled delivery of heat by focusing ultrasound energy inside a lesion using an external applicator under the guidance of MR thermography. MR-HIFU is non-invasive, non-ionizing, and enables precise tumor ablation sparing normal surrounding tissue. Given evidence favoring resection of DNL/ANF, MR-HIFU ablation may be a favorable alternative to surgery for treatment of DNL/ANF.

Methods: This is a retrospective review of MRI images from 28 patients with NF1 and PN from Children's National Medical Center and the NIH. DNL are defined as lesions >3cm, distinct from PN and lacking the "central dot" feature. Feasibility to treat with MR-HIFU ablation criteria include target being <8cm from skin surface; >1cm from plexus, spinal canal, bladder, bowel, growth plate and skin margin; not in skull or vertebral body and complete or partial ablation >50%.

Results: Among 28 patients, 122 DNL were identified. The majority of DNL was located in extremity (52%, n=64). Other sites included head/neck (n=8), chest (n=17), and abdomen/pelvis (n=33). HIFU ablation criteria was not met by 36% of lesions (n=44) primarily due to being <1 cm from skin surface (18.2%) or vital structures (33%), or lesions within the skull (6.8%). Complete and partial HIFU ablation was feasible for 42% and 22% of lesions, respectively.

Conclusion: MR-HIFU ablation may be feasible as a non-invasive alternative to sur-gery for DNL/ANF treatment. Further work is needed to determine if MR-HIFU ablation is safe and effective in DNL/ANF. A clinical trial involving surgical resection following MR-HIFU ablation would provide more information on its safety, histology, and effec-tiveness for DNL/ANF.

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Platform: Screening Whole Body MRI Using Diffusion Weighted Imaging in Neurofibromatosis Type 1 – Is Intravenous Contrast Medium Necessary?

Sunday, September 22, 2:25pm – 2:40pm

Shivani Ahlawat, MD, *Johns Hopkins University School of Medicine*

Purpose: To evaluate the added value of contrast enhanced (CE) sequences to a non-contrast (NC) whole body magnetic resonance imaging (WB-MRI) protocol using diffusion weighted imaging (DWI) with apparent diffusion coefficient (ADC) mapping for the detection and characterization of Neurofibromatosis (NF1)-related PNSTs as benign or malignant.

Materials and Methods: This IRB-approved, HIPAA-compliant study retrospectively reviewed 52 consecutive WB-MRIs (age range: 10-62 years, 24 women) with NF1 performed between 3/2013-3/2018 using short tau inversion recovery (STIR), T1-weighted (T1W), CE T1W, and DWI (b-values 50, 400, 800 s/mm^2 and ADC mapping). Two readers independently reviewed all imaging for the presence and character of distinct nodular lesions (DNLs, defined as PNST > 3cm) or plexiform PNSTs and recorded imaging features on all sequences including minimum ADC values (ADCmin) in two sessions (session 1: NC MR sequences, session 2: session 1 + CE MR Sequences). Readers recorded the potential of malignancy at each session according to a 4-point scale (0=definitely benign; 1=probably benign; 2=possibly malignant; 3=probably malignant). Clinical/imaging stability > 12 months for benign PNSTs and histology were used as reference standard. Descriptive, agreement, and diagnostic performance statistics were applied.

Results: 12(23%) WB-MRIs did not have DNLs and 5(10%) only had plexiform lesions without a DNL. 100 DNLs were characterized on 35 WB-MRIs. The median DNL largest lesional diameter was 4.6 cm (range 3.2-13.4 cm). Malignant transformation was detected in 4% ADCmin range: 0.3-0.9$\times$10^{-3}mm^2/s). For benign PNSTs, ADCmin ranged from 0.9-3.1$\times$10^{3}mm^2/s. There was 98% exact agreement in the potential of malignancy for each PNST between session 1 and 2 for both readers. The potential for malignancy was not changed from the probably or possibly benign category to probably or possibly malignant category with the addition of contrast material. Both sessions yielded high diagnostic accuracy with sensitivity of 100%, specificity of 94% and overall accuracy of 94%.

Conclusion: The addition of contrast enhanced MRI sequences to a non-contrast WB-MRI protocol including diffusion weighted imaging does not improve diagnostic performance for characterization of NF1-related PNSTs and as such, may not be necessary.

Full List of Authors: Filippo Del Grande, MD and Laura M Fayad, MD

Cranial Nerves Tractography: A Review

Sunday, September 22, 3:40pm – 4:00pm

Michel Kalamarides, MD, PhD, *Hôpital Pitié-Salpêtrière Paris*

Diffusion imaging tractography is used nowadays to "see" the brain white matter for planning before brain tumor resection. Because of limited spatial resolution, small fiber tracking is very difficult, in particular brainstem tracts and cranial nerves.

Predicting the displacement of cranial nerves by tumors could make surgery safer and the outcome better. Recent advances in imaging and processing have overcome some of the limits associated with cranial nerve tractography, such as spatial resolution and fiber crossing. Deterministic tracking methods have been reported but with noise/artefact. Probabilistic tracking is an alternative strategy with possibly, more accurate results.

The presentation will summarize the state of the art of cranial nerve fiber tractography and the challenges to be met.

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<u>Platform</u>: Non-Ossifying Fibromas in Patients Enrolled on the NCI NF1 Natural History Study

Sunday, September 22, 4:00pm – 4:15pm

Eva Dombi, MD, *National Cancer Institute, Pediatric Oncology Branch*

Background: Non-ossifying fibromas (NOF) are benign developmental bone lesions typically located around the metaphyseal region of long bones, involvement of the distal femur and proximal tibia is most common. The majority of NOF are asymptomatic and discovered incidentally. It is estimated that NOF are present in 30% of children. Diagnosis is established based on characteristic imaging signs, that include disruption of the cortical bone and well-defined lobulated fibrotic masses within the metaphysis, therefore biopsies are rarely indicated. Patients with NF1 have an increased incidence of NOF and lesions are frequently multifocal. The disease course is self-limiting, and the majority of NOF can be monitored without interventions. Symptomatic lesions limiting normal daily activity can be treated with curettage and bone graft.

Objective: To assess the incidence and clinical manifestations of NOF in patients enrolled on the NCI NF1 natural history study (NCT00924196).

Methods: We performed a retrospective review of all available imaging studies, including X-ray, regional and whole-body MRI, CT and FDG PET/CT exams to identify imaging findings consistent with NOF. Clinical symptoms were extracted from patient charts.

Results: 183 patients enrolled on the NF1 natural history study between February 2008 and March 2019. The region of the knees could be visualized on at least one imaging study in 173 patients, of whom 42 (24%) showed signs of NOF (23 males, 19 females). Imaging modality to detect NOF was MRI (N=34), FDG PET/CT (N=6), CT (N=1) and X-ray (N=1). Median age at the first imaging evaluation identifying NOF was 15.6 years (range 4.4-39.4). In 21 affected patients FDG PET was performed for unrelated clinical indications, and metabolic activity could be measured in 48 distinct NOF lesions. The median standard uptake value was 1.8 (range 0.5-8.2). On Lugano 5-point intensity scale 7 lesions were classified grade-1, 17 grade-2, 16 grade-3, 6 grade-4, and 2 lesions grade-5. Median follow up time with additional imaging was 5.7 years (range 0-11.6). Spontaneous resolution of NOF could be observed in 5 patients, after 4.7, 8.6, 9.2, 10.3 and 11.6 years respectively. Six patients reported intermittent pain in the affected area and one additional patient experienced prolonged severe pain following an injury in the location of the NOF. This patient was successfully treated with curettage and bone graft. No NOF related fractures were seen.

Conclusions: In our long-term observational study of NF1 patients we identified 42 individuals showing imaging characteristics of NOF. In the subset of patients who underwent FDG PET evaluation variable metabolic activity was detected in NOF, however based on anatomic location and CT appearance even lesions with high uptake could be identified as benign NOF. In the majority of patients, NOF were not associated with pain or limited mobility and only one patient required surgical intervention.

Full List of Authors: Anne Dufek, Andrea Baldwin, Jessica Dompierre, Andrea Gross, Mark Ahlman, Brigitte C. Widemann

KEYNOTE LECTURE: Recent Advances in the Neurobiology and Management of Neuropathic Pain

Monday, September 23, 8:00am – 9:00am

Allan Basbaum, PhD, FRS, *Department of Anatomy, University California San Francisco*

There are two major chronic pain conditions, nociceptive/inflammatory pain, which is provoked by tissue injury and neuropathic pain, which can follow from nerve injury to the peripheral or central nervous system. Because of the opioid epidemic, there is a now a growing effort to develop novel therapeutics to treat chronic pain, including neuropathic pain, which is not particularly responsive to opioids. This discussion will focus on new approaches to neuropathic pain management.

Local anesthetics block all voltage-gated Na channels and are remarkably effective in producing temporary relief in many peripheral injury-induced neuropathic pain conditions. For this reason, attention has focused on regulating the nociceptor. Unfortunately, the adverse side effects of systemic lidocaine administration limit their use to localized peripheral injection, or to topical patch administration. Based on evidence that the NaV1.7 subtype of Na channel is expressed almost exclusively in sensory neurons and that a loss of function mutation in NaV1.7 underlies a condition of congenital indifference to pain, efforts continue to develop selective NaV1.7 antagonists. The molecular heterogeneity of nociceptors also points to the utility of selectively targeting specific molecular entities (e.g. TRPV1, TRPM8). A gene therapy approach is also being assessed in preclinical studies. Here a virus is used to increase the sensory neuron expression of a novel receptor target. When engaged by a novel ligand, these receptors inhibit sensory neuronal activity and pain. As the novel ligand can only act at the expressed receptor, the therapeutic window is greatly increased. Finally, a very different approach attempts to alter the chemical milieu of the injury. Encouraging are clinical studies using antibodies directed against nerve growth factor, which have proven very effective against osteoarthritis and are being evaluated in neuropathic pain models.

Of course, opioids are highly effective analgesics, but have a poor therapeutic window, with many serious adverse side effects that undoubtedly contribute to their minimal efficacy in neuropathic pain conditions. For this reason, the effort to develop so-called biased opioid receptor agonists is of particular interest. The underlying principle is that after binding to the opioid receptor, opioids engage different downstream biochemical pathways. An ideal biased opioid receptor agonist would exert an analgesic action through one pathway, but not engage beta arrestin-mediated downstream effects that are implicated in respiratory depression, constipation and dependence. The search for effective biased opioid receptor agonists continues.

Taking a very different direction, our laboratory has introduced a therapeutic approach that considers that neuropathic pain is not a symptom of nerve damage, but rather represents a "disease" of the nervous system, comparable to epileptic conditions that result from reduced cortical GABAergic inhibition. In a neuropathic pain condition the loss of inhibition is in the spinal cord. The fact that the first line approach to the management of neuropathic pain involves anticonvulsant therapy is consistent with this parallel between epilepsy and neuropathic pain. Once again, however, adverse side effects limit the utility of many anticonvulsants. An alternative approach to treating the "disease" of neuropathic pain is to re-establish/rebuild the inhibitory tone. To this end we have demonstrated the utility of spinal cord transplants of embryonic progenitors of GABAergic interneurons. To date, in preclinical studies we have successfully reduced the mechanical allodynia and ongoing pain that occurs in peripheral nerve injury and chemotherapy models of neuropathic pain. As neurofibromatosis associated pain likely arises from damage to peripheral nerves, the potential adaptation of these new approaches deserves attention.

SESSION 5: Pain and Itch

Chairs: Michael Caterina, MD, PhD, Johns Hopkins School of Medicine; Vanessa Merker, PhD, ENRM Veterans Hospital, Boston; Shawn Kwatra, MD, Johns Hopkins University School of Medicine

The Role of Mrgprs in Itch Sensation

Monday, September 23, 9:00am – 9:30am

Xinzhong Dong, PhD, *Johns Hopkins University School of Medicine, Howard Hughes Medical Institute*

Chronic itch is a major clinical problem. Majority of chronic itch is mediated by non-histaminergic mechanisms. Therefore, it is crucial to identify cell surface receptors mediating non-histaminergic itch. During the past years, we have characterized a large family of G protein-coupled receptors in mice called Mrgprs. Several of these receptors are specifically expressed in distinct subsets of small-diameter dorsal root ganglion (DRG) neurons and function as novel itch receptors mediating histamine-independent itch. For example, we recently identified that MrgprA1 is a receptor for bilirubin and plays an important role in jaundice (a yellowing of the skin due to a buildup of bilirubin)-associated itch. Patients with jaundice commonly suffer from an intense non-histaminergic itch. We demonstrated for the first time the existence of bilirubin receptors (MrgprA1 in mice and MrgprX4 in humans) and that pathophysiologic levels of bilirubin elicit itch through MrgprA1 in primary sensory neurons in the DRG. In mouse models of cholestatic pruritus, we showed that genetic deletion of either *Mrgpra1* or *BVR,* the bilirubin-producing enzyme, attenuates itch. Plasma isolated from hyperbilirubinemic patients evoked itch in wild-type animals but not *Mrgpra1-/-* animals. Besides the sensory neuron specific Mrgprs, we and others discovered another member of the gene family, MrgprB2 in mice and its human homologue MrgprX2, are exclusively expressed in mast cells, a type of innate immune cells, which secret many pro-inflammatory mediators upon activation. We demonstrated that MrgprB2/MrgprX2 are receptors for various basic secretogagues and mediate IgE-independent mast cell degranulation. Recently we found that MrgprB2-associated itch functions independently of the IgE/FcεRI-histamine axis and Mrgprb2 plays an important role in allergic contact dermatitis. Therefore, we believe that targeting Mrgprs may lead to novel treatment of chronic itch in the future.

Complementary and Integrative Health Therapies for Pain

Monday, September 23, 9:30am – 10:00am

Stephanie Taylor, MD, *VA Greater Los Angeles Healthcare System*

Complementary and integrative health therapies such as acupuncture, mediation and Tai Chi are evidence-based therapies that can be used for pain and pain comorbid conditions such as symptoms of anxiety, depression or fatigue. This presentation will review the evidence for a variety of complementary and integrative health therapies and the national clinical and quality guidelines that include them. It also will review the efforts being conducted by the Veterans Administration, the nation's largest integrated healthcare system, to make these therapies available and study their effectiveness and implementation.

Platform: Sex-Dependent Differences in Pain and Sleep in a Porcine Model of Neurofibromatosis Type 1

Monday, September 23, 10:20am – 10:40am

Rajesh Khanna, *Department of Pharmacology, College of Medicine, University of Arizona*

Neurofibromatosis Type 1 (NF1) is an autosomal dominant genetic disorder resulting from germline mutations in the *NF1* gene, which encodes neurofibromin. Patients experience a variety of symptoms, but pain in the context of NF1 remains largely under-recognized. Here, we characterize nociceptive signaling and pain behaviors in a miniswine harboring a disruptive *NF1* mutation (exon 42 deletion). We present the first characterization of pain-related behaviors in a pig model of NF1, identifying unchanged agitation scores, lower tactile thresholds (allodynia), and decreased response latencies to thermal laser stimulation (hyperalgesia) in *NF1*$^{+/ex42del}$ (females only) pigs. Male *NF1*$^{+/ex42del}$ pigs with tumors showed reduced sleep quality and increased resting, two health-related quality of life symptoms found to be comorbid in people with NF1 pain. We explore these phenotypes in relationship to suppression of the increased activity of the N-type voltage-gated calcium (CaV2.2) channel by pharmacological antagonism of phosphorylation of a regulatory protein – the collapsin response mediator protein 2 (CRMP2), a known interactor of neurofibromin, and by targeting the interface between the α subunit of CaV2.2 and the accessory β-subunits with small molecules. Our data supports the use of *NF1*$^{+/ex42del}$ pigs as a large animal model for studying NF1-associated pain and for understanding the pathophysiology of NF1. Our findings demonstrate the translational potential of two small molecules in reversing ion channel remodeling seen in NF1. Interfering with CaV2.2, a clinically validated target for pain management, might also be a promising therapeutic strategy for NF1-related pain management.

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Platform: Schwann Cells with *LZTR1* and *SMARCB1* Mutations Have Different Secretomes That Influence Pain

Monday, September 23, 10:40am – 11:00am

Fatima Banine, *Oregon Health and Science University*

Schwannomatosis is characterized by multiple peripheral nerve tumors and intractable pain. In Schwannomatosis, mutation in the *NF2* gene, which encodes the merlin tumor suppressor protein, is accompanied by mutations in the SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 gene *(SMARCB1)* or by a mutation in the Leucine zipper like transcription regulator 1 gene *(LZTR1)*. Patients with *LZRT1* mutations may have more severe or different patterns of pain than patients with *SMARCB1* mutations. We found that both mouse Schwann cells and human schwannoma cells with *SMARCB1* mutations secrete elevated levels of several proteins including IL6 and CCL2 compared to wild type Schwann cells. Deletion of *SMARCB1* in Schwann cells up-regulates the pain mediators *Trpv1, TrpA1 and CGRP in vivo* in dorsal root and trigeminal ganglia, and the mice demonstrate increased sensitivity to capsaicin. Furthermore, conditioned media from *SMARCB1* mutant Schwann cell cultures, recombinant IL6 and recombinant CCL2 all up-regulate TrpV1 in wild type sensory neurons *in vitro*. Conditioned media from *LZRT1* mutant Schwann cells also up-regulates Trpv1 *in vitro*. However, unlike conditioned media from *SMARCB1* mutant Schwann cells, IL6 and CCL2 are not elevated. These studies indicate that Schwann cells lacking SMARCB1 or LZTR1 have distinct secretomes that can both influence pain phenotypes but through different mechanisms.

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SESSION 6: Metabolic Rewiring in NF2 Mutant Tumors

Chair: Filippo Giancotti, MD, PhD, MD Anderson Cancer Center

The Hippo Pathway in Cell Growth and Cancer

Monday, September 23, 11:00am – 11:20am

Kun-Liang Guan, PhD, *Department of Pharmacology and Moores Cancer Center, UCSD, La Jolla*

The Hippo pathway is crucial in organ size control and its dysregulation contributes to tumorigenesis. Core components of the Hippo pathway include the protein kinases of MST1/2, MAP4Ks, LATS1/2, the transcription co-activators YAP/TAZ and their DNA binding partners TEADs. LATS phosphorylates YAP/TAZ to promote cytoplasmic localization and degradation, thereby inhibiting YAP/TAZ and cell growth. The Hippo pathway is regulated by a wide range of signals, including cell density, GPCR, cellular energy levels, and mechanical cues. We will present our recent progresses on Hippo pathway regulation and its role in cancer. The emerging role of the Hippo pathway in tumorigenesis suggests potential therapeutic value of targeting this pathway for cancer treatment.

Ferroptosis, Mechanisms and Role in Disease

Monday, September 23, 11:20am – 11:40am

Xuejun Jiang, PhD, *Memorial Sloan Kettering Cancer Center*

Programmed cell death (PCD) plays important role in normal biology, and its deregulation contributes to the development of various diseases. In recent years, multiple forms of PCD that are distinctive from the canonical PCD mechanism – apoptosis – have been discovered. Among these non-apoptotic mechanisms, ferroptosis is an iron-dependent (thus the name) modality of PCD. Execution of ferroptosis involves an imbalance of cellular redox coupled with strong cellular metabolism, leading to a wave of iron-dependent cellular lipid peroxidation, and ultimate cell death. In this talk, our recent findings on the intimate communication of cellular metabolism and signaling with ferroptosis, particularly the role of NF2-Hippo-YAP signaling in the regulation of ferroptosis, will be discussed. The implication of ferroptosis in cancer and ischemic heart disease, and the potential of targeting ferroptosis for disease treatment, will also be discussed.

Harnessing Yap/Taz Signaling Network for Treatment of NF2 Tumors

Monday, September 23, 11:40am – 12:00pm

Chunling Yi, PhD, *Georgetown University*

Loss of NF2 causes a cascade molecular and cellular rewiring that contributes to NF2 tumorigenesis. We have examined the crosstalk and redundancies between the different branches of the signaling network downstream of NF2, which revealed novel synergistic combinations that we are currently exploring for treatment of NF2.

SESSION 7A (CONCURRENT): Cutaneous Neurofibromas – Bench to Bedside

Chairs: Jaishri Blakeley, MD, Johns Hopkins University Hospital; Matthew Steensma, MD, Van Andel Research Institute; Eduard Serra, PhD, The Institute for Health Science Research German Trias i Pujol

The Biology of Cutaneous Neurofibroma: Current Understanding and Future Perspectives

Monday, September 23, 1:00pm – 1:25pm

Lu Q. Le, MD, PhD, *Professor of Dermatology, Simmons Comprehensive Cancer Center, Hamon Center for Regenerative Science and Medicine, University of Texas Southwestern Medical Center*

Cutaneous neurofibromas (cNF) are multicellular tumors within the skin that affect most adults with Neurofibromatosis Type 1 (NF1). They are a major source of emotional and social distress as well as chronic physical symptoms. Therefore, individuals with NF1 often identify these tumors as their greatest burden within this complex syndrome. To date, there is no available medical treatment for cNF, no known way to prevent them from developing and the only current treatment are surgical removal, which is not possible for those with thousands of these tumors. The complexity of cNF biology stems from its heterogeneity at multiple levels including genetic, spatial involvement, temporal development, and cellular compositions. Despite multiple gaps remain in our knowledge of the associated pathogenesis, significant inroads have been made into cNF understanding in recent years, including its developmental origin and natural history. This knowledge combines with translational relevant models currently being developed will provide unique opportunities to unravel its biology to accelerate the development of prevention and treatment strategies for cNF. This talk will focus on our current understanding of cutaneous neurofibroma biology and highlight future goals and challenges.

Cutaneous Neurofibromas: Present, Future

Monday, September 23, 1:25pm – 1:50pm

Pierre Wolkenstein, MD, PhD, *Dept of Dermatology, Henri-Mondor Hospital, Assistance Publique – Hôpitaux de Paris, University Paris East*

Cutaneous neurofibromas constitute one of the main burdens for patients with neurofibromatosis 1. In this talk we will focus on their clinical presentation and their management at the present time. We will develop on current clinical research initiatives and lessons of translational research for future clinical trials.

The viewpoint of patients and their hope for treatments will be presented, the results of classical methods of destruction as well as new tools for their evaluation, report of a case of off label treatment will be shown.

We will conclude by a proposed strategy for ending cutaneous neurofibromas.

Platform: NF1 Patient Missense Variants Predict a Role for *ATM* in Modifying Neurofibroma Initiation

Monday, September 23, 1:50pm – 2:05pm

Yanan Yu, *Cincinnati Children's Hospital Medical Center; Graduate Program in Cancer and Cell Biology, University of Cincinnati, Cincinnati, OH*

In Neurofibromatosis type 1, *NF1* gene mutations characterize benign plexiform neurofibroma (PNF) Schwann cells (SC), and no other genomic changes have been identified that explain patient-to-patient variability in tumor numbers. Evidence from twin studies suggests that NF1 variable expressivity is caused by unidentified modifier genes (Sabbagh et al., *Hum Mol Genet.,* 2009). Whole exome sequencing data from SC and fibroblast DNA from the same resected PNFs confirmed SC biallelic *NF1* mutations, and that non-*NF1* somatic SC variants are variable, and present at low read number. We identified overrepresented germline variants, each present at low minor allele frequency in the general population, and many predicted as deleterious to protein function. Variants in these genes, including *OBSCN, PKHDL1, CUBN, CELSR2, COL14A1* and *ATM,* were also present in two additional, published, cohorts of NF1 patients (Gosline et al., *Sci Data.* 2017; Pemov et al., *Oncogene,* 2017). Many of these genes also showed decreased gene expression in neurofibromas versus human Schwann cells or nerves. Validating the relevance of identified variants, re-sequencing of tumor DNA confirmed *ATM* mutations. Also, *ATM*-relevant DNA repair defects were increased in the subset of neurofibromas with *ATM* variants, and in a subset of neurofibroma SC preparations and unselected tumors. In *Nf1-/-* mouse cells, genetic *Atm* loss promoted Schwann cell precursor self-renewal and increased tumor formation in Schwann cell precursor allografts, suggesting that reduced *ATM* expression can contribute to neurofibroma initiation. This was confirmed by intercrosses of *ATM* heterozygous mice with *DhhCre; Nf1fl/fl* neurofibroma-forming mice. Double mutants showed significantly increased numbers of plexiform neurofibromas. We conclude that *ATM,* and possibly other identified genes that show germline variants, are overrepresented in NF1 patients with neurofibromas, and are candidate modifiers of PNF pathogenesis.

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Platform: The Role of MicroRNA-155 in *Nf1* Neurofibroma Initiation

Monday, September 23, 2:05pm – 2:20pm

Jianqiang Wu, MD, *Division of Experimental Hematology and Cancer Biology, Cancer & Blood Diseases Institute, Cincinnati Children's Hospital Medical Center, University of Cincinnati, College of Medicine, Cincinnati, OH*

MicroRNAs (miRs) are small non-coding, endogenous, single-stranded RNAs that regulate gene expression mainly through post-transcriptional mechanisms. MiRs can have a large impact on many pathways, including pathways related to solid tumors. Several previous studies showed affected expression of miRs in dermal neurofibromas and malignant peripheral nerve sheath tumors. To study the role of miRs in plexiform neurofibroma formation, miR-microarray was performed in human plexiform neurofibroma Schwann cells (SCs) and normal human Schwann cells. Analysis of the miR-microarray data showed abnormal miR expression and differences in expression levels were confirmed by qRT-PCRs in mouse neurofibromas compared to normal mouse SCs. Among these miRs, miR-155 was identified as one of the highest overexpressed miRs. MiR-155 has not been studied in neurofibromatosis type 1 (NF1) related neurofibromas, the benign SC tumors that develop due to loss of *Nf1* function. In a model of tumor initiating cells, pharmacological and genetic inhibition of miR-155 decreased neurofibroma sphere numbers *in vitro.* In contrast, gain of miR-155 function increased cell proliferation in mature *Nf1-/-* mouse SCs compared to scramble controls. Global deletion of miR-155 in the *Nf1fl/fl;DhhCre* neurofibroma mouse model increased mouse survival and decreased tumor number and volume. We are testing nanoparticle encoded peptide nucleic acids (PNAs) treatment effects *in vivo*. These results suggest that miR-155 plays a role in tumor initiation and/or SC proliferation, and that targeting miR-155 might provide a novel therapy.

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<u>Platform</u>: Systematic Longitudinal Evaluation of Phenotype Development in a Swine Model of Neurofibromatosis 1

Monday, September 23, 2:20pm – 2:35pm

Katherine A White, BS, *Pediatric and Rare Disease Group, Sanford Research, Sioux Falls, SD*

As neurofibromatosis type 1 (NF1) is a heterogeneous disease with variable clinical presentation, large animal models of the disease are crucial to dissect molecular mechanisms, determine relevant biomarkers, and develop effective therapeutics. Recently we characterized a novel porcine model of NF1 with a heterozygous mutation in exon 42, replicating a common mutation from human patients. Here, we expand on this initial characterization by longitudinally monitoring, with non-invasive medical imaging, the first generation of naturally bred $NF1^{+/ex42del}$ animals along with wild type (WT) littermate control animals. Full body computed tomography (CT) scan at 4 months of age revealed evidence of short stature via significantly smaller long bones in $NF1^{+/ex42del}$ animals (femur, tibia, humerus, metacarpal), compared to WT controls. A large diffuse cutaneous neurofibroma was detected by CT and magnetic resonance imaging (MRI) on the shoulder of one $NF1^{+/ex42del}$ boar in the study, which subsequently grew in size and depth as the animal aged. Further, targeted MRI with gadolinium contrast showed progressive growth (>25%) and enhanced contrast uptake at each time point in all areas of the neurofibroma as the animal aged, indicating a progressive disease course. Histopathological analysis of neurofibroma samples across multiple regions of interest indicated inhomogeneity in neurofibroma presentation: Including an increase in tumor vascularization, increased infiltration of the tumor into the dermis/subcutis, and maturation of the tumor to a more fibrous appearance in the area of active tumor growth. Taken together, we present the first longitudinal assessment of tumor presentation in a swine model of NF1, providing a powerful tool to the NF1 community for future preclinical work. Additionally, we demonstrate detection of neurofibroma with a lower dose CT, suggesting that lower radiation scans may be a viable option for patients with NF1 in the future.

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Platform: Investigating the Role of *NF1*$^{+/-}$ Fibroblasts in Neurofibromatosis Type I-Associated Cutaneous Neurofibromas

Monday, September 23, 2:35pm – 2:50pm

Sara H. Osum, DVM, *Department of Pediatrics, Masonic Cancer Center, University of Minnesota, Twin Cities*

Individuals with Neurofibromatosis Type I syndrome (NF1) are born with heterozygous loss-of-function mutations in the *NF1* tumor suppressor gene, predisposing them to a variety of nervous system tumors and skin lesions. Cutaneous neurofibromas (cNF) are the most common tumor found in NF1 patients, presenting around puberty as small superficial growths that can number in the hundreds to thousands. These benign tumors are a major source of morbidity for NF1 patients, as they can cause itching, pain, and a cosmetic burden that has been linked to psychosocial issues. There are currently no approved therapies for cNFs aside from elective surgery, which is unrealistic for patients with thousands of tumors. A number of animal models have been developed to better understand neurofibroma pathogenesis and discover new NF1-targeted therapies. While these have led to important advances in our understanding of NF1 syndrome, there are still significant gaps in our knowledge of cNF pathogenesis, particularly regarding the role of the neurofibroma microenvironment. We have 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. 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. However, a direct functional relationship between haploinsufficient *NF1*$^{+/-}$ fibroblasts and tumor-initiating *NF1*$^{-/-}$ Schwann cells in cNFs has not been thoroughly studied. We hypothesized that fibroblasts may interact directly with Schwann cells through a paracrine signaling feedback mechanism to promote neurofibroma growth and maintenance. We performed RNA sequencing analysis and *in vitro* co-culture assays to determine whether neoplastic *NF1*$^{-/-}$ Schwann cells activate fibroblasts in the neurofibroma microenvironment through a paracrine signaling feedback mechanism. The results of these studies will enable researchers to better understand the complex mechanisms of neurofibroma maintenance and reveal new targets for therapeutic intervention. The NF1 minipig neurofibroma model provides a unique opportunity to investigate the role of the tumor microenvironment on neurofibroma growth and maintenance and could expedite the discovery and translation of new NF1-targeted therapies.

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SESSION 7B (CONCURRENT): Genetics of NF2 and Schwannomatosis

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

Implications of Mosaicism in Neurofibromatosis Type 2

Monday, September 23, 2:00pm – 2:20pm

Dorothy Halliday, FRCP, PhD, *Oxford University, UK*

Neurofibromatosis type 2 (NF2) is caused by both constitutional and mosaic NF2 pathogenic variants and testing using next generation sequencing (NGS) can now detect low levels of mosaicism in blood. Data from the South-West of England and the English NF2 cohort estimate the proportion of affected individuals with mosaic disease to be 57-58%. Severity of NF2 phenotype is reduced if the same pathogenic variant is present in mosaic form, especially if unidentifiable in blood, a feature utilised in the NF2 genetic severity score.

Two particular problems arise from a high rate of mosaicism. Firstly clarifying the diagnosis when genetic testing of lymphocyte DNA is normal; and secondly dealing with the implications of the diagnosis for close relatives where no mutation has been identified.

To assess the impact of mosaicism in NF2 we have assessed referral indications and outcome of all 392 new patients into the Oxford and South-West England NF2 service between April 2010 and April 2019 (excluding referral of established NF2 patients, patients at risk for schwannomatosis and patients referred to the satellite clinic in Plymouth). 38/392 (10%) were for a new diagnosis of NF2; 166/393 (42%) were patients at risk of NF2 due to the diagnosis in a close relative and 184/392 (47%) had potential NF2/schwannomatosis due to one or more clinical features. Of 184 with potential NF2, 20 were diagnosed with NF2 and 25 with schwannomatosis with molecular confirmation in 9 and 4 respectively; 83/184 had likely sporadic lesions but with the possibility remaining of mosaic NF2. For those at risk for NF2, in 71/153 (46%) molecular testing to exclude NF2 was possible, and for 82/153 (54%) clinical screening was offered in the absence of definitive genetic testing.

Within the south west NF2 service 62 patients have NF2 with no mutation present in blood and have in total 95 children, none of which are known to have clinical features of NF2; consistent with recently reported lower offspring risks in this patient cohort. Thus far tumour testing and repeat NF2 testing with NGS has enabled molecular confirmation in 16/62 patients with previously no pathogenic variant identified in blood, with 7/16 reclassified from one category (eg multiple meningiomas/schwannomatosis/NF2) to another; and the option of definitive genetic testing for 19 relatives.

This study has illustrated the high proportion of individuals referred and assessed compared to each new diagnosis of NF2 and the clinical uncertainty posed by the high proportion with mosaic NF2.

Platform: Higher Estimates of Mosaicism in *De Novo* Neurofibromatosis Type 2 Following Increased Use of Next Generation Sequencing

Monday, September 23, 2:20pm – 2:35pm

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

The *NF2* gene has a high *de novo* mutation rate and over 50% of neurofibromatosis 2 (NF2) patients have no clinically affected parent. Previous reports have assessed the frequency of mosaicism in NF2 to be 25-33%. Recently, newer sequencing techniques have improved the sensitivity of detection of NF2 mosaicism.

We assessed the incidence of mosaicism in *de novo* patients who fulfilled NF2 clinical diagnostic criteria, but who did not have a known affected relative from a previous generation. Lymphocyte DNA and, where available, tumour DNA were screened for *NF2* variants in 1055 unrelated *de novo* cases using next generation sequencing. We then surveyed the proportion of individuals with a proven or presumed mosaic *NF2* variant and the allele frequencies of identified variants.

The proportion of proven or presumed mosaicism was 232/1055 (22.0%). However, the proportion of non-mosaic heterozygous pathogenic variants was only 387/1055 (36.7%). When the known rate of mutation detection in second generation non-mosaic individuals was applied to *de novo* cases, we assessed the overall probable mosaicism rate to be 59.7%. This rate differed by age at presentation of bilateral vestibular schwannoma. In cases where a non-mosaic single nucleotide pathogenic *NF2* variant was detected, a mosaic variant was always detected in one of the parents.

Our study has identified a very high probable rate of mosaicism in people presenting with *de novo* NF2. This probably makes NF2 the condition with the highest expressed rate of mosaicism in a *de novo* dominant disease that is non-lethal in heterozygous form. The risk of inheritance to offspring from mosaic individuals is low and probably correlates with germline mutation allele frequency.

Full List of Authors: D Gareth Evans, Claire L Hartley, Philip T Smith, Andrew T King, Naomi L Bowers, Simon Tobi, Andrew J Wallace, Mary Perry, Raji Anup, Simon KW Lloyd, Scott A Rutherford, Charlotte Hammerbeck-Ward, Omar N. Pathmanaban, Emma Stapleton, Simon R Freeman, Mark Kellett, Dorothy Halliday, Allyson Parry, Juliette J Gair, Patrick Axon, Roger Laitt, Owen Thomas, Shazia K Afridi, Rupert Obholzer, The English Specialist NF2 research group, Chris Duff, Stavros M. Stivaros, Grace Vassallo, Elaine F Harkness, Miriam J Smith.

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Platform: A Phase 0 Pharmacodynamic and Pharmacokinetic Study of Everolimus in Vestibular Schwannoma (VS) and Meningioma Patients

Monday, September 23, 2:35pm – 2:50pm

Matthias A. Karajannis, MD, MS, *Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, NY*

Background: Inhibition of mTORC1 signaling has been shown to diminish growth of NF2 deficient tumors in preclinical studies, and clinical data suggest that everolimus, an orally administered mTORC1 inhibitor, may slow tumor progression in a subset of adult and pediatric NF2 patients with VS. To assess the pharmacokinetics, pharmacodynamics and potential mechanisms of treatment resistance, we performed a pre-surgical ("phase 0") clinical trial of everolimus in patients undergoing surgery for VS or meningiomas.

Methods: Eligible patients with meningioma or VS requiring tumor resection received everolimus 10 mg daily for 10 days immediately prior to surgery. Everolimus blood levels were determined immediately prior to and after surgery. Tumor samples were collected intraoperatively.

Results: Ten patients completed protocol therapy, including 5 patients with NF2-related meningioma, 3 patients with sporadic meningioma, and 2 patients with NF2-related VS. Median pre- and post-operative plasma levels of everolimus were found to be in a high therapeutic range (17.4 ng/ml and 9.4 ng/ml, respectively). Median tumor tissue drug concentration determined by mass spectrometry was 24.3 ng/g (range 9.2–169.2), and median tumor tissue to post-operative plasma drug concentration ratio was 0.39. We observed only partial inhibition of phospho-S6 in the treated tumors, indicating incomplete target inhibition compared to matched control tissues from untreated patients ($p = 0.005$). Consistent with prior observations that inhibition of mTORC1 may lead to MAPK pathway activation through a PI3K-dependent feedback loop, we observed a statistically significant increase of phospho-ERK ($p < 0.03$) versus untreated controls.

Conclusions: In patients with meningioma or VS, treatment with everolimus leads to incomplete inhibition of mTORC1 signaling and upregulation phospho-ERK. These data may explain the limited anti-tumor effect of everolimus observed in clinical studies for NF2 patients and identify upregulation of phospho-ERK as a likely resistance mechanism that could be addressed with combination therapies.

Full List of Authors: Shiyang Wang, Judith D. Goldberg, J. Thomas Roland, Chandranath Sen, Dimitris G. Placantonakis, John G. Golfinos, Jeffrey C. Allen, Erin Dunbar, Scott R. Plotkin, Srivandana Akshintala, Robert Schneider, Jingjing Deng, Thomas A. Neubert, Filippo G. Giancotti, David Zagzag, Jaishri O. Blakeley

SESSION 8: Targeted Gene Editing and Gene Therapy for Neurofibromatosis

Chairs: Rajesh Khanna, PhD, University of Arizona; Scott Plotkin, MD, PhD, Massachusetts General Hospital; Aaron Schindeler, PhD, University of Sydney, Australia

Introduction to Gene Therapy

Monday, September 23, 3:30pm – 3:42pm

Aaron Schindeler, PhD, *The Children's Hospital at Westmead, University of Sydney*

The emergence of new and efficient tools for gene editing has major implications for rare diseases research and medicine. CRISPR/Cas9 gene editing is a revolutionary technology, and over the past 7 years it has been improved upon with nickase, base-editing, and RNA-editing Cas9 variants, split-intein Cas9 variants, and homology-independent targeted integration (HITI) methods. Gene editing techniques have expanded the field of functional genomics, but will also enable cell and mouse models of individual patient mutations to be rapidly created. In the longer term, CRISPR-Cas9 has the potential to be curative for a range of genetic conditions, including NF. Critical to implementation are efficient ex vivo and/or in vivo delivery systems. Our team has developed a system for rapid gene editing restricted to the bone compartment, which could be considered a template for tissue-restricted high-efficiency gene editing.

Gene Replacement Therapy for NF2

Monday, September 23, 3:42pm – 3:54pm

Marco Giovannini, MD, PhD, *UCLA Medical School*

Gene therapy for hereditary diseases is becoming a promising new medical treatment, as in gene replacement therapy for immune deficiency diseases, hemophilia and vision loss, as well as in pre-clinical trials for a several neurologic diseases. In the nervous system AAV vectors can provide efficient gene delivery *in vivo* and long-term expression in non-dividing cells with a high degree of safety demonstrated in many clinical trials.

Our goal is to evaluate somatic gene replacement therapy for NF2. Overall, these studies serve as a proof of principle to support the notion that *NF2* re-expression in deficient schwannoma and meningioma cells can provide preclinical efficacy. Promising aspects of this approach are that it is effective over an extended period of months after a single injection, and that AAV vectors are proving beneficial without adverse effects in clinical trials for a number of human diseases. Given the limitations of surgery and radiotherapy to control these tumors, and the need for continuous treatment with drugs used to control schwannoma and meningioma growth, somatic gene replacement therapy might offer an additional tool for the clinical management of NF2, possibly changing its therapeutic landscape.

Genome-Guided Therapeutics for NF1

Monday, September 23, 3:54pm – 4:06pm

Bruce Korf, MD, PhD, *University of Alabama at Birmingham*

In spite of dramatic success in treating plexiform neurofibromas with inhibitors of Ras-mediated signaling, especially MEK inhibitors, not all patients respond and tumors do not completely disappear in those who do respond. In an effort to find additional therapeutic targets, our group has been looking for ways to restore function to the mutated gene or gene product. Given the wide diversity of pathogenic variants found in NF1, no single approach will work for all affected individuals. Our major current efforts are focused on stop mutation readthrough approaches, exon skipping, RNA editing, and CRISPR/Cas9 genome editing. We have developed a number of model systems to facilitate these studies, including customized mouse and rat models with human NF1 pathogenic variants, iPS cell lines, and a cDNA test system. Using these systems, a number of patient-specific mutations have been developed and are being used in testing candidate therapeutic approaches.

Dissecting Pain in NF1

Monday, September 23, 4:06pm – 4:18pm

Rajesh Khanna, PhD, *University of Arizona*

Neurofibromatosis type 1 (NF1) is a rare autosomal dominant disease characterized by tumors in the nervous system, cognitive disorders and by chronic idiopathic pain. Between 29% to upwards of 70% of NF1 patients report pain as a significant detractor of their quality of life. ~70% of NF1 patients take pain medications. Whether NF1 pain is different from normal pain is not known. The majority of NF1 pain is caused by spontaneous tumor generation on the nerves (i.e. malignant peripheral nerve sheath tumors; MPNSTs) inducing a neuropathy. In fact, it has become clear that increasingly severe pain should be heeded as a possible symptom of MPNSTs. To date, no disease-specific therapy is available to prevent or attenuate neuropathic pain in NF1. In preliminary experiments, we found that alterations in collapsin response mediator protein 2 (CRMP2), a protein implicated in axon guidance and regulation of neurite outgrowth, regulates pain. A mechanistic link between CRMP2 and NF1-related pain has never been investigated. CRISPR/Cas9 editing of $Nf1$ in sensory neurons leads to upregulation of voltage-gated Na$^+$ (NaV1.7) and Ca^{2+} (CaV2.2) channels, both of which are key in pain signal transmission. The increased activities of these channels augmented sensory neuron excitability and release of excitatory transmitters to the spinal dorsal horn to establish and maintain a state of central sensitization reflected by hyperalgesia (heightened sensitivity to pain). Remarkably, NF1-related pain in rats with MPNSTs (allodynia, painful response to an innocuous stimulus) was resistant to morphine. Overall, the literature and our preliminary data strongly suggest that decreased expression of neurofibromin, a protein encoded by the $Nf1$ gene, promotes opioid-resistant chronic pain. Our research asks two questions: (1) what is the nature of pain resulting from MPNST burden in the context of NF1? and (2) Can we target CRMP2 to reverse ion channel dysregulation and pain in the context of NF1?

Proactive Engagement of Stakeholders in Developing Clinical Trials for Gene Therapy in NF Patients

Monday, September 23, 4:18pm – 4:30pm

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

TBD

SESSION 9A (CONCURRENT): Basic Science Platform Presentations

Chairs: David Gutmann, MD, PhD, Washington University, St. Louis; Laura Papi, MD, University of Florence, Italy; Dawn Quelle, PhD, University of Iowa

Neurofibromatosis Type 1: A Sweet Story of Metabolism

Monday, September 23, 5:15pm – 5:30pm

Rebekah Tritz, MS, *Vascular Biology Center, Augusta University, Augusta, GA*

Neurofibromatosis type 1 (NF1) is an inherited autosomal dominant syndrome with a prevalence of 1 in 3000 persons. Manifestations of NF1 are varied in type, severity, and timing; however, persons with NF1 display distinct anthropometric and metabolic features including shorter stature, low BMI, and decreased risk for hyperglycemia and diabetes. The mouse and human form of neurofibromin, as well as its promoter sequences, have a 98% sequence similarity, suggesting that the biochemical and transcriptional functions are conserved. Based on this strong homology, we sought to identify whether a common Nf1-mutant murine line (Nf1+/-; Tyler Jacks MIT) recapitulated the anthropomorphic and metabolic phenotypes observed in persons with NF1. Male C57Blk6 and Nf1+/- mice were subjected to anthropometrics, indirect calorimetry, and glucose/insulin tolerance testing at 16 weeks. Nf1+/- mice recapitulated many of the characteristics observed in persons with NF1 including reduced fat, but increased lean body mass, lower fasting blood sugar and increased sensitivity to insulin. Compared to WT mice, Nf1+/- mice exhibited a diminished capacity to increase serum glucose concentrations in response to insulin as well as timed fasting. Therefore, we subjected WT and Nf1+/- mice to a diet including 30% glucose dissolved in drinking water for 16 weeks. In response to the high-glucose diet, Nf1+/- mice experienced more modest weight gain when compared with WT littermates, and Nf1+/- mice were completely protected from diet-induced hyperglycemia. Based on these observations, we used RNAi to knockdown neurofibromin expression in circulating human endothelial cells (EC). Glucose uptake and glycolysis were enhanced in neurofibromin-deficient EC. Surprisingly, Mek-Erk inhibition failed to blunt glycolysis in neurofibromin-deficient EC, which was effectively suppressed when EC were pre-incubated with wortmannin to block PI3K-Akt signaling. Glut 1, but not Glut 4 expression, was enhanced in neurofibromin-deficient EC via an Akt-dependent mechanism suggesting that constitutive neurofibromin-PI3K-Akt activation is principally responsible for the metabolic features associated with Nf1 mutations. Taken together, our data is the first to demonstrate that Nf1+/- mice mirror the metabolic features observed in persons with NF1 and identify a plausible mechanism for this phenotype. Further, our findings are relevant for understanding NF1-specific disease pathogenesis and inform novel therapy design as both are likely dependent on glucose metabolism.

MEK Inhibition Ameliorates Social Behaviour Phenotypes in a Spred1 Knockout Mouse Model for RASopathy Disorders

Monday, September 23, 5:30pm – 5:45pm

Sarah C. Borrie, *Department of Human Genetics, KU Leuven, Leuven, Belgium*

RASopathies are neuro-cardio-facio-cutaneous disorders stemming from mutations in genes regulating the RAS-MAPK pathway. Legius syndrome is a rare RASopathy disorder caused by mutations in the *SPRED1* gene. SPRED1 protein negatively regulates activation of Ras by inhibiting RAS/RAF and by its interaction with neurofibromin, a RAS-GAP protein. Incidentally, the phenotype of Legius syndrome is very similar to that of Neurofibromatosis type 1, another RASopathy caused by mutations in *NF1* gene that encodes neurofibromin. Common to both disorders are cognitive deficits and increased incidence of autism spectrum disorder (ASD). Mouse models for RASopathies exhibit cognitive deficits consistent with human phenotypes, but it is not known whether they also recapitulate ASD-like symptoms.

Here we examined autism-linked behaviours in *Spred1*-/- mice as a model for Legius syndrome, and probed the mechanisms underlying the behavioural phenotypes. *Spred1*-/- mice have deficits in social dominance in the automated tube test, and social communication deficits in their ultrasonic vocalisations, seen both in early postnatal stage and in adult mice. Assays of novelty investigation behaviours showed that *Spred1*-/- mice exhibit reduced nesting behaviour, marble burying and investigation of a novel object. Additionally, associative learning in an operant touchscreen battery was impaired in *Spred1*-/- mice. Targeting the RAS-MAPK pathway by treating adult mice with the specific MEK inhibitor PD325901 could reverse the deficits in social dominance and novelty investigation behaviours, but could not rescue the cognitive impairments. Together these findings demonstrate the specificity of dysregulation of Ras-MAPK pathway activity in mediating ASD-like social behaviour and novelty investigation deficits in *Spred1*-/- mice, and indicate the importance of correct regulation of the Ras-MAPK signalling for the maintenance of social behaviours.

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Macrophage Macropinocytosis: A Potential Target to Attenuate Tumor Vascularization in Neurofibromatosis Type 1

Monday, September 23, 5:45pm – 6:00pm

Pushpankur Ghoshal, PhD, *Vascular Biology Center, Augusta University, Augusta, Georgia*

Background: Neurofibromatosis type 1 (NF1) is a common genetic disorder characterized by a predisposition to multiple types of cancer. Somatic mutations of the NF1 gene are reported in 27.7% of all breast carcinomas and have been implicated as potential genomic drivers in the development of breast cancer. Despite the relatively high prevalence of breast cancers in NF1 patients, effective pharmacological therapies are not available largely due to a poor understanding of disease pathogenesis. This study was designed to investigate whether loss of NF1 in macrophages stimulates internalization of breast cancer cell-derived exosomes via macropinocytosis, leading to their pro-angiogenic differentiation and tumor vascularization.

Methods: Bone marrow-derived macrophages from LysM Cre[+]/Nf1[f/f] and LysM Cre[−]/Nf1[f/f] mice were treated with exosomes derived from the mouse breast cancer cell line 4T1 in the absence and presence of macropinocytosis inhibitor EIPA. The expression of pro-angiogenic receptors on macrophages was evaluated by flow cytometry. The Matrigel Plug assay and confocal microscopy were used to determine the role of macropinocytosis in macrophage differentiation to pro-angiogenic cells and angiogenesis *in vivo.*

Results: NF1 deficiency in macrophages stimulates actin polymerization via RAS/PI3K-mediated phosphorylation of $PI(4,5)_2$, leading to plasma membrane ruffling and macropinocytosis. NF1-deficient macrophages internalize breast cancer cell-derived exosomes via macropinocytosis and differentiate into pro-angiogenic cells *in vitro.* The pro-angiogenic differentiation of NF1-deficient macrophages is demonstrated by their increased expression of endothelial cell markers, such as Tie2, VEGFR2 and VE cadherin compared with no exosome-treated and EIPA + exosome-treated controls. The *in vivo* matrigel plug assay demonstrated increased exosome-stimulated angiogenesis by NF1-deficient macrophages, which was inhibited by EIPA treatment. Delving further into the mechanism, we found that GATA3, a well characterized transcription factor mediating Tie2 expression, was transported by exosomes into NF1-deficient macrophages.

Conclusion: The findings of the present study identify macropinocytosis as a new target to mitigate macrophage differentiation into pro-angiogenic cells and inhibit tumor angiogenesis in NF1 patients.

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Neurofibromin Also Has a GAP-Independent Activity, Acting as a Transcriptional Co-Repressor for Estrogen Receptor-Alpha

Monday, September 23, 6:00pm – 6:15pm

Eric C. Chang, PhD, *Baylor College of Medicine*

Germline inactivation of neurofibromin is responsible for neurofibromatosis, and patients suffering from this disease also have greater propensity for cancer, including breast cancer. Neurofibromin is also frequently somatically inactivated in cancer. It is well established that neurofibromin loss promotes Ras activity because it is a Ras GAP (GTPase Activating Protein). However, in a recent tumor DNA sequencing study, we reported that nonsense/frameshift *NF1* mutations strongly correlate with death/relapse after tamoxifen treatment in estrogen receptor-alpha positive (ER$^+$) breast cancer, even though no *NF1* missense mutations inactivating the GAP activity were found in such tumors. We later determined that most of the *NF1* nonsense/frameshift mutations create an NF1-null state, presumably by the nonsense mRNA decay pathway. Thus, only when the entire neurofibromin protein is lost will tamoxifen resistance occur, which suggested that besides the GAP activity, neurofibromin has other activities that are also required for promoting more aggressive tumor behavior.

Here we report that neurofibromin behaves like an authentic ER transcriptional co-repressor, containing leucine/isoleucine-rich co-repressor motifs that mediate ER binding. The binding between neurofibromin and ER is direct, and neurofibromin nuclear localization and association with ER at the estrogen-responsive elements are stimulated by tamoxifen but not by estradiol. Mutating the NF1 GAP domain does not impact ER binding, but when NF1's co-repressor motifs are mutated, its ER binding is lost without affecting GAP activity. Consequently, depletion of neurofibromin in ER$^+$ breast cancer cells releases repression of ER, causing estradiol hypersensitivity and tamoxifen agonism, and explaining the poor prognosis associated with neurofibromin loss in tamoxifen-treated ER$^+$ breast cancer. Preclinical modeling indicates that neurofibromin-deficient ER$^+$ breast tumors do retain sensitivity to selective estrogen receptor degraders (SERDs) such as fulvestrant, and that added targeting of Ras-effector kinases (e.g., MEK) suppresses SERD resistance when it develops and induces tumor regression.

Thus, neurofibromin is a dual repressor for both Ras and ER signaling, and both pathways must be properly co-targeted to efficiently treat neurofibromin-deficient ER$^+$ tumors. In ER$^+$ breast tumors, the finding of neurofibromin depletion identifies a distinct subset where the choice of endocrine treatment may be critical, and where MEK inhibitor combinations require clinical investigation. ER de-repression through neurofibromin loss may also explain the sexually dimorphic characteristics of neurofibromatosis, where tumorigenesis is promoted by female puberty.

Full List of Authors: Ze-Yi Zheng, Meenakshi Anurag, Jonathan T. Lei, Jin Cao, Purba Singh, Jianheng Peng, Nhu-Chau Nguyen, Philip Lavere, Yue Chen, Jing Li, Xin-Hui Du, Burcu Cakar, Hilda Kennedy, Wei Song, Beom-Jun Kim, Jiejun Shi, Sinem Seker, Doug W. Chan, Guo-Qiang Zhao, Xi Chen, Kimberly C. Banks, Richard B. Lanman, Maryam Nemati Shafaee, Xiang H.-F. Zhang, Suhas Vasaikar, Bing Zhang, Susan G. Hilsenbeck, Wei Li, Charles E. Foulds, Matthew J. Ellis

Screening for ASD Therapies Using a *Drosophila* Model of NF1

Monday, September 23, 6:15pm – 6:30pm

Alex Dyson, MSci, *University of Manchester, UK*

Background: Amongst children with neurofibromatosis type 1 (NF1), there is a substantially higher prevalence of autism spectrum disorder (ASD) compared to the general population. ASD is a neurodevelopmental disorder characterised by social and communication deficits, restricted interests, and repetitive behaviours. There are currently no FDA-approved therapies that target this core ASD symptomatology. A functionally conserved ortholog of *NF1* is present in the fruit fly, *Drosophila melanogaster,* deletion of which results in behavioural and cellular impairments reflecting those seen in human NF1 and ASD. We aim to develop a targeted screen for compounds that attenuate these phenotypes in a *Drosophila* model of NF1, in order to identify potential therapies for the treatment, or even prevention, of ASD in humans.

Methods: Grooming behaviour was characterised via the *Drosophila* Activity Monitor 5 (DAM5) from TriKinetics Inc. (Waltham, MA). Social interaction was examined by calculating inter-fly distance, using an assay adapted from Simon et al. (2012). To study synaptic transmission, we recorded excitatory junction potentials (EJPs) and mini EJPs (mEJPs) from the neuromuscular junction (NMJ) of third instar *Drosophila* larvae, a well-characterised glutamatergic synapse.

Results: We confirm previous findings (King et al. 2016) that $NF1^{-/-}$ flies exhibit increased grooming behaviour, which may correspond to the repetitive behaviours seen in children with ASD. Moreover, we report the novel observation that $NF1^{-/-}$ flies demonstrate an increased inter-fly distance, reflecting the social impairments present in ASD and NF1 individuals. We also replicate work (Tsai et al. 2012) showing an increased EJP, but not mEJP, amplitude at the $NF1^{-/-}$ larval NMJ, indicating enhanced glutamatergic transmission due to a pre-synaptic deficit. Excessive glutamatergic transmission has also been associated with ASD. Lastly, while attempting to identify a positive control for our screening assays, we found that methylphenidate hydrochloride, a dopamine reuptake inhibitor, reduced excessive grooming in $NF1^{-/-}$ flies when administered throughout development.

Conclusions: The simplicity of the DAM5 system for activity monitoring, together with the strength of the increased grooming phenotype in $NF1^{-/-}$ flies, render this assay appropriate for a targeted drug screen to identify ASD therapies. The assays demonstrating aberrant social interaction and altered synaptic transmission, respectively, will provide follow up screens to validate hit compounds for their ability to ameliorate ASD symptomatology. Supporting the translatability of our work, methylphenidate rescued increased grooming behaviour, and there is evidence that dopamine pathway disruption contributes to repetitive behaviours in ASD.

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Modelling the Development of NF1-Associated Malignant Peripheral Nerve Sheath Tumors Using Induced Human Pluripotent Stem Cells and Targeted Temporal Transforming Mutations

Monday, September 23, 6:30pm – 6:45pm

Garrett Draper, *University of Minnesota*

The genetic tumor predisposition syndrome Neurofibromatosis type 1 (NF1) results from the inheritance of a mutated copy of *NF1,* a Ras-GAP tumor suppressor gene. Subsequent loss of the remaining wild-type allele in Schwann lineage cells leads to hyperactive Ras signaling, cell proliferation, and the formation of benign plexiform neurofibromas (PNFs). These PNFs can progress through additional mutations in canonical tumor suppressor genes *TP53* and *CDKN2A* to develop into atypical PNFs. Furthermore, loss of function mutations in *SUZ12* which encodes a subunit of the Polycomb Repressive Complex 2 (PRC2) has been strongly implicated in the progression of atypical PNFs to lethal malignant peripheral nerve sheath tumors (MPNSTs). Although these and other mutations have been associated with MPNST formation, their temporal dependence during Schwann cell development using a human cell model has not been studied. To model the effects of these genetic mutations in MPNST development, we utilized induced human pluripotent stem cells (iPSCs) and specific differentiation protocols to obtain cells with a mature Schwann cell identity. Cells were subjected to targeted mutations at different stages of development. This model of induced MPNSTs (iMPNSTs) will allow for a better understanding of the timing at which these mutations occur during Schwann cell development to result in MPNSTs. Also, the results of these studies could help uncover additional gene mutations and pathways that are necessary or sufficient for the transformation of benign PNFs to MPNSTs and improve the effectiveness of future therapeutics.

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Conflict of Interest Statement: Dr. Largaespada is the co-founder and co-owner of several biotechnology companies including NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. (recently acquired by Biotechne Corporation) He consults for Genentech, Inc., which is funding some of his research. Dr. Largaespada holds equity in and serves as the Chief Scientific Officer of Surrogen, a subsidiary of Recombinetics, a genome-editing company. The business of all these companies is unrelated to the contents of this manuscript. Other authors have no conflict of interest to disclose.

Proximity Biotinylation Identifies a Set of Conformation Specific Interactions Between Merlin and Cell Junction Proteins

Monday, September 23, 6:45pm – 7:00pm

Robert Hennigan, PhD, *Cincinnati Children's Hospital*

Neurofibromatosis type 2 is an inherited neoplastic disease associated with schwannomas, meningiomas, and ependymomas that is caused by inactivation of the tumor suppressor gene *NF2.* The *NF2* gene product, Merlin, has no intrinsic catalytic activity; its tumor suppressor function is mediated through the proteins with which it interacts. We used proximity biotinylation followed by mass spectrometry and direct binding assays to identify proteins that associate with wild-type and various mutant forms of Merlin in immortalized Schwann cells. We defined a set of 52 proteins in close proximity to wild-type Merlin. The majority of Merlin-proximal proteins were components of cell junctional signaling complexes, suggesting that additional potential interaction partners may exist in adherens junctions, tight junctions, and focal adhesions. By using mutant forms of Merlin that cannot bind to phosphatidylinositol 4,5-bisphosphate (PIP$_2$) or constitutively adopt a closed conformation, we confirmed a critical role for PIP$_2$ binding in Merlin function and identified a large cohort of proteins that specifically interacted with Merlin in the closed conformation. Among these proteins, we identified a previously unreported Merlin binding protein, apoptosis-stimulated protein of p53-2 (ASPP2, also called Tp53bp2), that binds to closed-conformation Merlin predominately through the FERM domain. Our results demonstrate that Merlin is a component of cell junctional mechanosensing complexes and defines a specific set of proteins through which it acts.

Targeting Protein Translation with Rocaglamide and Didesmethylrocaglamide to Treat NF1 and NF2 Tumors

Monday, September 23, 7:00pm – 7:15pm

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

To sustain uncontrolled growth, cancer cells exhibit enhanced protein synthesis by upregulation of the protein translation machinery. Previously we reported that NF1-associated malignant peripheral nerve sheath tumors (MPNSTs) and NF2-related vestibular schwannomas and meningiomas exhibit elevated expression of eIF4A, eIF4E, and eIF4G, components of the eIF4F translation initiation complex, and that the eIF4A inhibitor silvestrol potently suppresses the growth of these tumor cells. Studies by others also show strong anti-tumor activity of silvestrol in several other cancer models, suggesting that it may be a potential cancer treatment. However, silvestrol has suboptimal drug-like properties, including a bulky structure, sensitivity to inhibition by the MDR1 multidrug-resistant transporter, and poor oral bioavailability. By screening 10 silvestrol-related rocaglates lacking the dioxanyl ring, we identified rocaglamide (Roc) and didesmethylrocaglamide (DDR) with potent growth-inhibitory activity comparable to silvestrol in MPNST cells. Structure-activity relationship analysis revealed that the dioxanyl ring present in silvestrol was dispensable for, but may enhance, cytotoxicity. Both Roc and DDR arrested MPNST cells at G2/M, significantly increased the sub-G1 fraction, and induced cleavage of caspases 3 and 7 and poly(ADP-ribose) polymerase, while decreasing total protein levels of these apoptotic markers and mitogenic kinases AKT and ERK1/2, consistent with translation inhibition. Additionally, these rocaglamides elevated the levels of γH2AX, a marker of the DNA damage response. Unlike silvestrol, Roc and DDR were not sensitive to MDR1 inhibition. Pharmacokinetic analysis confirmed that Roc had 50% oral bioavailability. Importantly, Roc, when administered intraperitoneally or orally, potently suppressed the growth of orthotopic NF1-deficient MPNST xenografts with no overt toxicity. Treated MPNSTs had abundant phospho-histone H3 labeling and more cleaved caspase 3-positive cells, consistent with G2/M arrest and indicative of increased apoptosis, respectively. In addition, Roc exhibited anti-tumor effects in patient-derived xenograft models for several types of sarcomas, including Ewing sarcoma, osteosarcoma, and rhabdomyosarcoma. Western blot analysis revealed that Roc and DDR decreased multiple oncogenic kinases, including IGF-1R, in sarcoma cells. Further, these rocaglamides potently inhibited proliferation of NF2-deficient schwannoma and meningioma cells. The more favorable drug-like properties and potent anti-tumor effects of Roc and DDR suggest that these rocaglamides have potential to become viable treatments for NF1- and NF2-associated tumors, including MPNST, as well as other sarcomas.

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SESSION 9B (CONCURRENT): Clinical Science Platform Presentations

Chairs: Justin Jordan, MD, PhD, Massachusetts General Hospital; Kaleb Yohay, MD, NYU Langone Medical Center; Sirkku Peltonen, MD, University of Turku, Finland

Prediction of Hearing Prognosis in NF2 Related Vestibular Schwannoma by Genetic Analysis

Monday, September 23, 5:15pm – 5:30pm

Yu Teranishi, MD, *Department of Neurosurgery, Faculty of Medicine, The University of Tokyo, Japan*

Introduction: Regardless of optimal treatment, hearing worsening due to neurofibromatosis type 2 (NF2) related vestibular schwannoma (VS) is irreversible. Aiming to improve their care and establish individual hearing prognosis the earliest possible, we performed a retrospective analysis of hearing outcomes due to NF2 related VS focusing on long term hearing prognosis and prediction of hearing outcome.

Methods: We analyzed germline DNA from a cohort of 48 patients by Sanger sequencing and multiple ligation-dependent probe amplification, reviewing their full clinical and imaging data follow-up of 15.0 ± 6.6 years. Their hearing outcome was reviewed using Gardner-Robertson Hearing Scale Grade. Tumor behavior including volume and growth rate, was also reviewed using tumor volumetry analysis. They were submitted to uni/multivariate analysis.

Results: The periods of preservation of hearing outcomes (age) depending on *NF2* germline mutation type were 26.5 years (Truncating), 34.0 years (Large Deletion), 37.0 years (Splice site), 43.5 years (Missense), and 49.5 years (Undetected *NF2* germline mutation) (log-rank test for totals: p<0.001, log-rank test for trends: p<0.001). "Truncating" (OR:4.242, 95%CI:1.13-15.92, p=0.0322), "Undetected *NF2* germline mutation" (OR:0.1167, 95%CI:0.03-0.43, p=0.001488) and "Onset age$\geq$25 (OR:0.1915, 95%CI:0.05-0.61, p=0.005528)" were significant predictors for their hearing outcome. VS growth rate also differed statistically significantly according to germline *NF2* mutation type and onset age$\geq$25 or not. The patients characterized by "Undetected *NF2* germline mutation" and "Onset age$\geq$25" had clearly different hearing prognosis compared to other NF2 patients.

Conclusions: Hearing outcome due to NF2 related VS are predictable by considering age at diagnosis NF2, and germline mutation of *NF2*.

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Early Development in Neurofibromatosis 1 (EDEN): Developmental Trajectory and Behavioural Phenotype of Infants with NF1

Monday, September 23, 5:30pm – 5:45pm

Shruti Garg, PhD, *University of Manchester, UK*

Background: Approximately half of all children with NF1 have comorbid neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) or Attention Deficit Hyperactivity Disorder (ADHD). However, little is known about the early emergence of the behavioural phenotype. The EDEN study is a prospective longitudinal study of a cohort of infants with NF1 from 5 months to 36 months. The aim of this study is to investigate the developmental profile of infants with NF1 as compare to infants at high risk of ASD or ADHD (by having an older sibling with ASD or ADHD respectively).

Methods: Data is presented from 30 infants with NF1 at 14 months as compared to infants who are at familial risk of ASD (n= 70), familial risk of ADHD (n=40) and infants with no familial risk (n=30; TD group). Behavioural observation and assessment was carried out using the Autism Observation Scale for Infants and the Mullen Early Learning Scale (MELS). Parent report on infant functioning and temperament was collected using the Vineland Adaptive Behaviour Scale (VABS) and the Infant Behaviour Report.

Results: At 14 months, infants with NF1 showed higher ASD symptomatology as compared to all of the other groups ($p<0.001$). In addition, on the MELS the NF1 group was significantly more impaired on all the five subscales (receptive language, expressive language, visual reception, fine and gross motor skills). ON the parent reported VABS, the NF1 group had significantly lower scores as compared to the ADHD risk and TD group but not the ASD risk group.

Conclusions: Using a prospective longitudinal design, this is the first study to report the cognitive behavioural phenotype in infants with NF1 using a comparative sample of cohorts with familial risk of neurodevelopmental disorders. The results suggest significant ASD symptomatology, motor and language impairment at 14 months.

A Longitudinal Analysis of Cognitive Predictors of Academic Achievement in Children and Young Adults with Neurofibromatosis Type 1 (NF1)

Monday, September 23, 5:45pm – 6:00pm

Taryn Allen, PhD, *Clinical Research Directorate, Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute*

Introduction: Youth with NF1 are at increased risk for cognitive and academic difficulties. Cross-sectional research has documented weaknesses in verbal and nonverbal skills, attention, memory, and executive functioning. Learning disabilities in reading, writing, and math are also common. Despite these well-documented vulnerabilities, less research has examined cognitive predictors of academic functioning and no known studies have looked at how cognitive skills impact academic achievement over time. In light of these gaps, we conducted a longitudinal analysis of cognitive and academic functioning in a sample of school-aged children and young adults with NF1, assessing the predictive impact of cognitive skills on achievement over time.

Methods: As part of a NCI natural history protocol, school-aged children and young adults (ages 8-22) with NF1 underwent neuropsychological testing at baseline, 3 years, and 6 years. The battery assessed cognitive abilities (e.g., IQ, attention, working memory, executive functioning), academic achievement, and behavioral functioning (e.g., social-emotional wellbeing; inattention; everyday behaviors related to executive functioning) using performance-based measures and parent-report scales. Multilevel growth modeling evaluated the impact of baseline cognitive abilities on academic functioning over six years. Each predictor was initially tested in a separate model to examine its independent effect. Then, all predictors were simultaneously included in a single model to examine their unique effects, above and beyond each other.

Results: Participants included 93 children and young adults with NF1 (age $M=12.84$, $SD=3.43$; 60% male; 95% with plexiform neurofibromas). Parent-rated inhibitory control and working memory were positively associated with initial levels of math performance [$t(59)=3.42$, $p<0.01$; $t(59)=2.36$, $p<.02$, respectively] and decline in math over time [$t(64)=2.01$, $p<0.05$; $t(64)=1.98$, $p=0.05$, respectively]. Working memory and cognitive flexibility exhibited significant positive unique effects on initial levels of reading [$t(61)=3.41$, $p<0.01$; $t(61)=2.10$, $p<0.05$, respectively], while working memory, cognitive flexibility, and attention had significant positive unique effects on initial levels of writing [$t(60)=4.13$, $p<0.001$; $t(60)=2.62$, $p<0.05$; $t(60)=1.99$, $p=0.05$, respectively]. Baseline cognitive skills did not have a significant effect on reading and writing over time.

Discussion: The current findings indicate that cognitive skills–specifically working memory and aspects of executive functioning–are uniquely related to initial levels of academic achievement in reading, writing, and math in youth with NF1. Further, initial weaknesses in these domains contribute to declines in math over time. Interesting, cognitive skills were not related to change in reading or writing in this sample. We have found that other factors (e.g., family and disease characteristics) play a more influential role in predicting achievement in reading and writing over time (unpublished data). Ultimately, by better understanding longitudinal patterns of academic functioning, and the underlying factors that contribute to achievement, we will be better able to develop appropriate interventions to support students with NF1 in the classroom.

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Cognitive Functioning in Adults with NF1: Preliminary Results of a Danish Population-Based Comparison Cohort Study

Monday, September 23, 6:00pm – 6:15pm

Karoline Doser, MA, *Survivorship Unit, Danish Cancer Society Research Center, Copenhagen, Denmark*

Background: Neurofibromatosis type 1 (NF1) is characterized by a number of somatic and psychiatric disease manifestations, and impaired cognitive function has been described in children with NF1. However, cognitive functioning in adults with NF1 has only sparsely been described.

Methods: We conducted a population-based comparison cohort study using a comprehensive neuropsychological test-battery to assess cognitive functioning in adults with NF1 compared to a control population. We included 103 adults randomly selected from a population-based sample of 244 adults diagnosed with NF1 at one of the two national Centers of Rare Diseases in Denmark between 1977-2016. Forty NF1-free matched comparisons are being recruited (ongoing) from a population-based randomly selected age- and gender- matched control sample obtained through the Danish Civil Registration System (N=300). The set of standardized neuropsychological measures assess: executive functioning (The Behavior Rating Inventory of Executive Function – BRIEF A), intelligence (Wechsler Adult Intelligence Scale -WAIS IV), visuospatial memory (Rey-Osterrieth Complex Figure - ROCF) as well as attention, executive planning, spatial span, working memory, visual information processing, and reaction time (Cambridge Neuropsychological Test Automated Battery - CANTAB) Analyses of variance models are being applied to investigate the level of cognitive functioning in comparison to NF1-free controls.

Results: Adults with NF1 had a mean age of 43 years (SD=16), with equal gender ratio and mainly severe levels of both disease severity (42%) and visibility (66%). Analyses are ongoing and will be presented at the conference for executive functioning, estimated IQ scores and visuospatial memory, as well as attention, executive planning, spatial span, working memory, visual information processing, and reaction time tasks in persons with NF1 compared to NF1-free controls.

Conclusion: This study is the first nationwide study focusing on adults with NF1 and will provide new evidence on the degree of impaired cognitive functioning.

Symptoms, Location and Management of 77 Children with Neurofibromatosis Type 1 Plexiform Neurofibroma in the National Complex Neurofibromatosis Service, Guy's Hospital, UK 2018-2019

Monday, September 23, 6:15pm – 6:30pm

Yoshua Collins-Sawaragi, MD, *London Children's Hospital*

Introduction: We report the symptoms, location and management of plexiform neurofibroma (PN) in children with Neurofibromatosis Type 1 (NF1) seen in the National Complex Neurofibromatosis Service at Guy's Hospital in London between April 2018 and April 2019.

Method: Review of our retrospective data collection and patient records.

Results: During the 13 months period, there were 77 NF1 patients with PN seen in the clinic (40 male, 37 female). Mean age was 9.7 years (SD ±4.2 years, range: 1.6 years -16.9 years). 21% (16) of patients were seen in the clinic for first time. The main location of the PN was craniofacial in 43% (33) followed by limb 18% (14), neck/ airway 14% (11), paraspinal/ spinal 12% (9). 13% (10) were in other locations including pelvis, chest, abdomen and buttock. Symptoms reported were disfigurement (defined as significantly changing appearance) in 60% (46), impairing function in 21% (16), pain in 18% (14) and posing a threat to function i.e. potential to cause neural/ airway compression in 10% (8). 27% (21) had more than 1 symptom. FDG PET CT examination was performed in 3 cases – 2 of these were negative and 1 patient is currently undergoing biopsy following an equivocal result. 45% (36) of our patients were managed conservatively. During the study period 11 patients had surgery for their PN. 15 patients had surgery prior to the study period and 9 are awaiting surgery. In total 35% (27) of patients have had or are awaiting surgery. 33% (9) of patients required more than 1 operation. 74% (20) of surgeries were performed in those with craniofacial PN. 18% (14) are receiving or about to receive a MEK inhibitor: 4 for Trametinib and 10 for Selumetinib (either as part of a clinical trial or on a named patient basis).

Conclusion: This study provides a comprehensive overview of the management of children with PN in an era where new therapies (MEK inhibitors) are becoming a mainstream treatment option. Our current figures suggest most patients are still being managed conservatively at 45% versus 35% surgically and 18% MEK inhibitors. We anticipate that there will be a shift to more patients receiving MEK inhibitor therapy and combination therapy (surgery and MEK inhibitor) in the future.

Characterizing NF1-Associated Skeletal Manifestations with Single-Cell Resolution and the Effects of MEK Inhibition on Bone Mineral Density in Children with NF1 and Plexiform Neurofibromas

Monday, September 23, 6:30pm – 6:45pm

Jonathan J Rios, PhD, *Texas Scottish Rite Hospital for Children*

Persistent fracture pseudarthroses in children with NF1 often result in amputation and are associated with somatic *NF1* mutations at the fracture site. Loss of *Nf1* in conditional mouse models led to multiple skeletal defects caused, at least in part, by impaired osteoblast differentiation of bone-derived stromal cells (BSCs). Mechanistic studies of human patient-derived BSCs, however, are lacking due to technical limitations of studying rare somatically-mutated cells from patient fractures.

We used time-series single-cell whole-transcriptome sequencing (scRNA-seq) to successfully characterize genetic dysregulation leading to osteogenic differentiation defects of somatically-mutated patient fracture-derived BSCs. Osteogenic trajectory analysis confirmed proper differentiation of NF1 patient iliac crest-derived BSCs, which express low levels of epiregulin (*EREG*[LOW]), and also identified multiple transient cell-fate transitions throughout differentiation. However, scRNA-seq of fracture-derived BSCs from the same patient identified two distinct cell populations, which we previously characterized as *EREG*[LOW] BSCs and somatically-mutated *EREG*[HIGH] BSCs. Further analyses confirmed that *EREG*[HIGH] BSCs failed to initiate an osteogenic differentiation response in culture.

We sought to identify genes whose expression was associated with osteogenic failure of *EREG*[HIGH] BSCs. Interestingly, alkaline phosphatase (*ALPL*) was constitutively over-expressed in *EREG*[HIGH] BSCs. Previous studies of the bi-phasic role of ALPL in osteogenesis suggest constitutive over-expression of *ALPL* in *EREG*[HIGH] BSCs may underlie their differentiation defect. Consistent with this, inhibition of ALPL during early phases of differentiation partially rescued osteogenesis of patient fracture-derived BSCs.

Loss of *NF1* activates MEK-ERK signaling, suggesting that inhibiting ERK may rescue osteogenic differentiation of fracture-derived BSCs. MEK inhibition with trametinib normalized *ALPL* expression but failed to rescue osteogenic differentiation of fracturederived BSCs. Furthermore, trametinib inhibited differentiation of control- and iliac crestderived BSCs, prompting further investigation of the potential effects of MEK inhibition on the skeleton in NF1.

The SPRINT phase II selumetinib (AZD6244, ARRY-142886) trial demonstrated plexiform neurofibroma (PN) shrinkage in children with NF1 and PN. This trial also prospectively evaluated the effect of selumetinib on bone mineral density (BMD). Of 12 patients with reduced BMD at baseline, follow-up scans after 12 months selumetinib treatment showed no clinically meaningful reductions in BMD. Median whole-body (less head) height-adjusted Z-scores at baseline and after 12 cycles of therapy were -1.34 and -0.955, respectively. In some patients, local BMD improved concomitant with decreases in the surrounding PN. These results provide novel mechanistic and therapeutic insight into the skeletal manifestations of NF1 and suggest that skeletal manifestations may benefit from therapies targeting non-skeletal manifestations.

Assessment of Pulmonary Function in Patients with Neurofibromatosis Type 1 and Airway Associated Plexiform Neurofibromas Before and After Treatment with Selumetinib

Monday, September 23, 6:45pm – 7:00pm

Andrea Gross, MD, *NIH, NCI Pediatric Oncology Branch*

Background: Airway associated plexiform neurofibromas (PN) in patients (pts) with neurofibromatosis type 1 (NF1) can be a significant source of morbidity, sometimes leading to complete airway obstruction. In our phase 2 study of selumetinib (AZD6244, ARRY-142886) for pediatric pts with NF1 and inoperable PN (NCT01362803), pts with airway associated tumors underwent standardized functional evaluations to assess for changes over time.

Methods: Pts received continuous dosing of selumetinib (25 mg/m^2 BID, 1 cycle = 28 days). Those with airway associated tumors based on MRI and clinical evaluation and no tracheostomy completed spirometry and impulse oscillometry (IOS) at baseline, and every 4 cycles with re-staging volumetric MRI of the target PN. Polysomnography was performed at baseline and after 12 cycles for all airway pts without tracheostomy, and those with an abnormal apnea hypopnea index (AHI) at baseline had sleep studies performed every 4 cycles for the first year and then annually. Key measurements included forced expiratory volume in one second (FEV1), airway resistance at 5 and 20 Hz (R5, R20) and total AHI, as per recommendations from the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) International Collaboration. Pts with evaluations at baseline and after 12 cycles on treatment were considered evaluable.

Results: Sixteen of 50 pts had airway-associated tumors (10 male; median age 10.4 yrs, range 5, 15.7). The median baseline PN volume was 680 mL (range, 80-3820 ml), with median PN shrinkage of -24% after 12 cycles on therapy (range -36.2, -13.9%). Five subjects (31%) had tracheostomies at baseline and 2 required nocturnal continuous positive airway pressure (CPAP). Airway-associated tumors were most commonly located above the bifurcation of the trachea, with 88% extending into the retropharyngeal space. All patients without tracheostomy (n=11) had evaluable spirometry results. Of these, there was a clinically meaningful ($\geq$12%) improvement in the FEV$_1$ % predicted in 3 pts (27%) and worsening in one (9%). Of the 10 evaluable pts for IOS, 5 had $\geq$ 20% decrease in R5 and none had worsening. However, for the R20, only 1 had improvement and 1 had worsening. There was no direct correlation between baseline tumor volume and baseline FEV$_1$, R5 or R20, nor was there a correlation between degree of tumor shrinkage and change in the airway functional measures. No evaluable pts had a baseline AHI > 5; therefore, meaningful changes in obstructive sleep apnea could not be assessed. Pts with tracheostomy could not complete these assessments, however one was able to be successfully decannulated during the study due to tumor shrinkage.

Conclusions: Assessment of airway function during this clinical trial for treatment of NF1 related PN was feasible and demonstrated meaningful improvement in some pts. Improvements meeting pre-defined thresholds of clinical significance were found without direct correlation between the functional changes and tumor volume. This finding underscores the importance of measuring functional outcomes for this population, as they may be a more direct indicator of a tumor's actual physiological impact through airway compression.

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Validating Measurement Techniques for Cutaneous Neurofibromas in Clinical Trials

Monday, September 23, 7:00pm – 7:15pm

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

Objective: To assess the feasibility and performance properties of digital calipers, 3D camera, and high frequency ultrasound (HFUS) to measure cutaneous neurofibromas (cNF) in patients with NF1.

Background: cNF affect virtually all patients with neurofibromatosis 1 (NF1) and are a major source of social/emotional distress. Treatment options for cNF are limited to surgical based interventions. Reliable measurement techniques to assess change in cNF are a critical gap for development of therapies for these tumors.

Methods: Adults with NF1 and cNF were recruited from Massachusetts General Hospital (MGH) to assess the intra- and inter-rater reliability of calipers, 3D camera, and HFUS to measure cNF. Six cNF on each NF1 participant's forearm were assessed independently by three different examiners using digital calipers (World Precision Instruments), 3D photography (Canfield Scientific Vectra H1), and HFUS (Fujifilm VisualSonics Vevo3100). 3D-photographs and ultrasound images were processed using manufacturer provided software, and tumors were measured via manual outlining by three independent assessors. Linear and volumetric assessments were compared using intraclass correlation coefficient (ICC) to determine the intra- and inter-rater reliability of each measurement technique.

Results: Ten subjects have participated to date with 57 cNF measured. Reproducibility of image acquisition and measurement was excellent within and across examiners for HFUS and 3D camera techniques (ICC > 0.97). In comparison, digital calipers provided moderate to excellent reproducibility but were notably the weakest technique (ICC ≤ 0.62- 0.88, depending on dimension analyzed). Examiners found HFUS and 3D camera easy to administer and images were rapidly acquired (< 30 seconds). cNF appear hypo-echoic and are easily visualized using HFUS. Both systems require minimal cooperation from study participants and were easily tolerated.

Conclusions: HFUS and 3D photography represent promising, non-invasive, methods for measurement of cNF and have higher inter-rater reliability than calipers. In addition to validating reproducibility, we are currently assessing each technique's coefficient of variation (to determine their accuracy) and ability to assess change in cNF size over time. This information will ultimately inform clinical trial design and facilitate the development of interventional treatments for cNF.

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KEYNOTE LECTURE: Perspectives of a "Long-Term" Recklinologist Focusing on the Future

Tuesday, September 24, 8:15am – 9:00am

Vincent M. Riccardi, MD, MBA, *The Neurofibromatosis Institute*

In my 42 years of devotion to Neurofibromatosis, with the last two decades more or less restricted to NF1, I have noted a remarkable "narrowness" in the topics that generate special research interest. In this regard, along the way, I have generated a list of General and Specific areas of focus that, additionally and alternatively, are liable to yield especially fruitful insights about NF1. For this presentation, I will be prepared to share *Sixty-Eight Areas of Focus for Future NF1 Research* (and I am sure the list is still missing some items). As time permits, I will proceed down the List as far as I can and share thoughts on how each discussed item might have useful yield in shaping and exploiting any of several levels of NF1 research. The Complete List will be made available to whomever wants it! Please join me in thinking out loud and, perhaps, opening new horizons.

SESSION 10: Animal Models

Chairs: Marco Giovannini, MD, PhD, UCLA School of Medicine; Nancy Ratner, PhD, Cincinnati Children's Hospital Medical Center

Modeling NF1 in Drosophila

Tuesday, September 24, 10:00am – 10:20am

James Walker, PhD, *Massachusetts General Hospital, Harvard Medical School*

The fruit fly *Drosophila* has often provided important insights into the functions of conserved genes involved in human disease. The *Drosophila* ortholog of *NF1* encodes a 2802 residue protein, 60% identical to human neurofibromin, with RasGAP activity. *Drosophila* models of NF1 using either loss-of-function *dNf1* mutants or RNA interference (RNAi) display a number of molecular, cellular and behavioral phenotypes. These have enabled us to capitalize on the power of genetics to probe the mechanisms underlying neurological deficits of NF1-deficient flies. While NF1-deficient flies do not develop tumors, they show increased Ras/ERK activity in neurons, reduced synaptic growth, olfactory associative learning and circadian rhythm deficits. We have delineated novel roles for neurofibromin in neurons to non-cell-autonomously control systemic growth and metabolism. Genetic screens have been used to identify a neuronal-specific receptor tyrosine kinase, Anaplastic Lymphoma Kinase (ALK), as a rate-limiting upstream activator of RAS signaling regulated by *NF1.* Biochemical studies have revealed metabolic defects caused by *NF1* deficiency in neurons. We have also conducted a genome-wide RNAi synthetic lethal interaction screen in NF1-deficient *Drosophila* cell lines to identify specific genetic vulnerabilities of *NF1* mutant cells.

Together these fruit fly model studies have identified novel roles of neurofibromin and signaling pathways that could inform further investigation in mammalian cell/animal models and could potentially represent therapeutic strategies for NF1.

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<u>Platform</u>: Inhibition of the Hippo Pathway in Schwann Cell Lineage Lead to Schwannoma Development

Tuesday, September 24, 10:20am – 10:35am

Zhiguo Chen, PhD, *UT Southwestern Medical Center, Dallas, TX*

Schwannoma is a major tumor type of Neurofibromatosis Type 2 (NF2) and the recently recognized schwannomatosis which is characterized by the development of multiple schwannomas in the absence of bilateral vestibular schwannoma. Patients with schwannoma suffer from chronic pain, numbness, and their physical impairment of vital organ or neural function can be life threatening. Despite the identification of NF2 (Merlin), INI1/SMARCB1 and newly identified schwannomatosis-predisposing gene LZTR1, the exact molecular pathogenesis causing schwannoma development is still poorly understood. Furthermore, although Merlin has been showed to regulate multiple pathways including the Hippo, MAPK and PI3K signaling, the exact dysregulated pathway leading to schwannoma development has not been confirmed. Therefore, there is currently no effective, specific medical treatment for these complex tumors. Here, we found that inhibition of the Hippo pathway in Schwann cell lineage lead to multiple cutaneous, subcutaneous and internal schwannoma development, which subsequently undergo malignant transformation. In these tumor cells, we observed the downstream inactivation of Hippo signaling and activation of MAPK signaling pathway. In addition, we found that YAP or TAZ is required for the schwannomagenesis and that MAPK pathway acts as a modifier of schwannoma formation. Our new model provides a framework to further clarify the mechanism of schwannoma development and identify potential therapeutic targets.

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Mouse Models of Neurofibromatosis type 1 (NF1)-associated Low- and High-Grade Glioma

Tuesday, September 24, 10:35am – 10:55am

Yuan Zhu, PhD, *Children's National Medical Center*

We have used genetically engineered mouse (GEM) models to investigate the pathogenic mechanism of NF1-associated brain tumors. Approximately 15-20% of NF1 children develop low-grade gliomas (Grade I), predominantly along the optic pathway, also known as optic pathway gliomas (NF1-OPG). Using multiple glial lineage-specific Cre drivers, we determined phenotypic consequences of Nf1 loss in optic nerve (ON)-derived astrocytic lineage as well as brain-derived oligodendrocytic lineage during neonatal development. We determined critical cellular and molecular events underlying NF1-OPG formation. In contrast to benign plexiform neurofibromas, NF1-OPG do not progress into malignancy and form high-grade gliomas (NF1-HGG), including glioblastomas (GBM, Grade IV). Instead, NF1-HGG/GBMs arise from *p53*-deficient neural stem cells located in the subventricular zone (SVZ) stem-cell niche, which inactivate Nf1 during tumor progression. Using cytogenetic alterations and whole-genome sequencing data, we built a single-cell phylogenetic tree of malignant gliomas and GBMs isolated from different regions of the brain. We show that a single SVZ cell-derived malignant gliomas/GBMs evolved in spatially segregated regions via two distinct patterns. Relatively quiescent glioma cells, maintained in the SVZ stem-cell niche, inactivated *Nf1,* while retaining normal *Pten* with near-diploid/2N genomes. In the brain parenchyma, highly proliferative gliomas underwent whole-genome doubling with unstable sub-tetraploid/4N genomes, loss of chromosome 19/*Pten* and PI3K/Akt activation. Akt inhibition by *Rictor*/mTORC2 deletion blocked distant glioma dissemination. The single-cell-derived model sequentially accumulates GBM-relevant driver alterations in the RAS/MAPK and PI3K/AKT/mTORC pathways, driving local expansion and distant dissemination during GBM initiation and manifestation, respectively. In summary, this presentation will discuss about the utility of GEM models of brain tumors to perform preclinical studies, which have the potential to translate laboratory findings into clinical application.

Development and Validation of a Novel NF2 GEMM to Facilitate Preclinical Studies

Tuesday, September 24, 10:55am – 11:15am

Joseph Kissil, PhD, *Department of Molecular Medicine, The Scripps Research Institute*

Neurofibromatosis type 2 (NF2) is a dominantly inherited autosomal disease with most common manifestation being the development of bilateral schwannomas of the 8th cranial nerve (Vestibular schwannoma). Although our understanding of the molecular mechanisms underlying NF2 has significantly improved over the past 2 decades, and a number of potential therapeutic targets have been identified, effective therapies remain lacking and there is an urgent need to develop therapeutic options for NF2 patients. Currently, one of the main obstacles towards the development of effective drug treatments for NF2 is the lack of appropriate animal models to enable efficient pre-clinical testing of drug candidates. To overcome these obstacles we employed a GEMM (Genetically Engineered Mouse Model) of NF2 (*Postn-Cre:Nf2flox/flox*) and incorporated a transgenic reporter allele (*Rosa26-LSL-GpNLuc*). The GpNLuc protein is a novel bioluminescence resonance energy transfer (BRET) reporter allele that can be conditionally turned on by Cre-recombinase. This reporter allele overcomes previous limitations associated with use of fluorescent or bioluminescent enzymes *in vivo* by using a fusion of enhanced GFP (eGFP; Gp) linked to an enhanced variant of luciferase isolated from deep-sea shrimp (NanoLuc; NLuc). The intra-molecular BRET occurring between Gp and NLuc generates the brightest bioluminescent signal known to date and improves spatiotemporal monitoring of small numbers of tumor cells using *in vivo* optical imaging. In this presentation, the characterization and validation of this new NF2 GEMM will be described as well as efforts to deploy the GEMM in preclinical evaluation of select drugs.

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Platform: The Role of Neurofibromin in Neurocognitive Impairment, CNS Cell Function, and Differentiation in a Porcine Model of Neurofibromatosis Type 1

Tuesday, September 24, 11:15am – 11:30am

Vicki J Swier, PhD, *Pediatric and Rare Disease Group, Sanford Research, Sioux Falls, SD*

Cognitive impairment is common among patients with neurofibromatosis type 1 (NF1), with as many as 70% of NF1 patients reporting difficulties with spatial/working memory, attention, and executive function. To examine how mutations in *NF1* can alter the structure and cellular composition of the brain and cognitive function, we longitudinally monitored CNS cellular composition and animal activity in our recently characterized *NF1 +/ex42del* miniswine model. Frontal lobe, anterior cingulate, corpus callosum, hippocampus, and amygdala from 14, 18, and 24 month old *NF1 +/ex42del* and WT miniswine were examined longitudinally using classic histopathological techniques. Cellular proliferation, mature oligodendrocytes, astrocytes, and microglia were monitored over time, revealing abnormalities in glial activation. Specific classes of neurons and synaptic markers were also monitored over time, with high performance liquid chromatography showing imbalances in GABA:Glutamate ratios in *NF1 +/ex42del* miniswine. Moreover, we describe disruptions in locomotor activity, learning and memory, and sensitivity to stimuli that were dependent on tumor burden and/or sex. Specifically, *NF1 +/ex42del* miniswine walk more carefully as analyzed by a kinematic gait analysis, with slower and shorter steps than their WT counterparts that are consistent with the attempt to preserve balance commonly seen in NF1 patients. *NF1 +/ex42del* miniswine are also more sensitive to various stimuli and have disruptions in their sleep cycles, and maintain memory/learning deficits as they age as measured by a simple T maze. Taken together, we confirm the utility of our *NF1 +/ex42del* miniswine model as a tool for monitoring cognitive impairment, dissecting the role of specific neural circuits and glia in neurological deficits, and assessing therapies for the improvement of cognitive dysfunction in NF1 patients.

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SESSION 11: Second NF Hackathon

Tuesday, September 24, 11:45am – 12:45pm

Salvatore La Rosa, PhD, *Children's Tumor Foundation Chief Scientific Officer*

This session will recap the NF Hackathon event that was held on Sept 13-15, 2019 in San Francisco. The Children's Tumor Foundation (CTF), the Neurofibromatosis Therapeutic Acceleration Program (NTAP) and Sage Bionetworks, in collaboration with Silicon Valley AI (SVAI) have organized this event that will host 120 participants among research scientists, computer scientists, engineers, software developers, and AI and ML programmers to (re)analyze a large and diverse set of NF data provided by many researchers through the NF Data Portal (nfdataportal.com). Participants will self-assemble in teams and will work on one or more of the proposed themes/tracks. During the week-end long event, teams will be asked to

• Use the data from pre-clinical systems of NF1 and NF2-associated tumors to identify validated and druggable targets that modulate disease
• Identify which genomic/transcriptomic features are most closely associated with the different NF tumor types; use these features to identify novel drug targets.
• Identify features of MRIs to diagnose specific tumor types or predict progression; Improve upon current segmentation algorithms.

You will hear from three of the top teams that tried to address the above questions and their approaches to finding a solution through data analysis.

POSTERS: Basic Science

Poster Presentation (odd numbers)

SUNDAY, SEPTEMBER 22, 2019 (5:05 – 7:05 PM)

LAST	FIRST	POSTER	TITLE
Allaway	Robert	1	Leveraging the Wisdom of the Crowd: Gaining New Insights into the Biology of Rare Tumors Using Transfer Learning Approaches
Arnold	Antje	3	Differential Gene Methylation and Expression of HOX Transcription Factor Family in Periocular Neurofibromas
Ayter	Sükriye	5	Telomere and Telomerase Activity in Cutaneous and Plexiform NF1 Tumors
Banerjee	Jineta	7	Supervised Classification of NF1 Tumor Types Based on Combinatorial Genetic and Protein Network Signatures
Beauchamp	Roberta	9	Dual mTORC1/2 Inhibition Negatively Regulates NRG1-ERBB3 Autocrine Signaling and Leads to Adaptive Feedback Response in NF2-Deficient Meningioma Cellular Models
Bedwell	David	11	Examining the Suppression of Nonsense Mutations as a Potential Treatment for NF1
Carnes	Rachel	13	Neurofibromin Binding Partners
Creus	Edgar	15	Testing Combination Therapy in an MPNST Precision Medicine Platform
Dave	Foram	17	Investigating the Role of TYRO3, AXL and MERTK (TAM) Family Receptors in Neurofibromatosis Type 2 (NF2) Associated Tumours: Schwannomas and Meningiomas
Farouk Sait	Sameer	19	Protein Tyrosine Phosphatase Inhibition Prevents Adaptive Resistance to MEK Inhibition in NF1 Associated Malignant Peripheral Nerve Sheath Tumors
Field	Jeffrey	21	Hippo-YAP Inhibitors Synergize with MEK and ERK Inhibitors in NF2 Deficient Schwannoma Cell Lines
Girish	Milind	23	Absence of Association Between the Size of Genome-Wide Copy Number Events and the Clinical Severity of NF1 Microdeletion Cases
Gosline	Sara	25	A Network-Based Workflow to Identify Drug Target Networks from Gene Expression Data
Grit	Jamie	27	Molecular Mechanisms of Targeted Therapy Resistance in Malignant Periphreal Nerve Sheath Tumors
Jung	Marie	29	Merlin's Role in Muscle Stem Cells
Kallionpää	Roope	31	Circulating Free Nucleic Acids in Neurofibromatosis Type 1
Keller	Bryant	33	*In Vivo* Targeting of Physical and Genetic Vulnerabilities in Malignant Peripheral Nerve Sheath Tumors (MPNST) and Neurofibromas
Kesterson	Robert	35	GABAergic Deletion of NF1 Induces Complex Metabolic and Behavioral Phenotypes
Ki	DongHyuk	37	Inhibition of 4E-BP1 Phosphorylation by DNA Topoisomerase I-Targeted Drugs Plus mTOR Kinase Inhibitors in Malignant Peripheral Nerve Sheath Tumors
Kohlmeyer	Jordan	39	Targeting RABL6A-RB1 Signaling Suppresses MPNST Pathogenesis
Lambert	Laura	41	Mammary Cancer and Behavior Characterization in a Patient Specific Neurofibromatosis Type I Rat Model
Lambert	Laura	43	Novel Patient-Specific Mutation Rodent Models of Neurofibromatosis Type I
Larsson	Alex	45	Modeling PRC2 Perturbation in *NF1*-Deficient Human Schwann Cells Reveals Potential Therapeutic Targets in Malignant Peripheral Nerve Sheath Tumors
Le	Kim My	47	Characterization of T Cells in Human Cutaneous Neurofibromas
Long	Ashlee	49	Repurposing CFTR Modulators for the Treatment of Neurofibromatosis Type I
Ma	Rufeng	51	Contractile Force Generation of Fibroblasts from Patients with Neurofibromatosis Types 1 and 2
Martikkala	Eija	53	Characterization of Mast Cells in Human Cutaneous Neurofibromas
Mazuelas	Helena	55	Identification of Pathways Triggered by Schwann Cell-Fibroblast Interactions Driving Cutaneous Neurofibroma Growth
McGivney	Gavin	57	Metabolic Profiles of MPNSTs Are Dependent Upon Tumor Suppressor Status
Mo	Juan	59	Humanized Neurofibroma Model to Delineate Tumor Pathogenesis and Preclinical Therapeutic Testing
Moon	Chang-In	61	Unmasking Intra-tumoral Heterogeneity and Clonal Evolution in NF1-MPNST
O'Loughlin	Evan	63	Modeling Nf2 Deficiency in the Liver
Oblinger	Janet	65	Brigatinib as a Potential Therapy for Malignant Peripheral Nerve Sheath Tumors
Robinson	J. Elliott	67	*Nf1* Haploinsufficiency Alters Mesoaccumbal Dopamine Dynamics and Responses to Salient Stimuli
Roy	Vincent	69	Increased Extracellular-Matrix Deposition in a Tissue-Engineered Skin Model Derived from NF1 Patients

POSTERS: Basic Science

Poster Presentation (odd numbers)

SUNDAY, SEPTEMBER 22, 2019 (5:05 – 7:05 PM)

LAST	FIRST	POSTER	TITLE
Smith	Abbi	71	A Novel Whole-Head Histopathological Approach to Evaluate CNVIII Architecture And Tumor Microenvironment in a Murine Model of Neurofibromatosis Type 2 Associated Hearing Loss
Sonawane	Poonam	73	Functional Analysis of *NF1*-Associated Low-Grade Glioma Co-Occurring *FGFR1* and *PIK3CA* Mutations
Sun	Daochun	75	Functional Heterogeneity Reveals a Hierarchical Organization of MPNSTs
van Minkelen	R.	77	Functional Analysis of Cases Suspect for Neurofibromatosis Type 1
Vogt	Daniel	79	Molecular and Cellar Changes in GABAergic Cortical Interneurons in Nf1 Conditional Mutants
Walker	James	81	Identifying Novel Therapeutic Targets for NF1-Deficient Tumors Using a Unique Combinatorial Screening Strategy
Wallace	Peggy	83	Creation of Immortalized Cutaneous Neurofibroma Schwann Cell Lines
Wallis	Deeann	85	Feasibility and Potential Impact of Exon Skipping as a Therapeutic for Neurofibromatosis Type I (NF1)
Watson	Adrienne	87	Preclinical Drug Evaluation in a Genetically Engineered Minipig Model of Neurofibromatosis Type 1
Wegscheid	Michelle	89	Human iPSC-Derived Cerebral Organoids Establish Mutational Specificity in NF1
Wu	Lai Man	91	Single-Cell Transcriptomics Resolves Cellular Heterogeneity and Oncogenic Networks During Malignant Transformation of Peripheral Nerve Sheath Tumors
Zhao	Fu	93	Novel Patient-Derived Xenograft and Cell Line Models for Therapeutic Testing of NF2-Associated Schwannomas
Zhou	Jiangbing	95	NF1 Gene Rescue via Polymeric Nanoparticles in Translational Animal Models

Leveraging the Wisdom of the Crowd: Gaining New Insights into the Biology of Rare Tumors Using Transfer Learning Approaches

Robert J Allaway, PhD, *Sage Bionetworks, Seattle, WA*

Applying computational methods to study neurofibromatosis (NF) is a challenge due to a rarity of amply-powered datasets. These approaches typically require a large number of observations to understand few variables. Datasets available in NF typically have few observations but many features, particularly in the "omics" era.[1] Among other things, this is a consequence of high-quality patient samples being difficult to acquire and expensive to characterize. To use machine learning methods to study NF, we must identify approaches that can tolerate the analysis of underpowered datasets. One potential avenue is to use transfer learning approaches to harness the power of non-NF datasets to infer meaningful biological processes in NF data. Broadly, transfer learning is the development of an algorithm on one group of data and the use of that algorithm to probe the nature of distinct data. Given that there is a large quantity of non-NF transcriptomic data available that characterize the human condition, transfer learning could move our understanding of rare diseases such as NF forward.

In this work, we analyzed transfer learning methods such as Multi-dataset Pathway Level Information ExtractoR (MultiPLIER) and Coordinated Gene Activity in Pattern Sets (CoGAPS) in NF datasets.[2,3] Specifically, we applied these methods to harmonized data derived from NF-related human tumors such as cutaneous neurofibroma (cNFs), plexiform neurofibroma (pNFs), malignant peripheral nerve sheath tumors (MPNSTs), as well as data from cell line models of schwannoma, meningioma, and others. By applying these methods to NF tumors, we uncovered latent variables that are differentially expressed either within or across gene sets. These latent variables can represent biological pathways, novel biological mechanisms, or other complex gene-gene relationships. We posit that the examination of latent variable expression in NF-related tumors could be used for therapeutic hypothesis generation in NF. For example, we found that a latent variable comprised largely of PLK1-AURKA signaling related genes was substantially more expressed in MPNSTs than in cNFs or pNFs; this signaling axis has been previously identified as a putative therapeutic target in MPNSTs.[4] Finally, we built an open-access web application to allow biologists to explore the full spread of latent variables identified using these transfer learning approaches and to discover how these variables relate to the NF tumors studied.

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4. Patel, A. V. *et al.* Ras-driven transcriptome analysis identifies aurora kinase A as a potential malignant peripheral nerve sheath tumor therapeutic target. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **18**, 5020–30 (2012).

Differential Gene Methylation and Expression of *HOX* Transcription Factor Family in Periocular Neurofibromas

Antje Arnold, *Johns Hopkins University*

Aims: The purpose of this study is to apply high throughput techniques to identify the biologic basis of the clinical behaviour of oculofacial neurofibromatosis, as well as novel prognostic markers and therapeutic targets for this disease.

Abstract: Although most commonly benign, neurofibromas (NFs) can have devastating functional and cosmetic effects in addition to the possibility of malignant transformation. In orbitofacial NF1 (OFNF), which occurs in 1-22% of patients, NFs may cause progressive, disfiguring tumors of the lid, brow, temple, face, and orbit. The purpose of this study was to identify biologic differences between periocular neurofibromas and those developing in other anatomic sites. We used Illumina Methylation EPIC BeadChip which interrogates 850,000 methylation sites on a genome wide basis at single nucleotide resolution. To study protein targets on a high throughput fashion, a tissue microarray was used. Expression of protein targets was evaluated with immunohistochemistry. We studied 20 periocular and 4 non-ocular neurofibromas through global DNA methylation profiling. We found global methylation differences between the two groups, and the top differentially methylated genes were part of the *HOX* family of transcription factors, which were hypomethylated in the periocular neurofibromas compared to the non-ocular neurofibromas *(HOXC8, HOXC4, HOXC6, HOXA6 and HOXD4)*. Next we compared 43 periocular and 26 non-periocular neurofibromas using immunohistochemistry. Protein levels of HOXC8 were higher in periocular neurofibromas (p=0.04). We found no differences in expression for HOXC4, HOXA6 or HOXD4 between the two groups (p>0.05). In summary, we identified gene methylation and expression differences in periocular neurofibromas when compared to neurofibromas occurring at other body sites (cutaneous, diffuse and plexiform). The findings deserve further investigation, given that the HOX family of genes play an important role during development and are dysregulated in a variety of cancers.

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Telomere and Telomerase Activity in Cutaneous and Plexiform NF1 Tumors

Sükriye Ayter, PhD, *TOBB ETU University , Faculty of Medicine*

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease that affects 1 in 3000 people worldwide. The disease is developed due to occurrence of mutations in NF1 gene. NF1 gene is coding cytoplasmic protein which is negative regulator of RAS proteins. The loss of neurofibromin results in activation of RAS cascade and cell proliferation. For this reason NF1 gene is categorized as tumor suppressor gene. It is clinically characterized by cafe-au-lait spots, Lisch nodules, axillary and inguinal freckling, multiple peripheral nerve tumors, bone lesions, and a predisposition to malignancy. Variations in NF1 mutations may not correlate with the variations in clinical phenotype. This unclear genotype–phenotype correlations is assumed to be due to modifier genes. One of these modifier candidates is telomer length and telomerase activity. The length of telomeres are shortening in each cell division. Nevertheless this shortening can be prohibited by the enzyme telomerase which adds a species-dependent telomere repeat sequence to the 3' end of telomeres. However, telomerase activity usually diminished after birth in somatic cells. Recent researches have shown the importance of telomer and telomerase activity and they are causally connected to human disease. However, the number of research on this concept for NF1 patients is very few. At the germline level, the current available literature does not allow to define if there is any association between telomere length and tumors therefore we used tumors.

Methods: We isolated DNAs and proteins of cutaneous and plexiform tumors from 20 NF1 patients (10 for each). The mutation profiles and pathological status of tumor tissues was confirmed from previous studies. DNA samples were used for telomere length measurements and telomerase activity were evaluated from proteins isolated from acquired tumor samples and analyzed by quantitative PCR based technics.

Results: Considering our preliminary results, higher telomerase activity is measured in cutaneous neurofibromas compare to plexiforms. Moreover, variations in telomere size were also detected. Comparing to the healthy DNA sample, although the telomere length of cutaneous neurofibromas are shortened, plexiforms show even smaller telomere length.

Conclusion: These data indicate that telomere length may also play an important role in NF1-associated tumor progression. Telomere length and telomerase activity measurements might be a potential biomarker for estimating tumor/cancer risk.

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Supervised Classification of NF1 Tumor Types Based on Combinatorial Genetic and Protein Network Signatures

Jineta Banerjee, *Sage Bionetworks, Seattle, WA*

Recent studies in precision medicine have confirmed the utility of patient-specific mutation profiles for informing clinical treatment, especially in cancer. A recent survey notes that identification of specific genetic mutations (for example: BRAF V600E mutation) causally associated with the disease help in designing effective treatments regardless of their cancer type. For rare and heterogeneous cancers like neurofibromatosis 1 (NF1), identification of key mutations that are strongly associated with specific tumor types can be helpful in designing patient-specific treatments as well as understanding progression of the disease. While some cancers can be causally linked to a specific mutation, others lack mutation "hotspots" and may even be polygenic in nature. A survey of NF1 suggests that while mutations in the NF1 gene are associated with the disease, over 1400 distinct mutations have been implicated and each manifests at low frequencies. Additionally, the rarity in occurrence of the disease (1 in 3000) and high levels of heterogeneity within the tumor types associated with NF 1 present significant challenges in proper analysis of patient samples. The rarity and heterogeneity of patient samples coupled with low frequencies associated with potentially deleterious mutations present a major obstacle in developing effective precision medicine treatments for NF1.

Here we present a harmonized dataset of genetic variants collected from 67 NF1 patients by various researchers across the world and shared with the aim of facilitating adequately powered analysis of the samples. Furthermore, we present a computational analysis pipeline to classify the different tumor types of NF1 (cutaneous neurofibromas, plexiform neurofibromas, lowgrade gliomas, and malignant peripheral nerve sheath tumors) based on variant signatures, and identify putative mutational signatures which may drive NF1 disease progression. Motivated by recent studies which have found cancer-relevant mutations by associating genetic variants with mutations in the encoded protein domains, we analyzed genetic variants that preferentially perturb important signaling pathways in specific tumor types. Our preliminary results confirm the perturbation of RAS-RTK and Notch signaling pathways in all NF tumor types. We then selected the variant signatures prevalent in specific tumor types and diffused the signal over a protein interaction network to compute high-dimensional featurization of the genetic variants. Finally, we used random forest classifiers to distill the feature set to a small set of proteins to identify how differences in those proteins may characterize differences among the different tumor types investigated in this study.

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Dual mTORC1/2 Inhibition Negatively Regulates NRG1-ERBB3 Autocrine Signaling and Leads to Adaptive Feedback Response in NF2-Deficient Meningioma Cellular Models

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Meningiomas (MN), the most common adult primary intracranial tumor, arise from the arachnoid layer of the meninges and are non-responsive to traditional chemotherapies, with ~50-60% showing loss of the *Neurofibromatosis 2 (NF2)* gene that encodes the tumor suppressor protein merlin. Our previous work showing that *NF2* loss activates mechanistic target of rapamycin complex 1 (mTORC1) and mTORC2 signaling has led to past clinical trials for NF2 using mTORC1-specific rapalogs, and two current clinical trials for NF2-related and sporadic MN using the dual mTORC1/mTORC2 inhibitor AZD2014. More recently, based on large-scale kinome and transcriptome studies, we reported activation of several EPH receptor kinases and Src family kinases (SFKs) upon *NF2* loss. Extending our transcriptome analyses of isogenic CRISPR-modified human arachnoidal cells (ACs), expressing and lacking *NF2,* and an *NF2*-null MN line Ben-Men-1, we have further identified increased expression of several ligands, particularly *NRG1*/neuregulin 1. *NF2*-null AC and MN cells showed NRG1 secretion along with ERBB3 receptor activation. Furthermore, conditioned-media from *NF2*-null ACs as well as exogenous NRG1-stimulation activated ERBB3 and EPHA2 receptors as well as mTORC1/2 signaling, suggesting pathway cross-talk. Treatment of *NF2*-null cells with an ERBB3 neutralizing antibody demonstrated partial downregulation of basally activated mTOR signaling but showed no significant effect on cell viability. We next raised the question whether activated mTOR signaling negatively regulates NRG1-ERBB3 signaling. However, unlike a previous report in breast cancer cells, mTORC1/2 inhibitor treatment decreased *NRG1* expression and downregulated the ERBB3 receptor in *NF2*-null AC and MN cells. Moreover, transcriptome analysis after dual mTORC1/2 inhibition revealed significant decrease in *ERBB3* and *ERBB4* with an increase in *IGF1R* receptor expression. Dual mTORC1/2 inhibitor treatment also led to re-activation of pAkt(T308), consistent with observations in other cancer cell types, but not pAkt(S473), suggesting a PDK1-dependent mechanism independent of NRG1-ERBB3 signaling. Interestingly, besides activation of upstream receptor kinases, SFK activation has also been proposed to activate PDK1-dependent pAkt(T308). Taken together, here we show potential existence of an autocrine loop where NF2 loss leads to NRG1 secretion that in turn activates ERBB3 and downstream pathways. Treatment of *NF2*-null cells with a dual mTORC1/2 inhibitor downregulated NRG1-ERBB3 signaling while increasing pAkt(T308) due to an adaptive feedback mechanism that may involve upregulation of IGF1R and/or activated SFK signaling, which is currently under investigation. These results thus provide new opportunities to co-target these pathways with combination drug therapy in *NF2*-deficient meningioma.

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Examining the Suppression of Nonsense Mutations as a Potential Treatment for NF1

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Germline nonsense mutations are found in 21% of NF1 patients. A nonsense mutation introduces an in-frame premature termination codon (PTC) into an mRNA. A PTC abrogates protein function via two mechanisms: 1) a PTC prematurely terminates translation of an mRNA, resulting in a truncated protein that lacks normal function, and 2) a PTC elicits nonsense-mediated mRNA decay (NMD), a cellular pathway that degrades PTC-containing mRNAs, preventing their translation. Translation termination and NMD represent two potential therapeutic targets for treating protein deficiencies caused by PTCs. Nonsense suppression is a therapeutic approach that utilizes small molecular weight compounds to suppress termination at a PTC, thereby restoring full-length protein expression. PTC suppression, also called "readthrough," occurs when an aminoacyl-tRNA becomes accommodated into a ribosomal A-site that is occupied by a PTC, allowing an amino acid to be incorporated into the nascent polypeptide at the site of the PTC. This readthrough event permits translation elongation to continue in the correct ribosomal reading frame, generating a full-length, functional protein. In addition, attenuating NMD can increase the pool of PTC-containing mRNA for translation, which enhances the amount of protein function that is restored by readthrough. In a project previously funded by the Cystic Fibrosis Foundation (CFF), we recently carried out a High-Throughput Screen (HTS) to identify new agents that suppress PTCs in the CFTR gene. We used a series of novel luciferase-based reporters to identify compounds capable of suppressing termination at PTCs and/or inhibiting NMD of PTC-containing mRNAs. Using these dual function reporters, we carried out an HTS of 771,345 samples, resulting in 157 confirmed hits. These hits consist of both readthrough inducers and NMD inhibitors and thus, have the potential to counteract the effects of NF1 nonsense mutations. We hypothesize that a subset of these compounds will also suppress NF1 PTCs and restore enough neurofibromin activity to correct NF1 phenotypes. We will test whether any of the 157 confirmed readthrough agents is capable of suppressing four different recurring nonsense mutations commonly found in NF1 patients with the goal of identifying molecules that can be developed into lead compounds. This approach could potentially have a significant impact on treating NF1 in patients who carry a nonsense mutation.

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Neurofibromin Binding Partners

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Background: NF1 is caused by mutations in the NF1 gene which encodes neurofibromin, a large multi-domain protein with several known binding partners. NF1 binding partners may play a key role in the pleomorphic NF1 phenotype. The BioGrid database summarizes NF1 interacting partners. Despite 37 independent studies, only 12 actually look at NF1 specifically. Although many studies utilize HEK293 or HEK293T cell lines, Biogrid studies do not overlap except at 3 of the 118 total unique proteins: FAF2, HTR6, and SPRED1. Lack of consistency makes it difficult to define relevant NF1 binding partners that might modulate its function. Reagents for studying neurofibromin and the affinity of neurofibromin antibodies has been limited.

Methods: We have created a tandem affinity purification (TAP) tag composed of a TEV cleavage site followed by a Strep II Tag and a 6X His Tag cloned in frame to the 3' end of the full-length mouse Nf1 cDNA. We have also created an empty vector incorporating this TAP tag system as a control. We transfected these into HEK293 cells and binding partners were purified utilizing Strep-Tactin®XT beads and identified via mass spectrometry.

Results: We show that our TAP tagged clones are functional and express His-Tag, neurofibromin, and can correct p-ERK/ERK ratios in NF1 null cells. Initially, we were able to pull down over 800 different proteins in HEK293 cells. We were able to refine the combined protein lists to discover approximately 22 proteins with high confidence of interaction with neurofibromin. We are able to compare this list of protein-protein interactions with the literature, global HEK293 proteomics data, and pseudo-validation approaches such as Reactome and Metacore. We chose to validate binding partners using a reverse immunoprecipitation approach and were able to pull down endogenous neurofibromin from HEK293 cells with antibodies to profilin-1, ephrin B1, and ran, amongst several others.

Conclusions: We identify several novel binding partners for neurofibromin. These proteins suggest that neurofibromin may take part in cellular processes other than inactivating Ras. Further characterization of these binding partners could aid in discovering new neurofibromin functions and drug targets.

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Testing Combination Therapy in an MPNST Precision Medicine Platform

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Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft-tissue sarcomas that are associated with Neurofibromatosis type 1 (NF1), as half of the cases are diagnosed in NF1 patients. Currently, the best therapy for MPNSTs is complete surgical resection with wide negative margins, but often it is not possible due to location or metastases. Chemotherapy or radiotherapy has not been proven effective and new compounds are needed to be tested as potential treatments. For many cancers, drug combinations are emerging as the therapeutic strategy of choice to overcome treatment failure and the appearance of drug resistance due to tumor cell heterogeneity with redundant relevant pathways.

A high-throughput screening of a library of compounds was performed using two different MPNST cell lines: MPNST-SP-01 (sporadic cell line, in-house derived from a sporadic MPNST) and S462 (NF1-derived cell line). Compounds that show reduce cell viability for the two cells were selected and tested in pairwise combinations for synergism. Eleven potential synergistic combinations were identified from the combination high-throughput screening.

From a total of eleven combinations, six showed synergism between compounds, being Combinations 1, 2 and 9 the most synergistic. These three combinations were further tested in 6 additional MPNST cell lines. At low concentrations, Combination 1 exhibited synergism in all cell lines and no toxicity in a non-tumoral cell line. An additive effect was identified in four out of six MPNST cell lines when using Combination 2. Combination 9 caused general cell toxicity. Thus, we selected Combination 1 as the best candidate to be tested *in vivo* in our patient derived orthotopic xenograft (PDOX) mouse model. This experiment is currently being performed.

In parallel, we aimed to study the stromal component of MPNSTs by characterizing five different MPNST-derived stromal fibroblasts (CAFs) cultures. We are planning to analyze cell morphology, DNA content and the expression of previously described CAFs markers like alpha smooth muscle actin, fibroblast activated protein, calponin and platelet-derived growth factor receptor. These analyses are still on going and will be presented in the meeting. In addition, we plan to use CAF conditioned medium to investigate their impact on combination treatment responses in different MPNST cell lines.

This work will provide valuable information on the use of potential new drug combination therapies for MPNSTs and will elucidate the role of stroma on treatment effectiveness.

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Investigating the Role of TYRO3, AXL and MERTK (TAM) Family Receptors in Neurofibromatosis Type 2 (NF2) Associated Tumours: Schwannomas and Meningiomas

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Background: Mutations in the *NF2* gene, which codes for the tumour suppressor Merlin, is responsible for the development of all Neurofibromatosis Type 2 (NF2)-related tumours including schwannomas, meningiomas and ependymomas. These tumours can also occur spontaneously in non-NF2 patients. The only available treatments for this group of tumours are surgery and radiosurgery. Hence, there is an urgent need for new effective drug-based treatments.

TAM family receptors are involved in tumour development, progression, metastasis and resistance to targeted therapies in several cancers. We have previously established that TAM family of receptors and their ligand Gas6 are overexpressed in schwannomas and AXL is responsible for promoting tumour development by altering proliferation, adhesion and survival of human schwannoma primary cells *in vitro* (Ammoun et al., 2014). Our recent study focus on exploring the role of TYRO3 and MERTK in schwannoma and all three TAM members in meningioma pathogenesis.

Methods: We investigated the expression and activation of TAM family receptors and ligand Gas6 in different grades of Merlin-deficient meningioma tissues and primary cells using western blotting. *In vitro* shRNA knock-down and pharmacological inhibition analysis were conducted using patient derived schwannoma and meningioma cells to understand the role of TAM receptors in the pathogenesis of tumour development.

Results: The results reveal overexpression and hyper-activation of AXL, MERTK, TYRO3 and their ligand Gas6 in Merlin-deficient grade-I, meningioma tissues and cells which decreases with progression to higher grades. The expression of AXL, MERTK and TYRO3, but not Gas6, appear to be Merlin-dependent in meningiomas. MERTK forms a complex with TYRO3 but not with AXL in both meningioma and schwannoma tissues, and its expression is mandatory to maintain AXL and TYRO3 levels in both cell-types. MERTK and AXL contribute to increased proliferation and survival of schwannoma and meningioma cells *in vitro.* Pathological proliferation and survival of both cell-types were successfully reversed by AXL inhibitor BGB324 (in phase-II clinical development) and MERTK inhibitor UNC2025 (pre-clinical) *in vitro*, with UNC2025 being more efficient. TYRO3 activation had no effect on cell proliferation of either schwannoma or meningioma cells.

Conclusion: AXL and MERTK are important in schwannoma and meningioma pathogenesis and are potentially good therapeutic targets. MERTK has the potential to be used as a common therapeutic target for the treatment of NF2-related tumours.

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Protein Tyrosine Phosphatase Inhibition Prevents Adaptive Resistance to MEK Inhibition in NF1 Associated Malignant Peripheral Nerve Sheath Tumors

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Neurofibromatosis type 1 (NF1)-associated malignant peripheral nerve sheath tumors (MPNSTs) are malignant sarcomas occurring in approximately 15% of NF1 patients. The mortality caused by these tumors is high, approaching 100% in patients with unresectable, metastatic or recurrent disease (1). Targeted inhibition of the MAP kinase (MAPK) pathway has been attempted in clinical trials using MEK inhibitors (MEKi). While emerging data are exciting, MEKi block MPNST progression only to a limited extent. Combination strategies targeting both the MAPK and PI3K/AKT pathways (horizontal inhibition) have had limited clinical success due to excessive toxicities which precludes delivery of optimal therapeutic concentrations. Thus, novel approaches and complementary treatment strategies that are tolerable and prevent adaptive resistance mechanisms in NF1 MPNST are urgently needed.

We have succeeded in establishing orthotopic *in vivo* models (patient-derived human xenografts-PDXs and genetically engineered mouse models- GEMMs) via implantation of tumor cells into the sciatic nerve, an approach that is critical, because it preserves the tumor microenvironment. Furthermore, we have optimized MPNST culture conditions, allowing us to establish robust primary cultures of MPNSTs while retaining key biological features of the original tumors. Using this preclinical testing platform, we demonstrate that –

1. A feedback loop mediated by induction of receptor tyrosine kinase (RTK) signaling becomes hyperactivated upon MEK inhibition (MEKi). This subsequently activates the MAPK pathway to such an extent that MEKi alone is unable to completely block signaling to ERK, thereby maintaining tumor proliferation.
2. SHP2 is a protein tyrosine phosphatase (PTP) that functions as a positive signal transducer, acting between RTKs and RAS and thus, functions as physiological mediator for RAS activation (9). A treatment paradigm where MEKi is combined with SHP099, an allosteric SHP2 inhibitor to counteract the rebound increase in RTK mediated signaling to RAS-MEK-ERK results in effective attenuation of MEK-ERK signaling. *In vivo* studies demonstrate that the drug combination exhibits synergistic effects on tumor growth inhibition and significantly prolongs survival.
3. SHP099 disrupts a functional complex (SOS1/GRB2) which is essential to RAS-GTP loading and inhibits RAS-mediated downstream MAP kinase signaling in NF1 MPNST.

Our results provide a mechanistic context for SHP2's precise role in the regulation of RAS-GTP and demonstrate that SHP2i can prevent adaptive resistance to MEKi. Given that SHP2i are currently in clinical trials (NCT03114319), our studies can be rapidly translated into a clinical trial to evaluate a combination of SHP2i and MEKi as a novel treatment approach in NF1-MPNSTs.

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Hippo-YAP Inhibitors Synergize with MEK and ERK Inhibitors in NF2 Deficient Schwannoma Cell Lines

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Neurofibromatosis type 2 (NF2) is an autosomal dominant tumor disorder caused by germline mutation in the tumor suppressor gene *NF2.* Individuals with NF2 primarily develop Schwannomas. However, standard treatments, including surgical excision and chemotherapeutic treatments, provide limited therapeutic effects and no effective targeted therapies for affected individuals are approved. A comprehensive pharmacological profiling of neurofibromatosis cell lines revealed little activity of most MEK inhibitors in human NF2-deficient tumor cells, HEI193 and KT21-MG1. A screen to a drug library of over 300 drugs found that knocking down Hippo-YAP signaling effector proteins YAP/TAZ sensitized NF2-deficient SC4 murine schwannoma cells to inhibitors of Ras, Raf, MEK, ERK, PI3K, mTOR, PAK, PLK, and others. We also tested novel Hippo-YAP signaling inhibitors that block auto-palmitoylation of the YAP partner TEAD. The Hippo-YAP signaling inhibitors were relatively inactive when used alone, but very active when they were used in combination with the MEK inhibitors, Selumetinib or Trametinib in NF2-deficient tumor cells. Less synergy was seen with related pathway inhibitors. These findings support studies with YAP TAZ knockdown cells suggest that suppressing Hippo-YAP signaling and MEK together may be an effective treatment for NF2 patients.

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Absence of Association Between the Size of Genome-Wide Copy Number Events and the Clinical Severity of NF1 Microdeletion Cases

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The most frequently observed *NF1* microdeletion is 1.4Mb in size, resulting from homologous recombination between two families of low copy repeats (NF1-REPs), and can be present in 5-10% of NF1 patients. The 1.4Mb copy number (CN) loss is typically associated with a severe clinical phenotype including cardiovascular malformations, learning disability, mental retardation, much earlier onset of development of cutaneous neurofibromas, skeletal abnormalities, dysmorphic facial features, and a much higher risk of developing a malignant peripheral nerve sheath tumour. It has been suggested that the deletion of genes adjacent to the *NF1* gene may act as disease modifiers and explain some of the clinical manifestations observed in 1.4Mb microdeletion patients, as NF1 patients with a 1.2Mb deletion have a milder phenotype and often show somatic mosaicism.

The aim of our study was to investigate whether the microdeletion phenotype might be associated with CN events elsewhere in the genome. DNAs from 22 *NF1* microdeletion patients (18 with a 1.4Mb CN loss, three with a 1.2Mb CN loss and one with a >5.5Mb CN loss) were analysed using Illumina Human 1M-Duo Beadchips. Five nuclear families comprising unaffected parents each with a proband carrying *de novo* 1.4Mb CN losses were analysed using Illumina Human Omni1-Quad Beadchips (Illumina). Common CN events overlapping with those noted in the Database of Genomic Variants, a comprehensive catalogue of structural variation in control data[1], or identified in a clinically normal parent were excluded from further analysis. The results did not reveal any additional CN events of significance in the five nuclear families' probands or in the 22 microdeletion patients. We then examined whether there was any association between microdeletion size and clinical features. Using the statistical software STATA 11 and *NF1* microdeletion length as a continuous variable, we show that learning difficulties are more likely to be associated with greater *NF1* deletion length ($p=0.025$; Mann-Whitney test). However, microdeletion length was not associated with any other clinical features. Because of the small sample size, this result must naturally be treated with caution, but our study demonstrates that CN events other than the primary *NF1* microdeletion are unlikely to be modifiers of clinical severity in NF1 patients with 1.4Mb deletions.

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A Network-Based Workflow to Identify Drug Target Networks from Gene Expression Data

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Identification of drug targets from preclinical 'omics data remains a chronic challenge within the NF research community due to inherent noise in genomic assays, inability to generalize experiment-specific data to a broader context, and difficulty in determining relationships between gene expression and pathway dysregulation. Network-based algorithms can be used to address these points[1] by integrating 'omics data with published molecular interaction data and selecting pathways that are relevant to the disease of interest. Integration of protein-protein, protein-DNA[2] or protein-RNA[3] interactions together with network refinement approaches such as those based on network flow[4], belief propagation[5], or other graph-theoretical constructs such as Steiner Trees, provide distinct interpretations of molecular data that can enable scientists to develop novel testable hypotheses.

In this work, we built upon existing network tools to create the Drug-Target Expression Network (DTEN), a tool that incorporates drug-target interactions into network-optimization algorithms to identify drugs for Neurofibromatosis Type 1 (NF1)[6]. Specifically, we built a scientific workflow comprised of existing network tools[7,8,9] and customizations to compare these data and identify specific drugs that putatively target these diseases.

We applied this approach to transcriptome data from plexiform neurofibromas (pNFs), malignant peripheral nerve sheath tumors (MPNSTs), and other NF1-linked tumor types to identify specific sub-networks that uniquely mapped to biological pathways in all NF1-related tumor types. Preliminary analysis has identified numerous interesting results. One network identified in MPNSTs was found to be enriched in proteins that partake in MAP kinase signaling and cellular stress responses, including DUSP22, a gene that was recently implicated in MPNST growth[10]. One of the small molecules identified in this network, PF-3758309, has been independently shown to inhibit MPNST cell lines. Similarly, a second network unique to pNFs had proteins involved in complement and NOTCH signaling pathways and contained ENMD-2076, a small molecule that was predicted to be efficacious in pNFs and also showed evidence of inhibiting pNF cell culture activity. We are currently enhancing the method to better rank DTEN-identified sub networks that are of interest to specific tumor types.

In summary, the integration of condition-specific gene expression data using protein-protein and protein-drug interactions in a single workflow is one approach to guide interpretation of rare disease gene expression datasets such as those in NF. The DTEN tool is currently under development at https://github.com/Sage-Bionetworks/dten and is available to the community.

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Molecular Mechanisms of Targeted Therapy Resistance in Malignant Periphreal Nerve Sheath Tumors

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Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are aggressive, highly chemoresistant sarcomas that are a leading cause of death in patients with Neurofibromatosis Type 1 (NF1). Deregulated RAS signaling caused by loss of neurofibromin, a RAS-GAP, is at the center of MPNST pathogenesis. MEK is a key RAS effector, and several clinical trials for MEK inhibitors in NF1-related tumors are underway. Unfortunately, we and others have shown that MEK inhibition alone is not effective at fully suppressing tumor growth in preclinical models of MPNST. Broadly, the goals of this study are 1) determine the genetic and molecular mechanisms that drive MEK inhibitor resistance and 2) identify and test novel drug combinations in preclinical models of MPNST. Resistance to kinase inhibitors is often promoted by adaptive kinome reprogramming of vital oncogenic signaling networks, such as receptor tyrosine kinase (RTK) or AKT/mTOR activation. We recently discovered that activation of the RTK MET promotes resistance to MEK inhibitors and that cotargeting MEK and MET prevents kinome reprogramming and tumor growth in MPNST mouse models. However, p53-deficient MPNSTs were less responsive to combined inhibition and upregulated AKT in response to drug treatment. We hypothesize that p53 deficiency promotes kinome reprograming and AKT activation in MPNSTs and that targeting these adaptive signaling changes will sensitize MPNSTs to MEK inhibition. To test this hypothesis, we have created several p53 deficient isogenic MPNST cell lines with varying sensitivity to MEK inhibition. We found that p53 deficient cells upregulate AKT by a MET dependent mechanism and that inhibiting mTOR sensitizes cells to MEK inhibition. Currently, we are characterizing the phosphoproteome and gene expression changes that drive resistance. Understanding how genetic alterations contribute to therapy response in MPNSTs is critical for the development of effective drug combinations as well as stratification of patients in clinical trial design and interpretation.

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Merlin's Role in Muscle Stem Cells

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NF2 patients often present a slow but progressive distal muscle atrophy and paresis in later stages of the disease. In a previous study we showed that Merlin-deficient neurons after sciatic nerve crush injury showed a proper reinnervation concomitant with no difference in muscle weight and a regular architecture of muscle fibers[1]. Therefore we started to investigate the role of Merlin in muscle stem cells (MSCs) after injury and during *in vitro* myogenesis. Strikingly Merlin isoform 2 expression increased during differentiation of myoblasts. Knockdown of Merlin expression during myogenic differentiation resulted in changed fusion index and myotube diameter. Moreover we could show that loss of Merlin results in deregulation of a major signaling pathway during *in vitro* myogenesis. To further investigate the functional relevance of Merlin in MSCs *in vivo* investigated the regeneration of the TA muscle of Pax7- creER;Nf2flox/flox mice after injury. Loss of Merlin expression in adult MSCs affected the regeneration and MSC numbers at 7 dpi.

This is the first study that could show that Merlin isoform 2 plays a crucial role in MSCs and during myogenic differentiation. Furthermore our study suggests that Merlin is important for the regeneration of the skeletal Muscle after injury and MSC function. Moreover we could already identify one crucial signaling hub involved in MSC activation and maintenance of muscle mass, to be regulated by Merlin in MSCs. This pathway could be one potential target for the treatment of muscle atrophy in NF2 patients.

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Circulating Free Nucleic Acids in Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is associated with a highly elevated lifetime risk of cancer. The syndrome is characterized by cutaneous neurofibromas which always maintain their benign phenotype, and plexiform neurofibromas that can undergo malignant transformation.

Measurements of circulating free plasma DNA (cfDNA) are gaining wider applicability in cancer diagnostics, targeting of therapy and monitoring of therapeutic response. The presence of malignant cells is known to increase the cfDNA concentration. As cfDNA assays are becoming part of routine oncology practice, also NF1 patients are likely to be followed up using this method. However, it is not currently known, whether the baseline levels of cfDNA differ between NF1 patients and healthy controls, e.g., due to neurofibromas.

Methods: The study was approved by the local ethical committee and it was pre-registered in ClinicalTrials.gov with identifier NCT02680431. All participants provided written informed consent. Peripheral blood samples were drawn from 21 patients with NF1 and 14 healthy controls. Plasma was isolated after Ficoll-Paque Plus (GE Healthcare) gradient centrifugation. Samples with visually detected hemolysis were excluded. Protein concentrations were assayed using Pierce BCA Protein Assay Kit (Thermo Scientific). The cfDNA was isolated with QIAamp Circulating Nucleic Acid Kit (Qiagen) and quantified using the Qubit 2.0 fluorometer and Qubit dsDNA HS Assay Kit (Invitrogen). The cfDNA concentration of each sample was normalized relative to the plasma protein concentration.

Results: The normalized median concentration of cfDNA in plasma was 19.3 ng/ml (range 6.6 to 78.8) among the NF1 patients and 15.9 ng/ml (range 4.8 to 47.1) in the control group. The plain difference between NF1 and controls was not statistically significant ($P=0.369$) but age-adjusted analysis indicated higher concentration in NF1 compared to controls ($P=0.023$). Among NF1 patients only, a moderate negative correlation between cfDNA concentration and age was observed (Spearman's $\rho = -0.55$). NF1 patients with a plexiform neurofibroma had non-significantly elevated cfDNA concentration compared to NF1 patients without known plexiform neurofibromas (median 25.5 vs 18.9, $P=0.12$).

Conclusions: The results suggest that NF1 is associated with increased plasma cfDNA concentration, possibly related to plexiform neurofibromas. The effect of NF1 on cfDNA is small compared to concentrations reported for patients with cancer, yet NF1 may need to be taken into account in reference values of cfDNA measurements. Moreover, patient age should be considered in cfDNA assays from NF1 patients.

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In Vivo Targeting of Physical and Genetic Vulnerabilities in Malignant Peripheral Nerve Sheath Tumors (MPNST) and Neurofibromas

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Neurofibromatosis Type 1 (NF1) predisposes individuals to developing an array of malignancies including Malignant Peripheral Nerve Sheath Tumors (MPNST). These aggressive soft tissue sarcomas have poor prognostic outlook in individuals where surgical resection is not feasible. While many therapeutic avenues have been explored, little improvement has been observed[1]. A multi-faceted approach is needed to determine the physical and genetic strengths/vulnerabilities of these tumors to provide clinicians with a more robust toolbox to treat these tumors.

As part of the CTF Synodos project, our lab implemented a high-throughput small molecule screen of *NF1* mutated and isogenic wild type human Schwann cells. The *NF1* and/or *SUZ12* mutant selective hits fell in to broad classes. Representative drugs were chosen for further *in vivo* study including: DMAPT (Parthenolide derivative), Selumetinib (MEK inhibitor), LB100 (Cantharidin derivative – PP2A inhibitor), Digoxin (Cardiac Glycoside – Increases intracellular calcium), and Vorinostat (HDAC inhibitor). These, along with some drugs chosen from the literature and additional screening (including Rigosertib - a microtubule destabilizer/potential RAS mimetic), were moved in to a variety of models for further validation. S462-TY human MPNST cell line flank-injected xenografts in immunodeficient NRG mice were enrolled on therapy. We also utilized our previously described genetically engineered mouse (GEM)-PNST model consisting of *Dhh-Cre;Nf1fl/fl;Ptenfl/fl.*[2] This model manifests multi-focal, 100% penetrant peripheral nerve sheath tumors with a median lifespan of 18 days that can be used for rapid readout of increased survival. Finally, we have established several Patient Derived Xenografts from MPNST samples collected at the University of Minnesota Hospital.

Promisingly, every monotherapy enrolled in the Xenograft or GEMPNST cohort displayed a marked and statistically significant increase in survival. Several of the targeted therapies including Digoxin, LB100, Rigosertib, and Vorinostat, increased median survival latencies in the Xenograft model by 6-9-fold. Similarly, the GEMPNST cohorts enrolled on DMAPT, Digoxin, LB100, or Selumetinib all showed statistically significant increased median survival. Continuing work pursuing these therapies in combinations to further control tumor growth and delay development of resistance and treatment escape is ongoing. We are currently enrolling PDX models of known genetic makeup to validate drug functionality in a larger representative sampling. Further work targeting physical barriers of the desmoplastic extra cellular matrix (ECM) in these tumors (specifically, high levels of Hyaluronic Acid leading to collapsed vasculature) will be pursued to improve the penetrance and efficacies of these drugs. Together, these approaches generate a cohesive and broad approach to treatment discovery in this aggressive sarcoma.

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Disclosures: Dr. Largaespada is the co-founder and co-owner of several biotechnology companies including NeoClone Biotechnologies, Inc., Discovery Genomics, Inc. (recently acquired by Immunsoft, Inc.), and B-MoGen Biotechnologies, Inc. (recently acquired by Biotechne Corporation) He consults for Genentech, Inc., which is funding some of his research. Dr. Largaespada holds equity in and serves as the Chief Scientific Officer of Surrogen, a subsidary of Recombinetics, a genome-editing company. The business of all these companies is unrelated to the contents of this manuscript. Other authors have no conflict of interest to disclose

GABAergic Deletion of NF1 Induces Complex Metabolic and Behavioral Phenotypes

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Children with NF1 often display cognitive deficits in visuospatial, motor, language, and memory associated with significant delays in neurodevelopmental milestones. Previous data from conditional knockout mice using the Dlx5/6-Cre driver line targeting GABAergic neurons of the forebrain indicated that neurofibromin plays a role in GABAergic neurons with selective deletion of NF1 in GABAergic cells leading to deficits in long-term potentiation (LTP) and spatial learning. Upon aging, we noted that conditional knockout of NF1 floxed alleles using Dlx5/6-Cre led to a rapid onset of obesity with both males and females weighing >55g by 20 weeks of age. Experimental NF1$^{fl/fl}$;Dlx5/6-Cre (DlxKO) mice when compared to wild type (WT) control animals display a complex metabolic phenotype wherein younger (<7wks of age) DlxKO mice are hyperphagic, yet also have increased energy expenditure (total and resting) and are hyperactive (both ambulatory and fine movements), particularly at the end of the dark cycle. DlxKO mice display a disturbed circadian feeding pattern with increased feeding bouts and continuous feeding even throughout the daytime period; however, older DlxKO mice are relatively inactive once obese per wheel running assays. DlxKO mice are polyuric and polydipsic due to overt diabetes with hyperglycemia (fasting glucose 138 mg/dL vs 95 mg/dL WT), hyperinsulinemia (20.1 ng/ml vs 0.6 ng/ml WT), and impaired glucose tolerance (GTT). Immunohistochemical analysis of pancreas indicated DlxKO mice have abnormal islets with beta cell hyperplasia accompanied by loss of alpha cells when staining for insulin and glucagon, respectively. DlxKO mice also displayed disturbed nest-building behaviors with two independent enrichment beddings. Overall, the data indicates that NF1 plays a previously unappreciated role in modulating energy balance due to expression in GABAergic neurons, and the relatively rapid and robust phenotype seen in the DlxKO model provides a new platform to assess both metabolic and behavioral functions of NF1.

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Inhibition of 4E-BP1 Phosphorylation by DNA Topoisomerase I-Targeted Drugs Plus mTOR Kinase Inhibitors in Malignant Peripheral Nerve Sheath Tumors

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are very aggressive and metastatic soft tissue sarcomas that frequently arise in patients with neurofibromatosis type 1 (NF1). Most of these tumors are unresectable at diagnosis and minimally responsive to conventional treatment. Therefore, identification of new genetic dependencies and drugs with anti-tumor activity will be critical to advance MPNST therapy.

Methods: To identify effective compounds, we examined a series of candidate agents for their ability to induce apoptosis in MPNST cells arising in *nf1/tp53-*deficient zebrafish. The compounds showing drug response in the embryonic implantation assay were evaluated by various experimental methods in human NF1-associated and sporadic MPNST cell lines.

Results: After testing a series of drugs, we found that DNA topoisomerase I-targeted drugs and mTOR kinase inhibitors were the most effective single agents in eliminating MPNST cells without prohibitive toxicity. In addition, three members of these classes of drugs, either AZD2014 or INK128 in combination with irinotecan, acted synergistically to induce apoptosis both *in vitro* and *in vivo.* In mechanistic studies, irinotecan not only induces apoptosis by eliciting a DNA damage response, but also acts synergistically with AZD2014 to potentiate the hypophosphorylation of 4E-BP1, a downstream target of mTORC1. Profound hypophosphorylation of 4E-BP1 induced by this drug combination causes an arrest of protein synthesis, which potently induces tumor cell apoptosis. Depletion of 4E-BP1 in NF1-associated MPNST cells abolished the synergy between DNA topoisomerase I-targeted drugs and mTOR kinase inhibitors in killing MPNST cells, establishing cooperative effects driving 4E-BP1 hypophosphorylation as the mechanism of synergy.

Conclusions: The synergy between DNA topoisomerase I-targeted drugs and mTOR kinase inhibitors in our MPNST model appears to be mediated through the mTORC1/4E-BP1 signaling pathway, which regulates the translation of cap-dependent RNAs. Our findings provide a compelling rationale for further *in vivo* evaluation of the combination of DNA topoisomerase I-targeted drugs and mTOR kinase inhibitors against these aggressive nerve sheath tumors.

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Targeting RABL6A-RB1 Signaling Suppresses MPNST Pathogenesis

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Background: Malignant peripheral nerve sheath tumors (MPNSTs) are deadly sarcomas that lack effective therapies. Greater insight into MPNST pathogenesis is needed to develop new, more targeted treatments. In most MPNSTs, the retinoblastoma (RB1) tumor suppressor is disabled by hyperactivation of cyclin dependent kinases (CDKs), commonly through loss of CDK inhibitors such as p27(Kip1). RABL6A is an oncogenic GTPase and newly recognized inhibitor of the RB1 pathway whose role in MPNST biology has not been studied. We hypothesized the RABL6A-RB1 pathway is an important, new therapeutic target and critical driver of MPNST pathogenesis.

Methods: RNA-Seq and immunohistochemical analyses of tissue microarrays (TMAs) containing human patient-matched PNFs and MPNSTs were conducted. RABL6A-RB1 pathway silencing was performed in multiple MPNST cell lines. Analyses included: western blotting of RABL6A-RB1 pathway, proliferation and viability, and drug response assays. Orthotopic xenografts were generated using MPNST cell lines in immunocompromised mice. Mice were treated with CDK inhibitors (alone or in combination) and tumor growth was monitored. Western blotting and immunohistochemical analyses were conducted on tumor samples.

Results: We discovered dramatic upregulation of *RABL6A* mRNA and protein in human MPNSTs compared to precursor neurofibromas. Remarkable overlap existed between the RABL6A gene expression signature and that found in the patient MPNSTs. Tumor expression of p27 and RABL6A proteins was inversely correlated. Silencing RABL6A in MPNST cell lines caused cellular death and G1 phase arrest that coincided with p27 upregulation, CDK downregulation, and RB1 activation. The growth suppressive effects of RABL6A loss, and its regulation of RB1, were largely rescued by p27 depletion. These findings establish a critical role for RABL6A-p27-RB1 signaling in MPNST pathogenesis. Indeed, reactivation of RB1 using a CDK4/6 inhibitor (palbociclib) killed MPNST cells *in vitro* in a RABL6A-dependent manner and suppressed MPNST growth *in vivo.* Resistance to CDK4/6 inhibitor monotherapy became apparent towards the end of treatment, which has been previously attributed to compensation via CDK2. Combination of palbociclib plus dinaciclib, a CDK2 inhibitor, showed that dual CDK targeted therapy prevented tumor growth more effectively than single agent treatment.

Conclusions: This work identifies RABL6A as a powerful driver of MPNST proliferation and survival that acts, in part, through p27-RB1 inactivation. Our findings suggest that RB1 reactivation in tumors using multiple CDK inhibitors may reduce drug resistance and effectively treat MPNSTs.

Mammary Cancer and Behavior Characterization in a Patient Specific Neurofibromatosis Type I Rat Model

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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 (KI), associated in humans with spinal NF1, as well as a 14 base pair deletion c.3661_3674del, p.P1220fs*1223 (KO) model were generated.

Methods: KO and KI rat models were created using two CRISPR guides and a dually compatible 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).

Results: Three of seven pups born were positive for CRISPR activity from which independent *Nf1* knock-out (P1220fs*1223) and missense knock-in (R1276Q) colonies were established. Unmated heterozygous *Nf1* R1276Q female rats do not display spontaneous tumors; however, mated females rapidly developed mammary tumors within two weeks of pregnancy. Heterozygous *Nf1* P1220fs*1223 females develop tumors spontaneously at sexual maturity with acceleration of tumor formation post-mating. Homozygous mutant offspring have not been detected in litters born from intercrossing of heterozygous *Nf1* mutant rats. Male rats do not display significant cognitive deficits or behavioral differences when assessed at 6 months of age with any of the tasks performed.

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. The mammary tumors are consistent with cribriform 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.

Novel Patient-Specific Mutation Rodent Models of Neurofibromatosis Type I

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Background: Animal models of human diseases such as neurofibromatosis type 1 (NF1) are essential for preclinical studies of therapeutics. Development of these models has accelerated greatly due to recent advances in genome editing technologies. Rodent NF1 models developed to date represent missense, nonsense, frameshift and splicing mutants to create a suite of models amenable to intervention with therapeutics of different classes (i.e. c.1466A>G; p.Tyr489Cys splicing modulation with antisense oligonucleotides, c.2542G>C; p.Arg681X with nonsense suppression). Models were selected from multiple genotype-phenotype correlation groups including less severe p.M992del and p.Arg1809Cys variants, and more pathogenic variants p.Gly848Arg and p.Arg1276Gln associated with familial spinal NF1.

Methods: Mouse and rat *Nf1* genes are targeted by pronuclear microinjection or electroporation of CRISPR/Cas9 and Cpf1 reagents with repair templates to generate founder animals. Both traditional targeting vectors and CRISPR reagents are used to modify mouse *Nf1* in embryonic stem cells. Founder animals or chimeras are outcrossed to establish germline transmission and independent colonies. Mutant alleles are tested for viability when homozygous as well as tumor formation when placed in the proper genetic context.

Results: Mouse models developed to date include c.2041 C>T (p.Arg681X), c.2542G>C (p.Gly848Arg), c.2393_2408del16, c.2919_2920insTT, c.2446C>T (p.Arg816X), c.5425C>T (p.Arg1809Cys), c.1466A>G (p.Tyr489Cys), c.2970-2972delAAT (p.M992del), and c.499_502delTGTT. Rat models created include the c.3827G>A (p.Arg1276Gln) mutation and a 14bp deletion model.

Conclusions: Multiple patient-specific alleles have been successfully generated in rodents using ES cell and CRISPR based approaches. Genotyped stillborn pups have shown high contribution of knock-in and knock-out mutant alleles, consistent with embryonic lethality from biallelic loss of NF1. The c.2041 C>T; p.Arg681X, c.5425C>T (p.Arg1809Cys), and c.2393_2408del16 mouse models are embryonic lethal when homozygous, whereas the homozygous c.2542G>C; p.Gly848Arg mice are viable with no gross phenotype despite reduced neurofibromin levels. Both the c.3827G>A (p.Arg1276Gln) mutation and 14bp deletion models in the rat appear to be embryonic lethal; however, these results may be confounded by severe mammary gland adenocarcinoma induced by pregnancy.

Modeling PRC2 Perturbation in *NF1*-Deficient Human Schwann Cells Reveals Potential Therapeutic Targets in Malignant Peripheral Nerve Sheath Tumors

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Neurofibromatosis Type 1 (NF1) patients are predisposed to development of many types of cancers and certain benign tumors. The most highly penetrant of these are tumors of Schwann cell in origin and located in the peripheral nervous system. These include malignant peripheral nerve sheath tumors (MPNST), which are among the deadliest of the soft tissue sarcomas, with 5-year survival rates of ~20 percent. Loss of function mutations in Polycomb Repressive Complex 2 (PRC2) genes, such as *SUZ12,* are commonly identified in NF1-associated and sporadic MPNSTs. This is highly suggestive that perturbation of epigenetic homeostasis plays a role in malignant transformation of neurofibromas. Dysregulated chromatin remodeling caused by the loss of PRC2 in MPNSTs likely confers novel vulnerabilities, that if identified could be exploited therapeutically. Using engineered human Schwann cells, our lab performed drug screens and discovered several compounds that selectively kill NF1- and/or SUZ12- deficient cells. Of those compounds, we chose a MEK and an HDAC inhibitor as well as a cardiac glycoside for further studies. To recapitulate *SUZ12* loss, we used siRNAs to target and knock down *SUZ12* expression. *NF1*-deficient cells with reduced *SUZ12* were more sensitive to our discovered drugs. Moreover, total loss of SUZ12 in CRISPR/Cas9-edited cells further sensitized them to these compounds. A third approach we took was to inhibit EZH2, the catalytically active subunit of PRC2. Here again, cells exposed to the EZH2 inhibitor were more sensitive to our compounds than cells that did not have the inhibitor present. In each of these MPNST-like genetic and epigenetic contexts, we performed combination drug studies to determine which showed synergism. The MEK inhibitor in combination with the cardiac glycoside exhibited high synergy and killed PRC2-perturbed cells more effectively than WT cells. Monotherapies commonly fail in clinical trials thus we believe a combination therapy is likely to be required to have effective and lasting results in reducing or eliminating MPNSTs. Testing cells of an appropriate genetic background is fundamental in assaying drug compounds that could have true translational relevance. Our isogenic cells and studies reflect such a need by recapitulating an MPNST-like genotype. Our initial drug discoveries of a cardiac glycoside in combination with a MEK inhibitor offers confidence that MPNSTs harbor specific vulnerabilities which can be targeted. We have moved the top compounds from our screens forward to testing in *in vivo* animal models of MPNST with the overall goal of informing future human clinical trials.

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Characterization of T Cells in Human Cutaneous Neurofibromas

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Background: Cutaneous neurofibromas (cNFs) manifest in almost every patient with neurofibromatosis type 1 (NF1). Emerging evidence suggests that *NF1* deficiency may affect immune cells, but the role of T cells in the cNF tumorigenesis remains to be elucidated. The aim of this study is to characterize and quantitate tumor-infiltrating leukocytes in cNFs.

Methods: The presence of CD3, CD4, and CD8 positive cells were studied by immunolabeling of cNF samples derived from NF1 patients. CD3 is expressed by all mature T-cells, CD8 is used as a marker for cytotoxic T cells while CD4 labels regulatory T cells, monocytes and macrophages.The quantities of cells positive for CD8, CD4 or CD3 were determined in 61, 56, and 43 cNF samples respectively. The immunolabeled tissue samples were digitized and the tumor area of each sample was annotated using a quantitative pathology software, QuPath. Detection of positive cells was conducted using the machine learning based tissue quantification implemented in Orbit Image Analysis software. In addition, the images were evaluated visually to detect clusters of positive cells.

Results: Intratumoral T cells were detected in all of the samples. The median cell densities (cells/mm^2) of CD3+ and CD8+ T cells were 119 (range 28.5–1053) and 75 (range 3.5–1220), respectively. The density of CD4+ cells in the samples was 849 (range 277–1676). CD8+ cells were scarce in tumors with <700 CD4+ cells/mm^2.

CD4 positive cell clusters were observed in 55/56 samples (98.2%), CD8 positive cell clusters in 34/61 samples (55.7%) and CD3 positive cell clusters in 29/43 samples (67.4%). The accumulation of CD4 positive cells at the border of tumor and skin was observed in 32/56 samples (57.1%), CD8+ cells in 24/61 samples (39.3%), and CD3+ cells in 18/43 samples (41.9%).

Conclusions: The results suggest that CD4+ cells are the most abundant leukocytes in cNFs. Since the total number of T cells, as demonstrated using antibody to CD3, is clearly smaller than the number of CD4 positive cells, the majority of CD4 positive cells in cNFs are monocytes or macrophages. The majority of T cells are CD8 positive cytotoxic T cells. Clusters of CD8 and CD3 positive cells were observed in over half of the samples and CD4+ clusters in almost all samples. The clustering of CD8+ T cells has been associated with cytotoxic activity towards tumor cells. Further studies will increase the understanding of the role of leukocyte subpopulations of cNFs.

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Repurposing CFTR Modulators for the Treatment of Neurofibromatosis Type I

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Background: Although MEK inhibitors have been partially successful for plexiform tumors, no single approach to treatment has been sufficient to achieve maximal clinical benefit. A mutation-targeted approach may therefore act in synergy with inhibition of Ras signaling. Determining how *NF1* pathogenic variants affect protein expression and thereby alter RAS-signaling can aid in developing future treatments. Small molecules that alter the function of the cystic fibrosis transmembrane conductance regulator (CFTR) have been successful in treating individuals with cystic fibrosis. Some of these drugs may be non-specific, however, and may work with other proteins, such as neurofibromin. Correctors function as protein chaperones to modulate protein conformation, enhance protein folding and assembly. Potentiators may act to increase conformational flexibility. Repurposing CFTR modulators is an enticing option for individuals with NF1 who have germline missense mutations or small in-frame deletions that may lead to full-length but unstable or non-functional protein (~18% of patients). **We hypothesize that CFTR modulators can be repurposed to stabilize and increase function of specific *NF1* mutations**.

Methods: We evaluated a panel of *NF1* missense and small in-frame indel variants for their effect on neurofibromin levels (protein stability) and Ras activity (GRD function) using our full-length cDNA expression system in neurofibromin-null HEK293 cells. We also treated cells transfected with these variant cDNAs with tezacaftor, lumacaftor, ivacaftor and combinations to determine effects on NF1 levels and Ras signaling.

Results: First, we classified specific *NF1* variants. Some variants, including C379R, W784R, G848R, and L1957P, lead to lower levels of NF1 protein, but retain GRD activity; these may benefit from treatment with correctors (tezacaftor and lumacaftor). Other variants, including DelM992 and R1809C, resulted in relatively stable NF1 protein levels but were incapable of inhibiting Ras-singling; these may benefit from treatment with a potentiator. Second, we analyzed mutations with and without modulators. While we see a spectrum of activity, lumacaftor increased G848R NF1 levels by 38%, and ivacaftor decreased delM992 pERK levels by 15%.

Conclusions: We suggest a new paradigm for thinking about *NF1* mutations that also includes several classes based on functional effects and allows for the development of personalized therapeutic strategies. Repurposing of CFTR modulators for NF1 therapy may be possible as correctors increase NF1 levels and potentiators increase NF1 function to suppress Ras activity.

Contractile Force Generation of Fibroblasts from Patients with Neurofibromatosis Types 1 and 2

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Introduction: Cells generate contractile forces via their acto-myosin cytoskeleton and transmit those forces to the extracellular matrix (ECM) via transmembrane proteins linked to the actin cytoskeleton by linker proteins. Contractile forces are necessary for reinforcing attachments with the matrix, migration, and ECM remodeling. The ability of cells to generate healthy magnitudes of contractile forces may be compromised when affected by genetic alterations associated with certain diseases. For example, neurofibromatosis types 1 and 2 (NF1 and NF2) are tumor predisposition genetic disorders in which the proteins encoded (neurofibromin and merlin, respectively) are both likely to effect force generation and transmission functionality. Here, we use collagen contraction assays and traction force microscopy (TFM) to measure and compare force generation and transmission ability between healthy, NF1+/-, and NF2+/- fibroblasts.

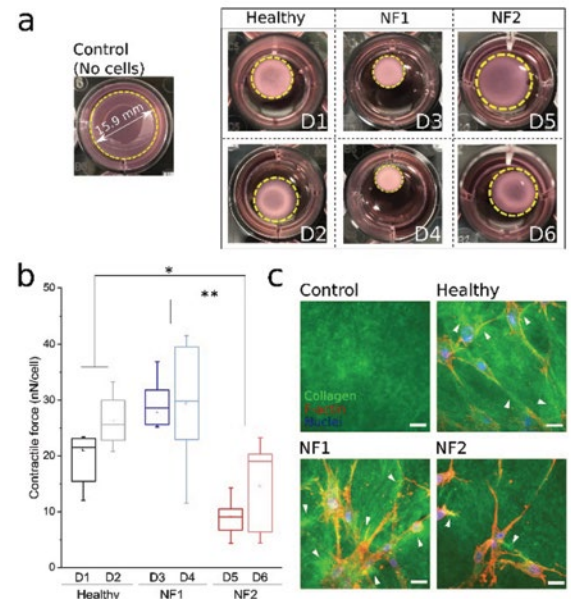**Materials and Methods:** NF1 haploinsufficient (NF1+/-) fibroblasts, NF2 haploinsufficient (NF2+/-) fibroblasts, and healthy fibroblasts, all isolated from patient tissues, were used in this study. Relative comparisons of force generation between these cell types were ascertained via collagen contraction assays. Briefly, cell-containing collagen solutions (2.4 mg/mL) were transferred into wells of a 24-well plate (0.5 mL per 15.9 mm-diameter well). After 24 hours of incubation, the diameters of the fibroblast-contracted collagen gel discs were measured. With the dimensions of the collagen discs before and after being contracted by the fibroblasts, we estimated the contractile force per cell for each of the conditions tested. Dynamic and subcellular localized force generation was studied with single cell 3D TFM. The actin of fibroblasts was labeled with CellLight Actin-RFP (Thermo) and then the fibroblasts were co-embedded with green fluorescent micro-beads (1 μm) in collagen I hydrogels. After 24 hours, time-lapse confocal images were captured every 15 minutes for 3 hours. Microbead displacements were analyzed and exported for analysis with internally generated Matlab codes.

Figure 1. Collagen contraction assay. (a) Images of the fibroblast cell-laden collagen disks. The three fibroblast types were from six donors (D1-D6). (b) The contractile force per cell was estimated based on the collagen gel deformation. (*$P < 0.01$, **$P < 0.05$, based on one-way ANOVA with Tukey's HSD post hoc). (c) Laser scanning confocal images of the cells in 3D collagen matrix. White arrowheads indicate local alignment of collagen fibers due to cell contraction. Scale bars are 25 μm.

Results and Discussion: The collagen contraction assay provided relative comparisons of force generating capabilities between the three cell types (healthy, NF1+/-, and NF2+/-). Here, we present the average contractile force per cell for two different donors per cell type and all three cell types (Figure 1). Our results indicate that NF2+/- fibroblasts generate significantly less force, on average, than healthy or NF1+/- fibroblasts. To accurately measure force generated by these fibroblasts and further relate cell traction force to cell migration behavior, we investigated the traction force of individual cells using advanced single-cell traction force microscopy. Our measurements enable us to characterize the force dynamics involved in cell migration process.

Conclusion: We presented the first comparison of force generation from NF1+/- and NF2+/- fibroblasts. From NF2+/- fibroblasts generated significantly lower traction force than NF1+/- and healthy fibroblasts. This result suggests that loss of merlin due to NF2 mutations decreases force transmission capability.

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Characterization of Mast Cells in Human Cutaneous Neurofibromas

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Background: Mast cells are recognized components of cutaneous neurofibromas (cNFs), yet systematic qualitative and quantitative characterization of the mast cell population in these tumors is incomplete. Since mast cells can have both anti- and pro-tumorigenic effects in cancer, they may be important players in tumor biology also in NF1. Studies of murine plexiform neurofibromas have shown that *NF1* deficiency affects the interplay between mast cells and tumor cells.

Mast cells can be divided into subtypes based on their protease content: the MC_T type contains only tryptase, MC_C only chymase while the MC_{TC} type produces both chymase and tryptase. Dermal mast cells are of MC_{TC} and MC_T type. CD117 (Kit) is a commonly used surface marker for mast cells. Protease-activated receptor-2 (PAR-2) is expressed by mast cells and plays a role in inflammation and immune response in many tissues including the skin. This study investigates the number of mast cells and characterizes them in human cNFs using antibodies to CD117, tryptase, chymase and PAR-2.

Methods: A total of 61 formalin-fixed, paraffin-embedded archival samples of human cNFs from 10 patients with NF1 (5 females, 5 males) were immunolabeled for the mast cell markers CD117, tryptase and chymase. The sections were digitized and positive cells were counted using Orbit image analysis software. Moreover, PAR-2 expression in fresh-frozen cNF samples was evaluated in combination with toluidine blue staining.

Results: The average numbers of cells positive for CD117, tryptase and chymase observed in cNFs were 299 (SD 177), 278 (SD 147) and 299 (SD 195) cells/mm^2, respectively. Small cNFs had higher mast cell content than larger tumors. The mast cell density varied 2-3 fold between tumors from different individuals, yet it did not correlate with patient sex. Tumors from patients <40 years of age had higher mast cell density than tumors from older patients. In preliminary studies PAR-2 expression was observed in some but not all cNF mast cells.

Conclusions: The results confirm that mast cells are an abundant component of human cNFs. The MCTC type is the predominant mast cell type in cNFs, similarly to healthy skin. Observing PAR-2 expression in only a subset of cNF mast cells indicates the presence of different subpopulations. Basic knowledge on cNF mast cells is important since it may be used further to elucidate whether cNF mast cells could be used as therapeutic targets.

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Identification of Pathways Triggered by Schwann Cell-Fibroblast Interactions Driving Cutaneous Neurofibroma Growth

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Background: Cutaneous neurofibromas (cNFs) are benign tumors that originate in the peripheral nervous system and are a hallmark of Neurofibromatosis Type 1 (NF1). Almost all NF1 patients develop from tens to thousands of cNFs constituting a great impact on their quality of life. There are currently no effective cNF therapies beyond their removal. cNF are composed by different cell types, Schwann cells (SCs), fibroblasts (FB), infiltrating immune cells, etc. They originate through the *NF1* inactivation in SCs although tumor microenvironment seem to play also a significant role. The importance of heterotypic interaction between SCs and FBs is not well understood.

Aim: To identify a cell type-specific expression profile in cNFs produced by heterotypic interactions between SCs and FBs. To translate this expression into signalling pathways and functionally test their role in cNF development.

Methods and Results: We set up an RNA-Seq experiment to obtain and compare the expression of cNFs, single SC and FB cultures and co-cultures of SC and FBs in order to identify a cell type-specific expression profile in cNFs produced by heterotypic SC-FB interactions. We characterized the *NF1* mutational status (Next Generation Sequencing, Microsatellite Multiplex PCR Amplification analysis) of 5 independent cNFs. We selectively cultured *NF1*(-/-) SCs and *NF1*(+/-) FBs from the 5 cNFs and sequenced and analyzed their exome to determine all coding genetic variation. To set up the RNA-Seq experiment, we previously defined SC-FB co-culture conditions, tested an efficient RNA-extraction methodology, used flow cytometry analysis for determining co-culture proportions of cells, etc.

Conclusions: We performed an RNA-seq experiment that consisted in obtaining and comparing the expression of cNFs, single SC and FB cultures and SC-FB co-cultures from different cNFs. We are in the process of obtaining NGS data and analysing results. Genetic variation in the coding region of the expressed genes in SC-FB co-cultures will allow us to identify a cell type-specific expression profile in cNFs produced by heterotypic SC-FB interactions.

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Metabolic Profiles of MPNSTs Are Dependent Upon Tumor Suppressor Status

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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; however, MPNST metabolism is not well-studied. The overall goal of this project is to define the unique metabolic pathways that are disrupted in MPNSTs and to understand the impact of distinct genetic mutations on these metabolic alterations. Mutations in the tumor suppressors *P53* and *CDKN2A* can alter cellular metabolism. 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. *P53* exhibits numerous roles in cellular metabolic regulation, while the metabolic role of *CDKN2A* is less understood. We used somatic CRISPR/Cas9 technology to generate primary mouse models of MPNST with disruptions in *Nf1/p53* or *Nf1/Cdkn2a*. These tumors are generated in paired littermate mice, allowing for precise comparison of metabolic profiles. Tumor initiation rates were different between the two models; however, tumor proliferation was not affected. Primary cell lines were generated from these tumors to confirm indels and downstream signaling targets. Mass spectrometry metabolomic profiling on tumor-derived cell lines identified tumor suppressor-dependent differences across multiple metabolic pathways, including glycolysis, the pentose phosphate pathway, the TCA cycle, and amino acid biogenesis. Taken together, these data suggest that *P53* and *CDKN2A* differentially alter metabolism in MPNSTs and demonstrate how CRISPR/Cas9 approaches can facilitate study of tumor suppressors that are commonly mutated across multiple cancers.

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Humanized Neurofibroma Model to Delineate Tumor Pathogenesis and Preclinical Therapeutic Testing

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Neurofibromatosis type 1 (NF1) is one of the most common tumor predisposition syndromes in which affected patients develop Schwann cell tumors (neurofibromas). Caused by a germline mutation in the *NF1* gene, neurofibromas arise following somatic loss of the remaining *NF1* allele. Recent studies have uncovered the role of the *NF1* gene in differentiation and cell fate decisions for different cell types, including neural stem cell and neuroglial progenitor *in vitro* and *in vivo*. To define the role of the *NF1* gene in Schwann cell lineage dynamics and neurofibroma pathogenesis, we utilized an isogenic series of patient-specific *NF1* mutant human iPS cells. We have successful established a robust protocol to differentiate isogenic *NF1* wild type, heterozygous and homozygous human iPS cells into Schwann cell lineage cells. Most importantly, when we implanted differentiated Schwann cell lineage cells from wild type human iPS cells into the left sciatic nerve versus differentiated Schwann cell lineage cells from *NF1*-null human iPS cells into the right sciatic nerve, bona fide neurofibromas only arose within the right sciatic nerve. These tumors were human-derived, based on immunopositivity for a human specific antigen, indicating that these Schwann cell lineage cells contained the cells of origin for human neurofibroma. We show here that the humanized neurofibroma model generated in this study can be used to study tumor pathogenesis, as well as serve as a tractable platform for preclinical therapeutic testing.

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Unmasking Intra-tumoral Heterogeneity and Clonal Evolution in NF1-MPNST

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Background: Sarcomas are highly aggressive cancers that invariably metastasize, fail to respond to conventional therapies, and carry a poor 5-year survival rate. This is particularly true for young adults with the Neurofibromatosis Type 1 (NF1) cancer predisposition syndrome, in which 10-13% of affected individuals will develop one subtype of sarcoma, the malignant peripheral nerve sheath tumor (MPNST). Despite continued research including genomic analysis of these tumors, no effective therapies have emerged from recent clinical trials based on the preclinical work. One explanation for these failures could be the lack of attention to intra-tumor heterogeneity. Prior studies have relied on a single sample from these tumors, which may not be representative of all of the clones present within the tumor.

Methods: Samples were taken from four distinct areas within a single tumor from a patient with an NF1-MPNST. Whole exome sequencing, RNA sequencing, and copy number analysis was performed on each sample and compared. A blood sample was obtained as a germline DNA control.

Results: Distinct mutational signatures were identified in different areas of the tumor as well as significant differences in gene expression among the spatially distinct areas leading to an understanding of the clonal evolution within this patient.

Conclusions: Significant intratumoral heterogeneity exists and may be a barrier in our ability to improve outcomes in patients with NF1-MPNSTs. These data suggest that multi-regional sampling may be necessary for driver gene identification and biomarker development in the future.

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Modeling Nf2 Deficiency in the Liver

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Mutations in the neurofibromatosis type 2 (NF2) tumor suppressor gene underlie familial NF2 and occur sporadically in multiple cancers. Work in the McClatchey lab has demonstrated a fundamental role for the *NF2* encoded protein, Merlin, a unique tumor suppressor that localizes to the interface between the cell membrane and the actomyosin cytoskeleton, in coordinating the architecture of individual cells and cell collectives to enable their functional specification. An important model for studying Merlin functions *in vivo* is the liver: *Nf2*^{+/-} mice develop both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), a phenotype that is recapitulated in mice harboring a liver-specific deletion of *Nf2*. These mice exhibit massive neoductular lesions, which we demonstrated surprisingly originate not from the proliferation of existing ducts but rather from the over-conversion of precursor cells to a biliary fate in the *absence* of proliferation. A deep investigation of this phenotype yielded fundamental insight into normal biliary development. In the normal liver, ducts arise via a *de novo* self-organizing process initiated by the polarization of a single cell, followed by lumen expansion and recruitment of neighboring cells. In the absence of Merlin, these polarized structures expand aberrantly and recruit excess cells to a biliary fate. Only after birth do these ectopic bile ducts proliferate abnormally to give rise to rampant ductular expansion and liver cancer. This study demonstrates how changes in cortical architecture resulting from Merlin loss can impact tissue patterning and cell fate, an understudied potential mechanism of tumorigenesis driven by NF2 loss. My follow up work aims to identify the mechanisms by which Merlin impacts tissue self-organization in development and cancer. Significantly, Merlin is known to regulate signaling from receptor tyrosine kinases and activating FGFR2 fusions are a prominent subtype of human ICC. In other model systems such as the zebrafish lateral line primordium, restriction of FGF signaling to luminal structures promotes self-organization and reinforces cell fate, suggesting a model for how NF2-driven disruption of tissue architecture may drive tumorigenesis. Through studies of receptor localization, splicing, and ligand expression, I have identified novel roles for FGFR signaling in biliary morphogenesis. I have also devised a 3D cell culture system that mimics biliary development and will allow me to test the hypothesis that unrestricted growth factor signaling in livers lacking NF2 drives biliary disorganization and tumorigenesis. Importantly, I intend to use knowledge obtained through studies of our highly tractable liver model to devise new ways to target NF2-deficiency in the cell types that initiate schwannomas and meningiomas in familial NF2 patients.

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Brigatinib as a Potential Therapy for Malignant Peripheral Nerve Sheath Tumors

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MPNSTs are highly aggressive soft-tissue sarcomas that have a high risk of recurrence and metastasis and are refractory to current treatment. These tumors can arise spontaneously or from pre-existing plexiform neurofibromas in patients with neurofibromatosis type 1 (NF1), a tumor predisposition syndrome caused by inactivating mutations in the *NF1* tumor suppressor gene which encodes a Ras-GTPase-activating protein. Importantly, even sporadic tumors often incur mutations in the *NF1* gene or the Ras pathway. As a consequence, MPNSTs exhibit upregulation of Ras downstream kinase signaling, including the phosphatidylinositol 3-kinase (PI3K)-AKT-mammalian target of rapamycin (mTOR) and Raf-MEK-ERK mitogen-activated protein kinases. MPNSTs also harbor other genetic alterations, such as aberrant activation of epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R), suggesting that these receptor tyrosine kinases (RTKs) may be therapeutic targets. Previously we showed that the FDA-approved ALK inhibitor brigatinib (ALUNBRIG™) suppresses multiple RTKs and non-RTKs, including focal adhesion kinase (FAK). Here we demonstrate that brigatinib exhibited growth-inhibitory activity in *NF1*-deficient ST8814 and *NF1*-expressing STS26T MPNST cells. Combination of brigatinib with the dual mTORC1/2 inhibitor INK128 (sapanisertib) yielded enhanced anti-proliferative effects. Treatment of ST8814 cells with brigatinib decreased p-EGFR and p-IGF-1R and their downstream p-AKT and p-S6. Treatment with INK128 also profoundly inhibited p-AKT and p-S6. Interestingly, combination of brigatinib and INK128 further reduced the phosphorylation of these signaling mediators and p-FAK compared to either monotherapy, suggesting cooperation in suppressing the AKT-mTOR pathway. However, we did not detect ALK expression in ST8814 and STS26T cells, indicating that brigatinib mediates growth inhibition via targeting of other tyrosine kinases. Experiments are in progress to investigate the anti-tumor activity of brigatinib in patient-derived xenograft (PDX) models for MPNST. Collectively, our data suggest that brigatinib should be further evaluated as a potential treatment for MPNST.

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Nf1 Haploinsufficiency Alters Mesoaccumbal Dopamine Dynamics and Responses to Salient Stimuli

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Recent advances in the development of genetically encoded neurotransmitter sensors has improved the ability to detect neurotransmission with high sensitivity and temporal resolution in freely moving rodents over months of testing. In these studies, we used the optical dopamine sensor dLight1.2[1] to monitor fluorescent dopamine dynamics in the lateral nucleus accumbens during motivated behavior in a mouse model of neurofibromatosis type 1 (NF1), which is associated with impaired executive function, attention and impulse control, and spatial learning. Previous studies suggest that these phenotypes are due to developmental perturbations in mesolimbic dopamine circuitry, however, these circuits have never been directly assayed *in vivo* in NF1 model mice. Using dLight1.2 and fiber photometry, we found that *Nf1*^{+/-} mice exhibit differences in spontaneous dopaminergic neurotransmission, which we further parsed with patch clamp electrophysiology, tissue clearing, and new systemic AAV vectors to provide sparse, multicolor labeling of dopaminergic neurons and facilitate morphological reconstruction. Our findings suggest that changes in spontaneous dopamine transients were due to excitation/inhibition imbalance in the ventral tegmental area and likely influenced by genotypic differences in cell morphology. Additionally, we observed that *Nf1*^{+/-} mice exhibit more robust dopaminergic responses to salient visual but not auditory or rewarding stimuli, which were correlated with behavioral phenotypes in a cued fear conditioning task. Overall, these studies provide the first ever *in vivo* characterization of dopaminergic circuit function in the context of NF1 and reveal novel pathophysiological mechanisms influencing cognitive sequelae of the disease.

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Increased Extracellular-Matrix Deposition in a Tissue-Engineered Skin Model Derived from NF1 Patients

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Background: Cutaneous neurofibromas are peripheral nerve tumors mainly composed of fibroblasts, perineural cells and Schwann cells embedded in an abundant extracellular matrix (ECM). Schwann cells secrete factors promoting matrix remodeling and collagen deposition by fibroblasts. *In vivo* studies using Nf1 knockout mice have demonstrated that fibroblasts cause abnormal collagen deposition in response to wound injury. Here we hypothesize that *NF1* haploinsufficiency in human dermal fibroblasts could enhance ECM secretion and assembly when cultured in 3D. The purpose of this study is to characterize the ECM protein content produced by dermal fibroblasts in a tissue-engineered skin (TES) model.

Methods: Our TES, free of exogenous materiel, was generated with dermal fibroblasts and keratinocytes, isolated from NF1 patients and control individuals. First, we measure the thickness of the dermis on histological cross-sections. Then, we evaluate the average number of cells by quantifying the DNA using the Quant-iT PicoGreen dsDNA Assay kit. Uniaxial tensile tests were performed to evaluate the mechanical properties of dermis constructs using an Instron Electro-pulse E1000 mechanical tester. Finally, we quantify different ECM proteins produced by dermal fibroblasts using dot blot analysis.

Results: Complete 3D reconstructed skins with a dermis and a stratified epidermis were successfully achieved. Interestingly, NF1-derived dermis were significantly thicker than the control-derived ones. DNA quantification indicate no significant difference between the NF1 and the healthy controls, showing a similar number of fibroblasts within the dermis. Mechanical analysis revealed that NF1 dermis have a higher maximum force to failure and an equivalent ultimate tensile strength. Finally, fibronectin and collagen I were found more expressed in NF1 fibroblasts when cultured in 3D.

Conclusion: Our study demonstrates that human dermal fibroblast haploinsufficient in *NF1* can alone secrete an abundant ECM when cultured in 3D. Fibroblasts could actively participate in matrix remodeling and tumor microenvironment modification. Our NF1-TES model could become a unique tool to better characterize the pathogenic mechanisms associated with skin tumor genesis. Ultimately, it could provide better tools to develop new therapies for patients through the development of personalized medicine strategies.

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A Novel Whole-Head Histopathological Approach to Evaluate CNVIII Architecture And Tumor Microenvironment in a Murine Model of Neurofibromatosis Type 2 Associated Hearing Loss

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Bilateral vestibular schwannoma development of cranial nerve VIII (CN VIII) is pathognomonic for NF2, leading to hearing loss and impaired balance. In human patients as well as in genetically engineered *Nf2$^{f/f}$;Postn-Cre+* mice, CN VIII tumor volume and degree of hearing loss are not strongly correlated, indicating that progressive hearing loss may be caused by mechanisms beyond simply tumor mass effect. We currently use the size and histopathology of the dorsal root ganglia as an estimation of tumor burden in the NF2 GEMM, as CN VIII is exceedingly challenging to dissect and measure on the numbers of mice needed to conduct therapeutics studies. Here we describe a new method to investigate the complex interrelationship of vestibular schwannoma, cochlear microenvironment and hearing loss.

Nf2$^{f/f}$; Postn-Cre+ and *Nf2$^{f/f}$; Postn-Cre-* mice were euthanized and heads were quickly removed. The end of the snout was trimmed off to enhance fixative penetrance, and the head was placed in 10% neutral-buffered formalin (NBF). After 48 hours the whole head was decalcified in 5% formic acid in 10% NBF. After 24 hours, the lower jaw was removed, and the head was divided by a midsagittal cut. The heads were decalcified for an additional 24 hours, rinsed, placed in cassettes, and transferred to 70% ethanol. The tissues were processed through graded alcohols, xylenes, and infiltrated with molten paraffin. The heads were embedded in paraffin by orienting each half with the skull base at the bottom of the paraffin mold. The blocks were cut on the microtome until the cochlea became apparent, chilled on ice, and 5-micron sections were mounted on slides. Unstained slides were examined under the microscope to ensure that the cochlear structures were captured prior to staining.

This method of fixation, processing and embedding allows us to histopathologically examine CNVIII architecture and tumor microenvironment of the vestibular schwannoma and cochlear structures in an unprecedented fashion. Further, the ability to preserve the cochlear structures without the need for cardiac perfusion will allow the harvesting of fresh tissues immediately after euthanasia for *ex vivo* protein analysis without contaminating fixative. This technique will facilitate future therapeutic studies that necessitate the collection of fresh tissues, while also preserving the cochlear architecture.

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Functional Analysis of *NF1*-Associated Low-Grade Glioma Co-Occurring *FGFR1* and *PIK3CA* Mutations

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Optic pathway gliomas occur in 15-20% of patients with neurofibromatosis type-1 (*NF1*), and result in visual deficits in up to half. Unfortunately, despite current frontline treatment with carboplatin and vincristine, 30-40% of *NF1*-LGG patients develop progressive disease after completion of chemotherapy. As part of the Synodos for *NF1* Glioma collaborative project, our colleagues at DKFZ recently completed comprehensive molecular profiling on 81 *NF1*-LGG samples and identified mutated genes in addition to *NF1* in a subset. Both *FGFR1* and *PIK3CA* gene hotspot point mutations were noted in several LGG samples, and *FGFR1^{N546K}* and *PIK3CA^{H1047R}* co-occurred in two samples. Our group hypothesized that these co-existing molecular alterations may differentially impact the aggressiveness of *NF1*-LGG behavior and signaling pathway adaptations that promote resistance to targeted agents. As there are no existing patient derived *NF1* LGG cell lines available, we aimed to functionally characterize these co-occurring mutations using heterologous model systems expressing *FGFR1^{N546K}*, *PIK3CA^{H1047R}* along with the loss of *NF1* gene. We used the gateway cloning system to generate myc tagged retroviral plasmids for each point mutation to create heterologous cell lines for biochemical analysis by infecting NIH/3T3 and primary mouse astrocytes to create stably expressing *FGFR1^{N546K}* and *PIK3CA^{H1047R}* and both combined heterologous cell lines. After addition of secondary concurrent mutations, the soft agar proliferation assay showed enhanced ability to form colonies (p<0.0005) in NF1$^{-/-}$ cells expressing *FGFR1^{N546K}* and *PIK3CA^{H1047R}* compared with the cells expressing *NF1$^{-/-}$ FGFR^{WT}* and *PI3KCA^{WT}*. Immunoblot analysis in *NF1$^{-/-}$ FGFR1^{N546K}* showed increased activation of the MAPK pathway whereas *NF1$^{-/-}$ PIK3CA^{H1047R}* cells showed significantly higher PI3K/AKT pathway activation compared to their *NF1$^{-/-}$ FGFR^{WT}* and *PI3KCA^{WT}* lines. We identified that NF1-/- cells expressing *FGFR1^{N546K}* and *PIK3CA^{H1047R}* lead to the increased phosphorylation of both PI3K/mTOR and MAPK pathways compared to control cells. *In vivo* studies using NSG mice are ongoing. Additionally, we are evaluating different kinase and non-kinase molecular components activated by these mutations using antibody array and Crispr/Cas9 screening technique. Overall our study suggests that mutations in both *PIK3CA* and *FGFR1* enhance RAS pathway activation and that this activation might contribute to tumor progression and resistance. Further functional and biochemical characterization of *FGFR1^{N546K}*, *PIK3CA^{H1047R}* and combination of *FGFR1^{N546K}* and *PIK3CA^{H1047R}* along with *NF1* loss may provide a better understanding of the acquired resistance and tumorigenic mechanisms to further guide combinatorial target specific therapeutic interventions for the *NF1*-LGG patients.

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Functional Heterogeneity Reveals a Hierarchical Organization of MPNSTs

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Plexiform neurofibromas (PN) and malignant peripheral nerve sheath tumors (MPNST) are cardinal features of the genetic autosomal recessive disease, Neurofibromatosis Type 1 (NF1). The high rate of tumor recurrence and treatment resistance of PN and MPNST are among the most challenging obstacles. These are highly heterogeneous tumors that have a rich microenvironment and complex intercellular dependencies. The biologic driving forces of tumor development and maintenance remain a mystery. By generating a transgene, *Nes-CreERT2-eGFP-DTR (CGD),* we can label the neural crest lineage and create new MPNST mouse models resembling human MPNSTs. This transgene includes the rat *Nes* promoter and its second intron, enhanced nuclear GFP and diphtheria toxin receptor, which enables us to sort out GFP+ and GFP- tumor cell populations and target the transgene expressing cells by diphtheria toxin (DT). The *CGD* transgene provides us a unique tool to explore the tumor heterogeneity, organization, and their biological relevance to tumorigenesis, relapse and metastasis.

Our preliminary data shows that 1) the *CGD* transgene labels a relative quiescent cell population *in vivo;* 2) the sorted GFP+ cells can continuously initiate new tumors and keep a homeostasis, while the sorted GFP- tumor cells fail at the tested cell number in the serial tumor transplantation; 3) DT treatment significantly inhibits the tumorigenesis of allograft model initiated by recombined cells from E13.5 boundary cap and dorsal root ganglia, and treatment strategy combining doxorubicin and DT demonstrates significant beneficial on inhibiting tumor progression; 4) RNAseq shows that signatures of Schwann cell progenitor and immature Schwann cell are significantly highly expressed in the GFP+ population comparing to the GFP- counterpart, suggesting a hierarchical organization of tumor cells.

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Functional Analysis of Cases Suspect for Neurofibromatosis Type 1

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Background: Neurofibromatosis type 1 (NF1) is caused by inactivating mutations in the *NF1* tumour suppressor gene. The broad phenotypic spectrum and age-dependent symptoms associated with NF1 makes clinical diagnosis challenging, particularly in young individuals. Mutation detection is therefore a useful part of the clinical work-up. However, the complexity and large size of the *NF1* locus makes not only mutation detection, but also variant interpretation, challenging. We performed *in vitro* functional assays for the re-classification of *NF1* variants of unknown clinical significance (VUS).

Methods: Routine genetic testing for *NF1* variants resulted in the identification of ~200 different VUS over the last 25 years in the Netherlands (http://www. LOVD.nl/NF1). We selected 27 variants for functional assessment. Expression constructs encoding *NF1* VUS were derived by site-directed mutagenesis and the RAS GAP activity of the neurofibromin variant proteins was estimated using a RAS-GTP pull-down assay. Secondly, we used coimmunoprecipitation to investigate whether the variants affected the interaction between neurofibromin and *SPRED1*. All patients fulfilled the NF1 NIH criteria. Variants in the Legius syndrome gene *SPRED1* were excluded. Re-classification was performed according to the guidelines of the American College of Medical Genetics (ACMG).

Results: In total we performed functional assessment on 27 different *NF1* variants. In 16/27 (59%) cases we obtained evidence to support variant re-classification according to the ACMG guidelines. Six (22%) VUS were reclassified as pathogenic and 10 (37%) as likely pathogenic. Eight (30%) variants could not be re-classified. The three (11%) remaining variants were reclassified as benign, however, this was not based on the functional tests but based on the fact that another pathogenic *NF1* variant was found in these patients.

Conclusions: Functional analyses were useful to help classify *NF1* VUS. Due to the clinical and familial consequences of an NF1 diagnosis, future studies should focus on optimizing assays of neurofibromin function to facilitate establishing an accurate molecular diagnosis.

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Molecular and Cellar Changes in GABAergic Cortical Interneurons in Nf1 Conditional Mutants

Daniel Vogt, PhD, *Michigan State University*

While Neurofibromatosis-1 (NF-1) NF-1 is often characterized by café-au-lait skin spots and benign tumors, the mechanisms underlying cognitive changes in NF-1 are poorly understood. A subset of those diagnosed with NF-1 will exhibit learning disabilities as well as autism spectrum disorder (ASD). While changes in both glial and GABAergic neuron populations have been implicated in NF-1 symptoms, the molecular and cellular alterations in these populations is still being uncovered. To address this, we conditionally deleted the mouse *Nf1* gene from the medial ganglionic eminence (MGE), which gives rise to the initial population of oligodendrocytes and the two largest groups of cortical GABAergic interneurons (CINs), i.e. express either somatostatin (SST) or parvalbumin (PV). Loss of *Nf1* led to a persistence of immature oligodendrocytes that prevented later born oligodendrocytes from occupying the cortex. Moreover, PV+ CINs were uniquely lost, without changes to SST+ CINs. Surprisingly, we discovered that loss of *Nf1* results in a dose-dependent decrease in *Lhx6* expression, the transcription factor necessary to establish SST+ and PV+ CINs, revealing a mechanism whereby *Nf1* regulates a critical CIN developmental milestone. Overall, these findings reveal new molecular and cellular targets that may have relevance for the cognitive changes in cohorts of those diagnosed with NF-1.

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Identifying Novel Therapeutic Targets for NF1-Deficient Tumors Using a Unique Combinatorial Screening Strategy

James Walker, PhD, *Massachusetts General Hospital, Center for Genomic Medicine*

Combinatorial screens to target genes that are specifically essential to the survival of tumor cells offer a potentially powerful tool for the identification of novel anti-tumor drugs. This concept takes advantage of a type of genetic interaction called synthetic lethality, where removal of either of two gene products alone has no effect on viability, but simultaneous removal of both genes results in cell death. Genes that have synthetic lethal relationships with a tumor suppressor are attractive drug targets because treatment is likely to have specific effects on tumor cells but have little toxicity on wild-type cells. However, despite the promise of synthetic lethal screens for drug target discovery, this approach has had limited success due to technical limitations, such as context dependency, which results in a lack of reproducibility of interactions between models systems.

We have recently developed new combinatorial screening methods that revolutionize the reproducibility of synthetic lethal screens. *Drosophila* cells have been used extensively for genetic screening and have several advantages over mammalian cell culture systems. For example, the lower complexity of the *Drosophila* genome reduces the effects of off-targets in RNAi screens, making interpretation of results rapid and reliable. In addition, *Drosophila* screens are a powerful method to identify context independent drug-targets as demonstrated by our recent screens targeting tuberous sclerosis complex (TSC).

Here we utilize these novel methods combining CRISPR and RNAi in *Drosophila* cells to identify potential new therapeutic targets for NF1. We generated a clonal *NF1*-deficient cell line using CRISPR, which was then used to perform genome-wide RNAi screens. By applying a network-based analysis approach to integrate protein-protein interaction data, we identified 46 high-confidence synthetic lethal interactions. We are prioritizing genes that can be targeted with existing drugs that are already in use for other diseases. Subsequent studies with available drugs have been used to validate several of these candidates in *Drosophila* cell lines, which are being pursued further using genetic and drug experiments both *in vivo* in the fruit fly and using human *NF1*-deficient cell lines. This study therefore has the potential to rapidly identify specific genetic vulnerabilities of *NF1* mutant cells and aid in the development of potential new therapies for NF1 tumors.

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Creation of Immortalized Cutaneous Neurofibroma Schwann Cell Lines

Peggy Wallace, PhD, *University of Florida*

Cutaneous neurofibromas are benign Schwann cell tumors that lie within the skin. These are nearly ubiquitous in neurofibromatosis type 1 (NF1), especially in patients after puberty. NF1 patients are constitutionally heterozygous for an NF1 gene mutation, and each neurofibroma is associated with an independent somatic mutation of the remaining NF1 allele in a Schwann cell that led to inappropriate proliferation. While predominantly consisting of these "two hit" Schwann cells, cutaneous neurofibromas also contain fibroblasts, nerve endings, vascular components, mast cells, macrophages, and variable amounts of extracellular matrix. Although tissue culture protocols can be used to coax Schwann cell-enriched cultures from these tumors, the cells typically senesce by passage 5 or 6. This property, along with presence of contaminating fibroblasts, limits the use of these cell cultures for research. Previously we have created immortalized Schwann cell lines from plexiform neurofibromas, separately heterozygous and "two-hit," which have enabled a number of *in vitro* and *in vivo* studies by labs around the world. We are now employing this same strategy with cutaneous neurofibroma Schwann cell cultures, by transduction with lentiviruses carrying a normal human telomerase gene (hTERT) and a normal murine cyclin dependent kinase 4 (mCdk4) gene, to bypass cellular senescence. Here we report the successful immortalization of 9 cultures (at least to passage 20), as well as germline and somatic NF1 mutations and cellular phenotypes such as dependence on substrate and/or growth factor, S100B staining, and proliferation/apoptotic indices. Additional characterization studies are also planned, including transcriptome analysis, and comparison with characteristics of the original tumor histopathology. These cell lines will provide novel new tools for basic research, and potential therapies, for cutaneous neurofibromas.

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Feasibility and Potential Impact of Exon Skipping as a Therapeutic for Neurofibromatosis Type I (NF1)

Deeann Wallis, PhD, *Department of Genetics, University of Alabama at Birmingham, Birmingham, AL*

Current therapeutics for NF1 primarily target the Ras pathway, but these are not always effective and are limited by toxicity. A mutation-targeted approach may therefore act in synergy with inhibition of Ras signaling. NF1 is a large protein and it is unclear how critical regions and domains outside the well described Gap-related domain (GRD) are for functionality. Potentially, some are dispensable and can be deleted or skipped through exon skipping which may lead to a truncated but still functional protein, despite genetic mutation. In silico analysis has identified that 45 of 57 total exons could be skipped either as a single exon or two consecutive exons while still maintaining the translational reading frame. We performed additional in silico analysis to predict which exons could be skipped with minimal loss of NF1 function and maximum therapeutic potential. In efforts to identify exon skips that are likely to be asymptomatic we prioritized exon candidates based on: known skips in unaffected individuals, lack of skips reported in NF1 individuals, and exons that are not within critical NF1 functional domains. Next, we performed a cDNA functional screen utilizing a novel Nf1 cDNA system to determine the effects of exon skipping on *in vitro* NF1 expression and GRD function. Both in silico and *in vitro* data will be presented. As anticipated, we find that some exons are critical for GRD function. In addition, we find that cDNAs representing loss of other exons are able to maintain GRD function. Furthermore, we note that deletion of some exons leads to lower levels of neurofibromin protein expression presumably due to loss of protein stability. We show that after both GRD function and NF1 expression levels are considered jointly, skipping of specific exons may be therapeutic as they are able to retain both NF1 protein levels and GRD activity. This represents a personalized medicine approach for the treatment of NF1.

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Preclinical Drug Evaluation in a Genetically Engineered Minipig Model of Neurofibromatosis Type 1

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We have employed gene-editing technology to create a Neurofibromatosis Type 1 (NF1) minipig that replicates the broad spectrum of disease that develops in NF1 patients and meets the National Institute of Health's diagnostic criteria for NF1. The NF1 boars are fertile and the NF1 mutant allele is transmitted at a Mendelian rate with no reduction in fitness of offspring that inherit this allele. To date, we have observed 100% penetrance of café au lait macules, a phenotype that occurs in nearly every NF1 patient, but has never been demonstrated in any other animal model. The NF1 minipig develops cutaneous neurofibromas and optic pathway glioma, that histologically resemble human tumors. Additionally, we have observed other NF1-associated phenotypes including Lisch nodules, tibial dysplasia, white matter decompaction, hypopigmentation, and freckling of the skin. The FDA has emphasized the need for development and testing of new therapies in large animal disease models prior to human studies. Therefore, we have conducted pharmacological studies in our NF1 swine to look at the pharmacokinetic and pharmacodynamic properties MEK inhibitors, currently 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. We envision this large animal model of NF1 will become a standard in the evaluation of the safety and efficacy of new drugs prior to Phase I clinical trials. Further, an NF1 minipig may enable researchers to better understand the biological and genetic mechanisms underlying this complex disease, detect NF1-related tumors earlier, identify biomarkers, discover novel drug targets, and test new drugs and combination therapies for safety and efficacy.

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Human iPSC-Derived Cerebral Organoids Establish Mutational Specificity in NF1

Michelle L. Wegscheid, BS, *Washington University School of Medicine, St. Louis, MO*

Background: Neurofibromatosis type 1 (NF1) is a common neurogenetic condition caused by germline mutations in the *NF1* gene. While all germline *NF1* gene mutations have traditionally been regarded as equivalent loss-of-function alleles, emerging evidence from population-based studies now suggests that the germline *NF1* gene mutation may be one factor underlying the clinical variability in this condition. In order to determine whether *NF1* gene mutations have mutation-specific effects on human brain development, we have employed an isogenic series of human induced pluripotent stem cell (hiPSC) lines CRISPR/Cas9-engineered to harbor distinct patient *NF1* germline gene mutations.

Methods: From these hiPSC lines, we derived three-dimensional cerebral organoids to analyze mutation-specific effects on neural progenitor cell, astrocyte, and neuron maturation. All experiments employed numerous biological replicates from two independently generated hiPSC clones.

Results: While all mutations resulted in increased glial cell growth and whole-organoid RAS activity, we observed striking *NF1* mutation-specific effects on neural progenitor cell proliferation, death, and neuronal differentiation within the ventricle-like zones of developing cerebral organoids.

Conclusions: These findings provide the first evidence of *NF1* mutational specificity operative at the cellular and tissue levels, supporting population-based genotype-phenotype correlations, relevant to their applications to risk assessment and targeted therapeutics for a disorder characterized by clinical heterogeneity.

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Single-Cell Transcriptomics Resolves Cellular Heterogeneity and Oncogenic Networks During Malignant Transformation of Peripheral Nerve Sheath Tumors

Lai Man (Natalie) Wu

Malignant peripheral nerve sheath tumors (MPNSTs) are highly aggressive Schwann cell (SC)-derived sarcomas with strong metastatic proclivity and resistance to radiation and chemotherapy. Molecular events driving SC-to-MPNST transformation are incompletely understood.

MPNST are heterogeneous tumors with a distinct microenvironment that contributes to the establishment of a tumor niche. Cancer behaviors including initiation, progression, metastasis and relapse cannot be fully defined by genetic mutations alone, but are critically dependent on intercellular communication between tumor cells and the microenvironment. However, currently, a comprehensive understanding of tumor cell lineage evolution, heterogeneity and cell-cell communication network in MPNST is lacking. A better understanding of cellular heterogeneity and tumor initiating cells during tumorigenesis at a single-cell level will facilitate identifying molecular and cellular determinants underlying malignant transformation in MPNST.

We have established a murine model of aggressive peripheral nerve malignancy with HIPPO-TAZ/YAP activation (Lats1/2-def mice), which provides a unique opportunity for dissecting mechanisms of SC malignant transformation. To identify potential PNST-driven progenitor populations, we compare single-cell transcriptome profiles of normal peripheral nerves and Lats1/2-def sciatic nerve tumors at different stages of progression during pre-neoplastic and advanced stages. Our data reveal that early sciatic nerve tumors contain primarily mature Schwann cells, whereas full-blown tumors harbor neoplastic SCs composed of different subsets: fast proliferating, mesenchymal-like and neural crest stem cell-like cells, indicative of a developmental trajectory from differentiated SCs to tumor-like SCs with distinct molecular signatures. We also identify key transcriptional networks governing SC transition to cancerous cells. Using single-cell transcriptomics to characterize cellular heterogeneity in early-stage and full-blown MPNST-like tumors, we further identify distinct cell-cell communication networks between SC-derived tumor cells and cancer-associated stromal cells within the tumor microenvironment. In summary, our studies using single-cell transcriptome profiling in clinically relevant mouse models of PNST reveal tumor cellular heterogeneity and the microenvironment niche for driving SC tumorigenesis. It will provide principles for identifying potential targets or treatment strategies to cure MPNST.

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Novel Patient-Derived Xenograft and Cell Line Models for Therapeutic Testing of NF2-Associated Schwannomas

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Background: Neurofibromatosis type 2 (NF2) is a rare genetic syndrome that predisposes individuals to multiple schwannomas of the central nervous system. The development of effective therapies for NF2-associated schwannomas is hindered by the lack of valuable experimental animal models. This study aimed to establish patient-derived tumor xenograft (PDTX) models of NF2-associated schwannomas for preclinical assays.

Methods: We established an improved schwannoma cell culturing method and an engraftment technique to model a panel of patient-derived tumor xenografts (PDTXs) from NF2-associated schwannomas. We utilized H&E and immunohistochemical staining for histological characterization, RNA sequencing for gene expression profiling. We adapted the schwannoma cell culturing method to derive novel immortalized NF2-associated schwannoma cell lines from the xenografts, and then performed drug screen to identify the pharmacologic characterization.

Results: The PDTXs recapitulated the histologic, genetic, and biological characteristics of the corresponding primary tumors. Furthermore, the gene expression profiles of cell lines derived from xenografts closely resemble those of primary culture cells. Upon screening 156 inhibitors of PI3K/AKT/mTOR pathways, twelve inhibitors were significantly active to suppress cell proliferation. Targeted therapy of PDTXs with the dual mTORC1 and mTORC2 inhibitor—AZD8055 resulted in the significant growth inhibition *in vitro* and *in vivo*.

Conclusions: This is the first report on the establishment of patient-derived NF2-associated schwannoma xenograft and cell line models. These novel models provide reliable tools to develop patient-specific therapies for this rare syndrome.

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NF1 Gene Rescue via Polymeric Nanoparticles in Translational Animal Models

Jiangbing Zhou, PhD, *Department of Neurosurgery, Yale University, New Haven, CT*

To assess new potential treatment strategies for NF1, we aim to restore the function of defective NF1 alleles at the genome level through two approaches using polymeric nanoparticles as a delivery vehicle. First, we are exploring CRISPR/Cas technologies for somatic gene editing. Currently, the most effective CRISPR/Cas9 editing with homology driven repair that minimizes nuclease activity is a three-component system including Cas9 protein and synthetic nucleotides (gRNA and repair templates). To maximize gene editing efficiency and reduce off-target effects, we will deliver a combination of Cas9 protein and sgRNAs/repair templates using engineered nanoparticles. CRISPR-mediated gene editing of NF1 mutations poses unique problems as it is not yet clear which cell populations must be edited and when in order to prevent disease manifestations in numerous tissues. To this end, we will test the hypothesis that rodents harboring patient-specific mutations can be corrected at the genome level by nanoparticle delivery of CRISPR-Cas reagents, and prevent or mitigate NF1 pathologies. Mice harboring the c.2041C>T; p.R681X nonsense mutation in NF1 exon 18 will be treated with nanoparticles encapsulated with CRISPR/Cas9 complexes along with repair templates to convert the mutant allele to wild-type, and then assessed for a) rescue of lethality due to loss of NF1 gene function, b) rescue and/or treatment of developing plexiform tumors, and c) rescue and/or treatment of developing optic gliomas. Secondly, we are developing gene replacement therapy through targeted delivery of a full-length mouse NF1 cDNA using nanoparticles and test it in the same animal model as above. Nanoparticles will be synthesized with encapsulation of plasmid DNA for expression of mouse *NF1* gene and tested in NF1 null cell lines *in vitro,* and assessed for ability to restore NF1 activity to: a) rescue lethality due to loss of NF1 gene function; b) rescue and/or treat developing plexiform tumors, and c) rescue and/or treat developing optic gliomas.

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A Single Center Study to Assess the Intra-Observer Reliability of Measuring Muscle Strength Using a Hand-Held Dynamometer in Children and Adults with Neurofibromatoses (NF)

Srivandana Akshintala, MBBS, MPH, *New York University Langone Health, Division of Pediatric Hematology-Oncology*

Background: Generalized or focal muscle weakness secondary to tumor or non-tumor manifestations is a significant concern in NF. Reliable and sensitive measures of muscle strength are necessary to study functional outcomes in NF clinical trials. Hand held dynamometry (HHD) is a convenient technique that provides a quantitative measurement of strength, however, the reliability of this technique is unclear. The objective of this study is to assess the reliability of HHD in measuring strength in NF and to evaluate its utility as an outcome measure in clinical trials.

Methods: Patients >5 years with neurofibromatosis type 1 (NF1) or type 2 (NF2) and at least 1 muscle group weak by manual muscle testing (MMT) were eligible. Maximal isometric muscle strength of a weak muscle group and the biceps of the dominant arm was measured using the Ametek Chatillon® DFE2 HHD per a standardized protocol. An average of 3 readings per session was used as an observation and 3 sessions with a minimum of 15-minute rest period between each were performed on the same day by a single observer. Intrasession and intersession intraclass correlation (ICC) were calculated to assess reliability.

Results: Of the first 19 patients (12 male/ 7 female, 14 NF1 and 5 NF2) who underwent testing, median age was 13 years (range 6-45). Weak muscle groups tested included iliopsoas (n=3), quadriceps (n=5), gluteus medius (n=8), shoulder external rotators (n=2), and deltoid (n=1) and their strength ranged from 2-/5 to 4+/5 by MMT. For all the weak muscles combined (n=19), the first session intrasession ICC was 0.98 and intersession ICC was 0.99. For the subset of 14 NF1 patients, the intrasession and intersession ICC were 0.97 and 0.98 respectively. Biceps strength was measured in 18 patients: the first session intra-session ICC and intersession ICC were 0.97 and 0.98 respectively; the intra-session and intersession ICC were 0.97 in the subset of 13 patients with NF1.

Conclusion: Preliminary results of our ongoing study show that HHD appears to be a reliable technique to measure muscle strength in NF. The study is currently ongoing and additional analyses are planned to further assess the reliability and measurement error of this technique to help determine its utility as an outcome measure in clinical trials.

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Awareness of Breast Cancer Risk and Screening Guidelines Among Women with Neurofibromatosis Type 1

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Since germline mutations in *NF1* were established as a risk factor for early onset breast cancer, the National Comprehensive Cancer Network (NCCN) released guidelines in 2017 for women ages 30-50 years with Neurofibromatosis Type 1 (NF1). However, awareness and implementation of the guidelines has not been explored. An online survey was sent to 2,617 participants in the Children's Tumor Foundation (CTF) registry who indicated they were female, diagnosed with NF1, and aged 18 or over. 409 participants completed the survey (15.6% response rate), with 338 of these residing in the United States (US). The majority of participants were White and not of Hispanic, Latino, or Spanish origin, had a high school or higher degree, and relatively equally distributed across 5 geographical regions of the US. Many participants were seeking care for their NF1 at an NF clinic (27.5%) or their primary care provider (26.6%), while 27.2% indicated they were not seeing any provider for NF1. 69.4% (114/163) of US participants aged 30-50 had undergone a mammogram, with the most common frequency being 1 in the past 5 years (29.0%), while only 20.3% had a prior breast MRI.

72.7% of US participants stated women with NF1 have increased risk of breast cancer, and 65% reported they were aware of early onset breast cancer risk, however only 24.3% reported awareness of the NCCN guidelines. Factors that positively correlated with awareness of risk included receiving care at an NF clinic, and a history of breast cancer. Care at an NF clinic also positively correlated with awareness of the guidelines. The most common information sources about screening guidelines were: NF provider, social media, and CTF event or presentation.

At survey conclusion, 15.7% of participants indicated their screening met NCCN guidelines, and 37.3% of participants were interested in changing their screening practices to meet them. Amongst participants aged 30-50, 20.3% were screening according to guidelines, and 47.2% were interested in changing their screening.

This study indicates that though many women with NF1 are aware of their increased risk of breast cancer, they are not aware of the NCCN guidelines or receiving screening in accordance with them. Upon learning of the NCCN guidelines, many desire to change their screening to meet them. Additional efforts in patient and provider education are needed to increase breast cancer screening awareness and thus implementation amongst women with NF1.

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Microsurgery of Peripheral Nerve Sheath Tumors (PNST) in Neurofibromatosis Type 1 and 2: A Retrospective Analysis

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Introduction: Peripheral nerve sheath tumors (PNST) are frequent both in NF1 and NF2 and may cause cosmetic problems, neurological morbidity, and chronic pain. The optimal treatment of these NF1 and 2 manifestations was not yet clearly defined.

Objectives: To evaluate the clinical spectrum of PNST and the results of microsurgery in NF1 and 2 patients.

Patients and Methods: We retrospectively reviewed clinical data of NF1 and 2 patients who underwent microsurgery for PNST in our hospital in the last 15 years. All the patients were operated by the same neurosurgeon (SR). Files of patients who underwent surgery for PNST were thoroughly assessed, and functional outcome of the surgery was evaluated.

Results: We identified 34 NF1 patients and 10 NF2 patients who underwent microsurgery for PNST. The median age of NF1patients was 25.5 years (10-56 years), the median age of NF2 patients was 24.5 years (10-53 years). In the NF1 group, chronic pain was the most common cause of surgery (in 34 patients) followed by neurological deficit (8 patients) and cosmetic problems (2 patients). All ten NF2 patients suffered from chronic pain, and 5 of them demonstrated as well progressive neurological deficit. Ten NF1 patients underwent second surgery for another PNST, and 2 patients were operated for the third time. In the NF2 group, one patient required second surgery, and one additional patient underwent three removals of PNST. We will present the results of functional outcome of microsurgery for PNST removal in both groups.

Conclusion: PNST is a common manifestation of both NF1, and NF2 in children and adults. Microsurgery is treatment of choice for this kind of tumors associated with chronic pain, neurological deficit and cosmetic problems.

Cutaneous Neurofibroma-Related Intimacy Concerns in Adults with NF1

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Previous studies have shown that cutaneous neurofibromas (cNFs) negatively impact quality of life for individuals with Neurofibromatosis type 1 (NF1). An important aspect of quality of life is the presence of intimate relationships. Little research has been performed to assess the impact of cNFs on intimacy for individuals with NF1. The purpose of this study was to determine if adults with NF1 experience cNF-related intimacy issues and if this is being addressed by healthcare providers (HCP). To evaluate this, paired patient and HCP surveys were developed that were adapted from the *Dermatologic Intimacy Scale.* The patient survey was disseminated to adults with NF1 enrolled in the NF Registry. Patients were eligible if he/she was an adult, had at least one cNF, and able to read English. The HCP survey was disseminated through the NF Clinical Network provider listserv. HCPs were eligible if he/she was an adult, was working with the NF1 community through the NF Clinical Network, and able to read English. Two hundred sixty-four adults with NF1 and 15 NF1 providers responded to this survey. Although patients indicated they feel anxious and isolated due to their cNFs, both HCPs (100%) and patients (85%) reported infrequently discussing intimacy in a clinical setting. For the patients, cNF burden inversely correlated with how often providers asked about intimacy concerns (p = 0.0082). For HCPs, lack of time and resources, and the need to discuss other features of NF1 were barriers in clinic. Many HCPs indicated they were uncomfortable with discussing intimacy concerns (60%) and felt like support resources were important (80%). Seventy-three percent of NF1 patients responded that it was less important for HCPs to raise cNF-related intimacy concerns in a session; however, 63% reported one-on-one support to address these concerns. Thus, cNF-related intimacy concerns are present in the adult NF1 community and HCPs are not addressing these concerns. Intimacy concerns should be addressed in the NF1 community, although the exact role of providers has yet to be fully explored. The development and utilization of support resources, whether that be written materials or patient support groups, may be a good starting point.

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Independent Mutations in *NF1* in Two Families with Neurofibromatosis Type 1

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For an autosomal dominant condition as Neurofibromatosis type 1 (NF1), the coexistence of two independent mutations in the same gene within a family is considered highly improbable. It is currently assumed that the clinically affected members of the same family share an identical molecular aetiology.

We present the case of two families in which two independent mutations in *NF1* have been identified in first-degree relatives. In the first family, the affected father carried the familial c.6792C>A mutation in *NF1* and his daughter a different pathogenic *NF1* mutation, c.2990G>A. In the other family, the father harboured the familial c.5839C>T *NF1* mutation, while his son carried a distinct pathogenic mutation, c.1466A>G. In both cases, the affected progenitor is the father.

Polymorphisms analysis of the region adjacent to each *de novo* mutation allowed us to determine that inherited mutations were originated in the paternal allele. Although a paternal origin of *de novo* mutations does not discard the possibility of being a random event, it opens the hypothesis of a higher *NF1* mutation rate in these families.

Other families with two or more independent mutations in *NF1* have been reported in the literature (Martín Santo Domingo Y et al, 2017; Upadhyaya M et al, 2003; Klose A et al, 1999). Moreover, we have identified two additional families with independent *NF1* mutations in distant relatives in our cohort of more than 500 families with NF1.

Nowadays, preconceptional genetic counselling in NF1 patients is focused on the 50% of risk of transmitting the familial mutation assuming that the risk of *de novo* mutations is the same as in the general population. However, the data presented here together with other reported NF1 families presenting the same phenomena suggest that NF1 individuals might have a higher risk of experiencing *de novo NF1* mutations than the general population. This fact could have important implications for genetic counselling.

International studies involving a larger number of NF1 families are needed in order to determine the real frequency of *de novo* mutations in NF1 families.

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Evaluating the Performance of the NF2 Genetic Severity Score and the Potential Impact of Using Simplified Clinical Criteria and Functional Assays in a Cohort of Spanish Patients

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Background: Neurofibromatosis Type 2 (NF2) is an autosomal dominant genetic disorder characterized by the development of multiple benign nervous system tumors, particularly, bilateral vestibular schwannomas (1). A genotype-phenotype association concerning the type of *NF2* mutation is well established. A Genetic Severity Score (GSS) for NF2 mutations was developed using an English cohort of patients to help predicting the course of the disease (2). In the present study, we evaluated the performance of the GSS in our Spanish cohort of NF2 patients and analyzed the potential added value of using simplified clinical criteria and functional molecular information.

Methods: Patients: GSS was validated using the Spanish Reference Centre (CSUR) on Phakomatoses HUGTiP-ICO cohort of 25 NF2 patients. **Clinical evaluation:** Two different clinical criteria were used: 1) the same criteria as the English study; 2) a simplified clinical criteria (SCC) that took into account the number and localization of tumors, as well as the age at diagnosis. **Functional assays:** Merlin, YAP, ERK and their phosphorylated forms were measured by Western Blot using patients' cultured 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 the most severely affected group, while most of the patients presenting other types of mutations presented a milder form of the disease. These results are in accordance with the English study, validating the GSS in our cohort. However, for some mosaic patients and those presenting splicing mutations, GSS did not predict their clinical phenotype as expected. Thus, we decided to evaluate Merlin levels and the active forms of ERK and YAP proteins in the performance of GSS to predict NF2 patient's phenotype. We identified a correlation between the phenotype and the degree of activation of these pathways. In addition, applying the SCC and together with the genetic study and functional analysis allowed the better classification of some patients.

Conclusions: We validated the GSS in the Spanish cohort although a significant phenotypic variability was identified in certain groups of patients. To improve the performance of GSS in these groups, we evaluated the additional use of functional assays together with a simplified phenotype classification that allowed a better classification of mosaic patients and those with a milder phenotype.

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Cyclin-Dependent Kinase (CDK)4/6 Inhibition for Neurofibromatosis Type I (NF1) Related Atypical Neurofibromas: Phase I/II Trial in Development

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Neurofibromatosis type 1 (NF1) is a genetic tumor predisposition syndrome that results in the development of progressive tumor and non-tumor manifestations, the majority of which have no effective medical therapies. 25-50% of individuals with NF1 develop histologically benign plexiform neurofibromas (PN), which can cause substantial morbidity. Through a longitudinal natural history study (ClinicalTrials.gov ID: NCT00924196) and a series of clinical trials of targeted agents, the NCI POB recently identified the MEK inhibitor selumetinib as the first active therapy for children with NF1 and large PN. Use of whole body MRI with volumetric analysis of tumor burden allowed for characterization of PN growth and for response assessment on clinical trials. In addition, lesions with distinct imaging characteristics called "distinct nodular lesions" were identified. Based on pathology review, many of these lesions are atypical neurofibromas (ANF) that develop in preexisting PN. In contrast to PN, ANF develop after early childhood, show faster growth independent of age, and are FDG-avid on FDG-PET. Furthermore, based on preliminary observations, ANF appear to be less responsive to MEK inhibition than PN. We and others have described ANF as precursor lesions to malignant peripheral nerve sheath tumors (MPNST), which are chemotherapy resistant and have poor survival. The management of ANF includes close observation and imaging with MRI and FDG-PET. Given the observation of increased risk for transformation of ANF to MPNST, a recent consensus conference recommended surgical resection of ANF if feasible, without substantial morbidity. However, many ANF cannot be easily removed by surgery due to anatomic location, and a subset of patients have multiple ANF.

No clinical trials specifically targeting ANF have been conducted to date. ANF are characterized by heterozygous loss of *CDKN2A/B* as the only somatic change in addition to biallelic *NF1* deletion. *CDKN2A* is the primary inhibitory brake on CDK4/6-driven signaling and is commonly deleted in adult cancers. The CDK4/6 inhibitor abemaciclib received FDA approval in 2017 for some women with advanced or metastatic breast cancer. ANF is a prototypic premalignant lesion for testing experimental intervention, as these lesions are at risk for malignant transformation and share a potentially druggable genomic alteration (*CDKN2A/B* deletion). We have developed a phase I/II clinical trial of abemaciclib in children and adults with NF1 and unresectable ANF. Enrollment is projected to begin in October 2019. The aim of this trial is to characterize the safety profile of abemaciclib and the objective response rate of NF1 related ANF.

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Psychosocial Outcomes in Children with Neurofibromatosis Type 1 and Plexiform Neurofibromas

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Objective: This case series seeks to examine neurocognitive outcomes, social-emotional functioning, and family burden in young children diagnosed with Neurofibromatosis, type 1 (NF1) with early growing plexiform neurofibromas (PNFs).

Background: Neurofibromatosis, type 1 (NF1) is a common predisposing chronic disease arising in early childhood, with an incidence of approximately 1:3000. Though NF1 displays a wide range of phenotypic variability, the primary feature of the disease is peripheral nerve sheath tumors called neurofibromas. Less is well known regarding the broader neurocognitive and social-emotional profile in presentations with more complex tumor growths, namely PNFs, which are present in at least half of the NF1-affected population.

Methods: Participants with NF1 and PNFs (n=2) aged 6-7years completed comprehensive neuropsychological evaluations and parents completed measures of quality of life, social-emotional/behavioral functioning of child, parental stress, family adaptability, and family cohesion.

Results: Outcomes suggest broad neurocognitive dysfunction (e.g., executive functioning deficits, attention problems, visual-motor delays, and poor motor coordination), social-emotional challenges (e.g., symptoms of anxiety and depression, and poor social skills), and familial distress.

Conclusions: Findings indicate the value of early and frequent monitoring of children with PNFs in medical systems and multi-disciplinary teams, and the importance of early intervention for both children and families.

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Trametinib Reduces 18-Fluorodeoxyglucose (FDG) Uptake in Positron Emission Tomography (FDG-PET) in a Patient with NF1

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Background: With an incidence of approximately 1:3000 Neurofibromatosis 1 (NF1) is considered one of the more common cancer predisposition syndromes caused by a germline mutation in the NF1 gene. Benign tumors that are associated with NF1 are plexiform neurofibroma (pNF) and atypical neurofibroma (aNF), and there is a lifetime risk for developing a malignant peripheral nerve sheath tumor (MPNST) often arising within a pNF or aNF of around 10%. Clinical symptoms as newly developed pain, neurological deficit and rapid tumor growth are suggestive for malignization. Unfortunately, prognosis is still poor. Progressive fluorodeoxyglucose- (FDG-) avidity in positron emission tomography (PET) may indicate a change in dignity. What are the therapeutical alternatives if surgery is not an option?

Case Presentation: We report from a 15 yr. old boy with NF1 and a progressive life-threatening inoperable pNF. FDG-PET at the beginning revealed elevated standardized uptake values (SUV) in 3 locations suspicious of MPNST with a SUVpeak of 2.8, 5.0, and 3.4, respectively. A biopsy of the most intensive uptake lesion was performed and histopathology reported aNF. We started the boy on Trametinib, a 1/2 Methylkinase (MEK) –Inhibitor with 1mg daily. Due to side effects we had to adjust dosage to 0,5mg daily after 3 month. Imaging studies were performed on a regular basis in order to document tumor response. FDG-PET showed a markedly regressive metabolic activity (SUVpeak 2.6, 3.3, and 2.6) during therapy with low dose Trametinib one year later, which can be regarded as therapeutic response.

Conclusions: NF1 Patients with inoperable pNF and aNF and elevated SUV´s in FDG-PET where complete resection is not feasible may benefit from a MEK-Inhibitor such as Trametinib. Further studies are needed to confirm the benefit, define the optimal dosage and find out the duration of tumor control.

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NF1 and Me: Negative and Positive Perceptions of NF1 of Affected Adolescents

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Adolescence is a crucial transitional stage that comes with numerous physical, mental, cognitive, emotional and social changes. It involves the construction of the self, the development of self-confidence and the establishment of relationships with peers. Late adolescence (15 to 19 years old) is a particularly vulnerable period. In general, it is also the period in which teens experience sexual relations and feelings of love for the first time. All these changes can seem overwhelming during this sensitive phase marked by physiological, psychological and social pressure. Navigating these issues is even more difficult for late adolescents with Neurofibromatosis type 1 (NF1). In this mind and in partnership with the Quebec Association of Neurofibromatosis (ANFQ), we initiated the "NF1 & Me Study" to better identified and understand the needs and concerns of adolescents affected by NF1. The findings presented here relate their perceptions of the impact of this genetic condition on their lives, as well as their coping mechanisms.

Methodology: A qualitative approach allows us to obtain a more complete picture as possible of the adolescents' perceptions. Anonymous asynchronous online textbased interviews were conducted with 12 Canadian adolescents (9 girls, 3 boys), aged 15 to 19. The recruitment stopped when data saturation has been reached. The data were analysed by using a general inductive analysis.

Results: For the participants, the NF1 negatively affects various aspects of their life given its physical consequences, the uncertainty about its evolution and its impact on the relationships they have with others. It impacts the school, career choice, social life and romantic relationships. However, if the adolescents have adequate support at school, friends and pleasant social life, as well as a romantic relationship, they may have more positives perceptions of NF1. All participants provided insight as to how they accept their diagnosis. Although some have a generally negative perception of their situation, the majority described coping mechanisms such as seeking support and focusing on the positive aspects of NF1.

Conclusion: "NF1 & Me" generated transferrable and innovative results on the psychosocial aspects of NF1, from the experiences of Canadian affected adolescents. This study allowed a better understanding of their concerns, but particularly to identify factors and contexts that can improve their living conditions and well-being. These findings could help adolescents with NF1 and their families, as well as teachers and health professionals to develop more effective approaches and optimize intervention strategies.

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Phenotype-Genotype Correlations with Noonan Syndrome-Like Features in Neurofibromatosis Type 1

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Introduction: Neurofibromatosis type 1 (NF1) is a relatively common, well-characterized genetic condition with clear clinical diagnostic criteria and high sensitivity and specificity molecular testing options. Over time, clinicians are increasing likely to seek out genetic testing—earlier and in more patients—for a variety of reasons. Nevertheless, robust genotype-phenotype correlations are currently scarce. Similarly, while the NF1-Noonan syndrome and Watson syndrome phenotypes were linked to the NF1 locus some time ago, there is a relative paucity of recent data to either clarify the phenotypes of these conditions, or to guide management. The common RasMapKinase pathway has been suggested as a possible mechanism for overlap, and it thereby follows that we might expect that certain *NF1* variants be enriched in the NF1-Noonan population. Here we present clinical data to suggest that the *NF1* c.4180 A>G (p.Asn1394Asp) variant is highly associated with the NF1-Noonan phenotype, focusing on detailed phenotyping of one of our patients in the context of laboratory reported vs. overall population incidence.

Conclusion: The c.4180 A>G variant in *NF1* is confers a risk for the NF1-Noonan phenotype, and may provide an avenue to better explore the basic science, phenotypic features, and clinical management recommendations for this subset of patients in the future.

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Features of Diffuse Plexiform Neurofibroma on the Body Surface in Patients with Neurofibromatosis 1

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Background: Neurofibromatosis 1 (NF1) is a genetic disease characterized by cutaneous, neurological and osseous complications. About 30% of NF1 patients develop plexiform neurofibroma (PN) that often causes cosmetic and functional problems. However, the predilection site of diffuse PN on the body surface (superficial type) has not been clarified. Therefore, we studied the features of superficial diffuse PN in NF1 patients.

Methods: A retrospective study was conducted for 354 NF1 patients referred to our institution from 2007 to 2018. We investigated a total of 40 NF1 patients (17 men and 23 women; median age, 32.5 years, age range, 0-65 years) with clinically apparent superficial diffuse PN (47 PNs in 40 patients) according to analysis of photographic images. All of the patients met the diagnostic criteria by National Institutes of Health for NF1.

Results: In the cases evaluated, 57.4% of diffuse PN were located on the trunk, 21.3% were on the head and neck, 12.8% were on the lower limbs and 8.5% were on the upper limbs. By mapping the distribution of diffuse PN on the body surface per patient, we evaluated the predilection sites of the trunk and the head and neck in detail. Remarkably, 73.0% of diffuse PN were located on the dorsal side. The frequency was significantly higher on the trunk than on the head and neck (p = 0.016).

Conclusions: Our findings provide useful information for the early detection of diffuse PN before serious cosmetic or functional problems occur. The process of Schwann cell migration from the neural crest might be related to the distribution of diffuse PN on the dorsal side of the trunk.

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Case Report: Possible Diagnosis of Neurofibromatosis Type 2 Based on Raw-Data Analysis of Direct-to-Consumer Genetic Testing

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A 22-year-old male presented to Neurofibromatosis clinic with suspicion for Neurofibromatosis Type 2 (NF2) after running his raw genetic data through the popular software program (Promethease) after doing direct-to-consumer testing through 23andMe in 2014. His mother also did testing through 23andMe and her Promethease results did not note any mutations in NF2. Of note, there was no known family history of NF2 and the patient lacked symptoms associated with the disorder. His results, therefore, were unexpected.

Worried that he might have NF2, patient engaged care with a neurologist near his home with neurological exam notable for hyperreflexia, otherwise normal. He underwent contrast-enhanced MRI of the brain, IAC and total spine as well as audiology exam, all normal. The possibility of genetic testing through a commercial laboratory was suggested but not pursued as the patient was planning to relocate.

The patient presented to our clinic for second opinion and to pursue further genetic testing. Neurological exam again demonstrated hyperreflexia but was otherwise normal. Physical exam was notable for a few small café au lait macules thought consistent with his mixed-race background. His previous imaging studies were reviewed and demonstrated no NF2-related findings. His hearing was normal. During the appointment, he met with our Genetic Counselor, who reviewed his genetic testing results and discussed his case with the laboratory director at the medical genomics laboratory at the University of Alabama-Birmingham (UAB).

It was decided that full gene sequencing was unnecessary and targeted testing was undertaken at UAB. Results were significant for the absence of the previously reported pathogenic variant c. 1387G>T and instead the presence of the likely benign variant c.1378G>A at the same site. In light of this test result in conjunction with normal brain and spine imaging and lack of family history of NF2, patient was informed there is no evidence at this time to suggest that he has NF2. He was understandably relieved.

This case study demonstrates the risk for false-positive results based on direct-to-consumer testing and raw-data analysis and its impact on the individual and the healthcare system in general. Due to these erroneous results, the patient experienced significant anxiety and underwent visits with two different neurologists, had contrast-enhanced MRI of the brain and spine, and required further genetic testing.

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Benefits and Challenges of a NF Clinic Model including a Geneticist, Genetic Counselor and Genetics Nurse at Mayo Clinic

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Background: Mayo Clinic's Department of Clinical Genomics has established a weekly Neurofibromatosis Clinic that provides clinical evaluations for patients with Neurofibromatosis type 1 (NF1), Neurofibromatosis type 2 (NF2), and Schwannomatosis. The current clinic model incorporates appointments with a genetics nurse, geneticist, and genetic counselor. When creating this clinic model there was a focus on allowing each provider to practice at the top of their scope when considering allotted times, roles, and responsibilities of each individual.

Methods: Each contributing provider was asked to compile a list of the benefits and challenges of having a geneticist, genetic counselor, and genetics nurse involved in the practice.

Results: Common benefits included (1) increasing patient access, (2) having allotted time for each provider to complete the appropriate tasks that are within their scope, (3) improving quality of appointments, (4) having multiple expert clinical providers available for patient contact, and (5) facilitating engagement in clinical trial recruitment and database curation. Common challenges included (1) scheduling difficulties, (2) patient fatigue in extended appointments, (3) communication between providers, (4) sustainable billing practices.

Conclusion: The incorporation of a geneticist, genetic counselor, and genetics nurse in a clinic model to care for patients with NF1, NF2, and Schwannomatosis has presented both benefits and challenges. This model allows for each provider to practice at the top of their skill set, increase efficiency, and to provide high quality patient care. By identifying our successes and areas for growth we are able to continue to improve upon a model for a successful genetic Neurofibromatosis Clinic.

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NF1 Associated with Breast Cancer in the Cases of Tokyo Jikei Medical University

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Background: Women with neurofibromatosis type 1(NF1) are reported to have a higher risk of breast cancer than the general population in the European countries. Our aim is to make sure if it is true in Japanese NF1 patients with breast cancer.

Methods: The database of Japanese National Cancer Center was analysed to determine age-specific rates of women with NF1 patients and controls. Archival NF1 breast cancer samples were examined for histologically and immunohistochemically.

Results: Out of 421 cases of women with NF1 who have visited our facility in 2018, 4 patients were diagnosed with breast cancer. The standardized incidence ratio (SIR) for breast cancer in the Japanese women with NF1 population was 8.17(CI: 2.62-22.47) based on incidence rates of breast cancer in the general population taken from the database of Japanese National Cancer Center.

A trend of higher SIR for breast cancer in the young women with NF1 was observed. Archival 8 NF1 breast cancers were more often associated with poor prognostic factors, which are 4 triple negative ;ER(estrogen receptor) negative, PR (progesterone receptor) negative and HER2 (human epidermal growth factor receptor 2) negative, 3 HER2 amplification and 1ER,PR positive.

Conclusions: Likewise in European countries, Japanese women with NF1 have a higher risk of breast cancer than the general population. The results need active follow up for breast cancer in women with NF1.

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Spinal Lesions in Neurofibromatosis Type 1 in Adults

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Learning Objective: To characterise the clinical and radiological features of a large cohort of adult patients with Neurofibromatosis type 1 and see if there are any associations between various features.

Objectives: Neurofibromatosis type 1 (NF1) can manifest itself in many ways in the spine. This study aims to report the types of spinal lesions, symptoms and demographic data in a large cohort of NF1 patients from the Manchester NF1 centre and make comparisons between those with classical NF1 and a subtype called spinal neurofibromatosis.

Methods: This is a descriptive study of retrospectively collected data from 303 adult patients reported from the Manchester NF1 centre and is one of the largest reported series of NF1 patients. Prevalence of each symptom and lesion was calculated and statistically significant associations were established.

Results: The most reported symptoms were cutaneous lesions (44.9%) and pain (37%). 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 most progressive type of lesion was that of spinal nerve root tumours (16.8%). The only statistically significant association between lesions was dural ectasia and spinal deformity (P < 0.003), where dural ectasia was associated with a 32.6% increase in spinal deformity.

Conclusion: This is one of the largest reported descriptive study 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. Spinal neurofibromatosis patients are likely to have surgery, suggesting closer surveillance is required.

Longitudinal Examination of Social Skills of Children with Neurofibromatosis Type 1 Beginning in Early Childhood

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Background: Social difficulties are commonly reported by parents and teachers of children with neurofibromatosis type 1 (NF1) (Barton & North, 2004; Huijbregts & de Sonneville, 2011). However, there are no investigations of the longitudinal pattern and most studies focus on the school age years. This study aims to examine social skills longitudinally in children with NF1, including examination of ADHD symptomatology and cognitive functioning as contributing factors.

Methods: Participants were 25 children with NF1 assessed longitudinally from early childhood (ages 3-6; $M=4.12$, $SD=1.09$) to school age (ages 9-13; $M=10.40$, $SD=1.35$) and their parents. The following measures were used: Social Skills scales on the Social Skills Rating System (SSRS) and Social Skills Improvement System (SSIS) for social functioning; Conners Parent Rating Scale–Revised (CPRS-R) and Conners-3 for ADHD symptomatology; and Differential Ability Scales-2nd Edition (DAS-II) for cognitive ability.

Results: Early childhood and school age social skills did not differ significantly ($t(24)=0.97$, $p=.34$, $d=0.23$) and were not significantly correlated ($rho=.30$, $p=.08$). Social skills of 3- and 4-year-olds were not significantly correlated with school age social skills ($rho(17)=.32$, $p=.104$) but social skills of 5- and 6-year-olds were significantly correlated with school age social skills ($rho(24)=.56$, $p=.002$). Social skills difficulties were observed for 32% of young children and 24% of school age children with NF1 with no significant difference in proportion ($p=.69$). Early childhood parental rating of inattention was significantly negatively correlated with school age social skills ($rho(25)=-.39$, $p=.026$), while ratings of hyperactivity were not significantly related to social skills ($rho(25)=-.05$, $p=.42$). Early childhood cognitive functioning was not significantly correlated with school age social skills (GCA: $rho(25)=-.06$, $p=.39$; V: $rho(25)=-.19$, $p=.18$; NV: $rho(25)=.15$, $p=.24$; S: $rho(21)=.15$, $p=.26$).

Conclusions: Early social skills predicted school age social skills for children with NF1 and no difference in the frequency of social skills difficulties was observed, suggesting social skills remain stable over time. Early inattentive symptoms contributed to later social skills while cognitive function was not a significant factor in social skills outcomes. Overall, these findings add to the limited social skills literature in children with NF1 which may be useful for the development of targeted interventions, with implementation at a young age, to support optimal functioning.

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Transition Readiness Assessment in Adolescents and Young Adults with Neurofibromatosis Type 1 (NF1)

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Neurofibromatosis type 1 (NF1) conveys significant disease morbidity and lower quality of life (QoL) compared to the general population. Research has shown that inadequate development of health-related self-management skills is directly correlated to decreased positive health outcomes in similar patient populations. Thus, the process of healthcare transition (HCT) is necessary to improve the provision of care and health outcomes in adults with NF1. In order to design an informed HCT intervention for NF1, baseline transition readiness must be assessed. A survey distributed by the Children's Tumor Foundation (CTF) was developed to assess transition readiness and the impact of NF1 on factors of young adult life. Final statistical analysis of the data collected with this study is complete and recommendations for improving health outcomes are documented.

A total of 101 participants aged 14-26 years completed the survey with a mean age of 19.14 (SD 3.41). The majority of participants were female (59.4%), white (83.2%), lived in or near a city (60.4%), and had a high school education or less (61.2%). Participants generally reported that NF1 had significant or some impact on all assessed factors of young adult life including education, career, relationships, and family planning. The median Transition Readiness Assessment Questionnaire (TRAQ) score for individuals with NF1 in this study (3.50/5.00) was significantly lower than the median TRAQ scores in individuals without chronic medical conditions (3.93/5.00) ($p < 0.0001$). Females with NF1 had an estimated 0.51 point increase of their total TRAQ score than males (p = 0.00785). Increased participant age was associated with a higher total TRAQ score (p < 0.0001). Individuals with NF1 who scored higher on the TRAQ score also scored higher on NF1-specific transition questions ($r = 0.632$). Results show that teens and young adults with NF1 self-report general knowledge of NF1 and comfort talking to providers, and discomfort with appointment keeping, insurance related tasks, addressing NF1 emergencies, and discussing NF1 with non-medical providers and peers. Our data show that TRAQ scores and NF1-TRAQ scores were significantly lower in individuals who reported that their diagnosis of NF1 had some or significant impact on education, career, and relationships. This demonstrates that decreased transition readiness in individuals with NF1 is associated with a negative impact in many areas of young adult life. Data from this study supports the need to develop NF1-specific HCT intervention tools to increase QoL and to standardize care across NF clinics.

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Quality of Life Patient-Reported Outcomes in Neurofibroma Patients Undergoing Surgery

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Introduction: Surgical excision of dermal neurofibromas and peripheral nerve sheath tumors in neurofibromatosis and Schwannomatosis patients represent a therapeutic challenge since no evidence-based guidelines exist on criteria for these patients to undergo surgery. Treatment is considered to be symptomatic, focused primarily on pain management. We report our neurofibroma center's experience with surgical excision of neurofibromas and peripheral nerve sheath tumors by describing patient demographics, indications, and patient-reported outcomes on the impact of surgery on their quality of life.

Methods: Patients with neurofibromatosis type 1, type 2, and Schwannomatosis undergoing surgery for excision of neurofibromas or peripheral nerve sheath tumors were enrolled in a prospective database as approved by our institutional review board. Demographics, medical history, radiology and surgical pathology reports were collected from the electronic medical record. A survey performed either in-person or over the phone was applied, which consisted of 10 questions on interference of symptoms with daily activities (e.g., dressing, ambulating), pain scale, impression of overall state of health, and experience with surgery. Survey questions were answered using a Likert scale. Primary outcome was comparison of these quality of life survey questions before and after surgery. Secondary outcomes were post-operative complications (e.g., wound complications, hematoma, re-operations). Measures of central tendency and descriptive statistics were used to describe absolute and mean results. Student's t-tests were used to analyze binary data sets. Statistical significance was predetermined at $p < 0.05$.

Results: Ninety-six patients were enrolled in the study with a mean age of 32 years, 51% of the patients were female. A diagnosis of NF type 1 was carried in 80% of patients, NF type 2 in 11% of patients, while Schwannomatosis in 9%. Surgical pathology reported tumor histology as neurofibromas (84%) (of which 29% were plexiform), Schwannomas (10%), mixed tumors (4%), and malignant peripheral nerve sheath tumors (1.7%). The most common indication for surgery was symptoms other than pain interfering with daily activities (e.g., pruritus, interference with dressing, interference with ambulation, cosmesis)(49%), followed by pain (24%), concerning interval growth of lesion (21%) and other concerning features of lesions (5%). Patients' reported overall state of health improved from an average of 2.9 (of 5)(pre-operative) to 3.9 (of 5)(post-operative)($p < 0.05$), interference of symptoms with daily activities improved from 4.1 (of 5) (pre-operative) to 2.0 (of 5)(post-operative)($p < 0.05$), 95% of the patients responded that they would undergo surgery again. A post-operative complication was reported in 9.7% of patients, with wound complications (e.g. delayed wound healing, superficial surgical site infection) being the most common.

Conclusion: In the absence of evidence-based guidelines on surgical indications for excision of neurofibromas and peripheral nerve sheath tumors, we present a case series' data supporting patient-reported symptoms as an indication to undergo surgery. Such intervention translates into significant improvement of daily activities, pain scores, and patients' perception of their overall state of health. The post-operative complication rate is comparable to that of other soft tissue surgeries, maintaining these interventions under a "low risk" category. These data can guide clinicians caring for neurofibroma and Schwannomatosis patients on counseling them when considering surgery.

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Morphology of the Corpus Callosum and Neuropsychological Development in Children with Neurofibromatosis Type 1 (NF1)

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Background: Children with NF1 experience an elevated incidence of cognitive impairment and learning difficulties. To date, a biomarker that identifies children who are at greatest risk for these does not exist. Studies suggest that corpus callosum (CC) morphology may serve as an anatomical marker of cognitive ability. This retrospective study explores whether CC size, measured during early childhood, can predict which children with NF1 are at greatest risk for impaired cognitive development.

Methods: Children who had MR images obtained during early childhood and subsequent neuropsychological testing were identified using a pre-existing registry of NF1 patients. Parent questionnaires (e.g., Behavior Rating Inventory of Executive Functioning), patient performance-based measures (e.g., Wechsler scales), and MR images were obtained from medical records. CC parameters were measured using methods previously described by Garel et al (2011). The thickness of the body of the CC (BT), thickness of the isthmus of the CC (IT), and thickness of the splenium of the CC (ST) were compared to the neuropsychological results using Spearman's rho correlations.

Results: The sample consisted of 54 children (40.7% male, age at MRI M=6.87 years, age at neuropsychological evaluation M=10.44 years, years elapsed between MRI and neuropsychological evaluation M=3.58 years). Approximately 80% had a psychiatric diagnosis and 60% received special education services. Neurocognitive functioning was consistent with existent literature (Full scale IQ M=94.02; Digit span forward M=8.25; Digit span backward M=8.87). Using reference biometric data, the majority of children (74% to 96%) fell within the upper 50th percentile for all CC parameters assessed.

There were significant negative associations between measures of CC thickness and measures of working memory, including between the Wechsler Working Memory Index, and BT (rho=-0.34, p=0.03) and IT (rho=-0.31, p=0.04). There were also significant positive associations between ST, and teacher-reported externalizing behavior (rho=0.38, p=0.02) and parent-reported hyperactivity (rho=0.37, p=0.03), such that as corpus callosum thickness increased, children's externalizing behavior and hyperactivity also increased.

Conclusion: Corpus callosum morphology, as measured during routine screening MRI scans, may be an important early structural biomarker for identifying children at risk of developing cognitive difficulties. This data provides critical information needed to design prospective cognitive intervention studies for NF1 patients who are at greatest risk for delayed cognitive development.

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Non-Literal Language Skills in Children with Neurofibromatosis Type 1

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Background: Children with neurofibromatosis (NF1) commonly experience cognitive deficits, including language deficits. While expressive and receptive language difficulties are well documented, social communication abilities are less well understood. We investigated non-literal language (NLL) skills, including simile, metaphor, and sarcasm, as well as literal language comprehension, in children with NF1 relative to typically developing controls (TDCs). We also examined the association between NLL skills, general intellectual functioning and behaviour difficulties, specifically attention deficit hyperactivity disorder (ADHD).

Methods: This multisite cross-sectional prospective study compared NLL abilities in 4 to 12 year old children with NF1 (N=45, M age = 7.8) and TDCs (N=23, M age = 8.0). Participants with NF were recruited from the Neurofibromatosis Clinic at the Royal Children's Hospital in Melbourne and the Neurogenetics Clinic at The Children's Hospital at Westmead in Sydney, Australia. TDCs were recruited from the general community and matched to NF1 participants on mean chronological age. NLL skills were assessed using a novel task consisting of 13 short stories read to participants. Children were then asked questions which required them to understand sarcasm, metaphor, and simile to answer the questions correctly. Literal language probes were also asked as control questions. The Wechsler Intelligence Scales were used to assess general intellectual functioning. The Conners-3 parent questionnaire was used to rate ADHD symptomatology.

Results: Children with NF1 demonstrated significantly poorer sarcasm abilities than TDCs ($p < .05$), but did not significantly differ from TDCs on the metaphor, simile and literal language conditions. In both groups, older age and higher IQ were significantly associated with better NLL comprehension. Within the NF1 group, total attention deficit-hyperactivity symptom severity scores and verbal comprehension index scores were not related to performance on the NLL task.

Conclusions: Children with NF1 demonstrate a reduced ability to identify sarcasm than typically developing peers. These findings have implications for social interactions and pragmatic communication in children with NF1, particularly those with lower general intellectual functioning. Interventions aimed at training children with NF1 to better understand sarcasm would be beneficial for affected children and may help to improve social communication in this group.

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Enhancing Patient Care: Role of Nurse Care Coordinator in a Multidisciplinary Neurofibromatosis Type I Program

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Our facility has a comprehensive Neurofibromatosis Type 1 (NF1) program, which has tripled in growth to include several NF1 multidisciplinary (MD) clinics designed to meet the medical complexities of individual NF1 patients. Initially, a genetic counselor (GC) was designated as the clinic coordinator. As the clinics grew, demands were being placed on the GC beyond their expertise/scope. The NF1 program decided to hire a Nurse Care Coordinator (NCC) to improve efficiency and help address the complex medical care needs of this population. Responsibilities were then divided between the NCC and the GC. The GC role provides initial screening, diagnosis counselling, and assistance with genetic testing. The NCC role provides documentation of patient status and medical needs, pre-clinic summaries for team members, development of ongoing care plans, medical education for patients, triaging individual medical concerns, assistance with coordination of multiple appointments, pre-clinic testing (MRI), program development, research data collection and communication with community providers. The addition of this NCC role has resulted in improved efficiency of the clinics, reduced appointment access times, improved communication among team members, and increased patient satisfaction regarding medical care coordination needs. The NCC is the primary contact for patients, families, and community providers. The NCC works closely with team members to determine the specialties needed for the monthly clinics, ongoing medical care coordination needs, and the infrastructure necessary to put program goals into action. Two specialized clinics exist to provide individual care for patients with NF1. Our NF1 MD clinics combine providers from 6 specialties and are designed for patients who have either complex symptoms or vascular complexities. The collaboration of the NCC and GC within the program produced a balance of addressing genetic counseling and medical needs all within each scope of practice. Further progress is needed to ensure proper follow-through of medical needs, education and access for patients and community providers, continued development of individual specialized care plans. The nurse care coordinator role is crucial in improving and developing a NF1 multidisciplinary program within any care facility.

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Clinical Characteristics of 427 Patients from 389 Korean Families

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Neurofibromatosis type 1 (NF1; OMIM#162200) is one of the most common genetic diseases affecting 1 in 3,000 people. Although NF1 is highly penetrant in the affected patients, it holds a wide range of expressivity. In the current study, we evaluated the clinical and genetic characteristics of a large Korean NF1 cohort.

A total 389 out of 424 patients (91.7%) fulfilled the NIH diagnostic criteria and 381/424 patients (89.9%) fulfilled the NIH diagnostic criteria when family history of NF1 was excluded. The NIH criteria were fulfilled in nearly all patients at age 8 years or older (98.3%). Among the 35 patients without NIH criteria fulfilled, 34 patients with NF1 were diagnosed by genetic testing.

Patients with NF1 experience a life-long clinical course and phenotypes progress with aging. In this respect, the detailed clinical and radiological findings at evaluation were compared among three different age groups; prepuberal age (<8 years), adolescent age (8-18 years), and adulthood (>18 years). As patients get older, the cutaneous manifestations became more evident. In addition, hypertension (15%), hearing difficulty (5%), arrhythmia (15%), and plexiform neurofibromas (50%) were more commonly observed in the adult patients. On the other hand, attention deficit, brain tumor, and FASI were more common in patients under 18 years of age. Regarding the mode of NF1 inheritance, cutaneous neurofibromas, widely spread cutaneous neurofibromas, plexiform neurofibromas, and vertebral dysplasia were more prevalent in familial cases than in sporadic cases. Between male and female patients, plexiform neurofibromas, severe plexiform neurofibromas, and brain tumors were frequently reported in male patients.

As an age-related condition, several phenotypes became more prevalent in older patients, and some phenotypic differences in terms of inheritability and sex was found as well. Further study is required to understand these phenotypic differences among the patient subgroups.

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Treatment of Pain and Tumor Growth in NF2

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Background: Neurofibromatosis Type 2 (NF2) is an autosomal dominant disorder caused by mutations to the NF2 tumor suppressor gene, characterized by multiple nervous system tumors. Chronic pain affects the majority of patients with NF2 and is the primary factor that contributes to the decreased quality of life (QOL). Patients with chronic pain usually require pain treatment, which often includes opiates. There are limited additional therapies that can effectively reduce pain in patients with NF2 but intravenous (IV) bevacizumab has been reported to provide significant relief to patients suffering from debilitating pain.

Case Study: James is a 24-year-old young man who initially presented with manifestations of NF2 at age 10 and by 15 years old had developed daily pain symptoms including neck, back, and lower extremity pain. He has multiple CNS schwannomas, meningiomas, and neurofibromas. His pain was poorly controlled with multiple oral medications, including opioids. A trial of imatinib was completed without benefit. James started IV bevacizumab at age 16 and his pain was under better control, but he was critically dependent on bevacizumab. He had a break from bevacizumab for wisdom teeth extraction and was hospitalized in the intensive care unit for pain management. Following five years of bevacizumab he developed worsening toxicities including hypertension, proteinuria, and elevated hemoglobin. James transitioned to therapy with trametinib, a MEK inhibitor, and was able to wean off bevacizumab six months later. Treatment of NF2 related pain with trametinib has significant benefits. He no longer needs to travel over 140 miles every two weeks for a bevacizumab infusion. James is a college graduate, has a long-term partner whom he lives with, works full time, volunteers in his community, and participates in musical groups, martial arts, and dancing. The improvement in his QOL while taking trametinib is significant, both with increased pain management, decreased side effects, and time to complete therapy.

Future Implications: Treatment of NF2 tumor related pain can be managed with MEK inhibitors. Discussion of the pathology of pain in NF2 and how to manage it continues to be developed. The effectiveness as well as the positive impact on QOL need to be considered when treating patients with NF2-related pain.

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Update: Neurofibromatosis Therapeutics Program Nurse Care Coordinator Role

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Background: Neurofibromatosis (NF) therapeutics is a vital field in the care of children with NF. Recent developments in the treatment of plexiform neurofibromas (PN) have increased the numbers of patients seen for therapy. The Neurofibromatosis Therapeutics Program (NFTP) provides high quality care to patients receiving therapy for brain tumors and PNs. The Nurse Care Coordinator (NCC) is the key player on the team managing all patients under the care of the NFTP.

Methods: The program at Children's Hospital Colorado (CHCO) includes a physician, nurse practitioner, nurse care coordinator, and family navigator. The NCC collaborates with other disciplines in the care of the NF patient with plexiform neurofibromas and/or CNS tumors. Key players are identified in each subspecialty and communicated with regularly. Often patients will be seen in other clinics to increase continuity of care and ensure patients comply with treatment surveillance. The NCC completes triage, determines subspecialties to be seen and timing of visits, educates patients and families about treatment, and completes insurance authorization. The NCC assists with the tissue collection study of plexiform neurofibromas and brain tumors which examines epigenetics research on tissue obtained during surgery. Weekly review meetings both with the Neurology NF NCC and with the NFTP team improve continuity and timeliness of care for patients. CHCO cares for patients in a seven-state region and the NCC communicates regularly with local providers caring for our patients. Most importantly, the NCC ensures that patients and families maintain close contact with the NFTP regarding side effects and compliance while on therapy.

Results: The NCC has increased adherence to required studies and monitoring, earlier identification and treatment of side effects associated with treatment, better enrollment on the tissue collection study, increased patient satisfaction, improved intradisciplinary collaboration, faster insurance authorization of medications, and streamlined referral and second opinion process.

Future Areas of Growth: Future goals include: NFTP website with education and skin care pathways, quarterly newsletters, continuing education offerings, tracking tool for annual patient visits, development of skin assessment tool and response to skin care interventions, research into who develops side effects to treatment, and education materials for adolescents with the risk of malignant transformation of tumors, and transition to adult care.

Conclusions: The NCC ensures that the NFTP continues to be a strong and vibrant area of growth with the aim to provide high quality of care for the whole child and conduct lab and clinical research.

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Impaired Theory of Mind in Youth with Neurofibromatosis Type 1

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Objective: Children and adolescents with neurofibromatosis type 1 (NF1) are at increased risk for social adjustment difficulties (e.g., peer acceptance), which adversely impacts quality of life across the lifespan. Deficits in social information processing skills, including the ability to understand the mental states/intentions of others, or Theory of Mind (ToM), likely contribute to these difficulties yet little research has evaluated these abilities. The current study compared ToM performance in youth with NF1 to typically-developing peers.

Participants & Methods: Youth with NF1 (n=20; *M* age=12.55 years, *SD*=1.67) and unaffected healthy control (HC) participants (n=14; *M* age=12.66 years, *SD*=1.75) completed a computer-based ToM task. The Jack & Jill (J&J) task consists of a series of three-part sequenced pictures in which one character (Jack) is depicted switching or not switching (Switch) the location of a ball from one colored hat to another, which is either witnessed or unwitnessed (Witness) by the other character (Jill). Participants were asked to take Jill's perspective and report on her beliefs of the ball's location across four conditions (Switched/Witnessed; Switched/Unwitnessed; Unswitched/Witnessed; Unswitched/Unwitnessed). Eight trials of each condition, for a total of 32 trials, were administered in a counterbalanced fashion. A three-way mixed repeated-measures analysis of covariance (ANCOVA), controlling for age and IQ, evaluated accuracy scores between groups across conditions.

Results: An aligned rank transformation was applied to accuracy scores across conditions due their non-normal distributions. Repeated measures ANCOVA revealed a significant main effect of group, with the HC group outperforming youth with NF1 across conditions, $F_{(1,30)}=29.95$, p<.001. There was a statistically significant three-way interaction among group, Switch condition, and Witness condition, $F_{(1,30)}=13.53$, p=.001. Post-hoc Mann-Whitney U tests revealed significant group differences on the Switched/Unwitnessed condition (*p*=.016), to the disadvantage of the NF1 group (*M*=70.00, *SD*=39.40) relative to the HC group (*M*=98.21, *SD*=4.54). Wilcoxon signed-rank tests showed a significant decline in accuracy on the Switched/Unwitnessed condition relative to the Switched/Witnessed condition for the NF1 group (*p*=.026) but not the HC group (*p*=.705). Groups did not differ across other component conditions (*ps*>.05).

Conclusions: Participants with NF1 study demonstrated ToM impairments relative to unaffected peers. Conversely, they had similar accuracy to peers on task conditions that did not require ToM. Difficulties with understanding the mental states and intentions of others may contribute to the social difficulties of youth with NF1. Additional research is needed to establish these social information processing abilities as targets for screening and intervention in NF1.

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Academic Achievement and Cognitive Functions of Children and Young Adults with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PN): Stability and Change Across Six Years

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Background: Prior studies have demonstrated that individuals with NF1 often exhibit learning disabilities and cognitive impairments, typically using a cross-sectional design to compare an NF1 group with a control group. There is a lack of longitudinal studies examining the academic and cognitive development of individuals with NF1. To fill this gap, the current study aims to document how academic and cognitive outcomes of children and young adults with NF1 and PNs develop across time and what individual factors predict initial levels and change of academic and cognitive outcomes.

Methods: Participants were 88 children and young adults with NF1 and PNs (8-22 years, *M* age 12.90+3.50, 61% male) enrolled in a natural history study. Comprehensive neuropsychological assessments were conducted at baseline, 3 years, and 6 years. Participants' academic achievement (math, reading, and writing), IQ, and attention were assessed using standardized tests. Executive functioning (inhibitory control, working memory, and cognitive flexibility) was assessed using standardized tests (ST) and parent-reported surveys (PR). Individual factors included age, sex, parental education, parental NF1 status, and NF1 disease-related complications. Multilevel growth modeling was used.

Results: Children and young adults with NF1 and PNs had significantly lower mean scores on almost all academic and cognitive outcomes compared to normative scores. Z-scores of participants' math, working memory (ST), and inhibitory control (ST) declined over time, suggesting that the gap between participants and their normative peers on these outcomes widened across time. Older (vs. younger) participants were more likely to experience decreasing math and writing but increasing IQ, inhibitory control (PR), and working memory (PR), suggesting potential curvilinear relationships between age and these domains. Males exhibited worse reading but better inhibitory control (ST) and working memory (PR) than females at baseline. These sex differences persisted across time except in the context of working memory (PR). Participants whose parents had higher (vs. lower) levels of education had higher initial levels of academic achievement, IQ, working memory (ST), and cognitive flexibility (ST); an advantage that lasted across time. Participants who have parents with (vs. without) NF1 were more likely to experience a decline in reading, writing, and attention. More NF1disease-related complications were related to lower initial levels of reading and writing.

Conclusions: There are considerable individual differences in the academic and cognitive development of children and young adults with NF1 and PNs. Patients with low parental education are particularly at risk for academic and cognitive impairments. Certain academic and cognitive functions get worse across time, especially for older youth and those who have a parent with NF1. These findings indicate the importance of providing resources and interventions to support children throughout their development. In particular, attention should be given to children with hereditary NF1 and those with parents that did not graduate high school, as these individuals are at greater risk for cognitive impairment.

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In-Hospital Clinical Network for NF1 in Nagoya University Hospital: Roles of Orthopaedic Oncologists

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Background: Owing to the variety of clinical features in patients with neurofibromatosis type 1 (NF1), a multidisciplinary approach is recommended for their health surveillance. We have managed in-hospital clinical care network for NF1 since January 2014. A multidisciplinary team was composed of specialties including orthopaedic surgery, neurosurgery, pediatrics, ophthalmology, dermatology, plastic surgery, psychiatry, and genetic counseling etc. The aim of this study is to examine the characteristics of our patients treated under in-hospital clinical network.

Methods: A total of 152 patients were enrolled in this network until March 2018. All patients less than 16 years old were under the care of pediatrics, and clinical examination by orthopaedic surgeons, neurosurgeons, and ophthalmologists was essential. In orthopaedic surgery, we have routinely evaluated the presence of deep-seated tumors and their potential of malignancy with whole-body MRI when patients consulted us. Diffuse neurofibromas and plexiform neurofibromas were excised in cases that they could cause pain, disfigurement, and dysfunction.

Results: The median age of the patients at a first visit was 22.0 years, and 61 (40%) patients had an NF1 family history. One hundred and thirty-nine patients were referred from local clinics and general hospitals. Fifty-six (40%) patients were referral from orthopaedic surgery, 25 (18%) from pediatrics, 20 (14%) from dermatology, 10 (7%) from plastic surgery, and 18 (13%) from the others. Of the 152 patients, 17 (11%) in 8 families were treated together with affected family members. The most common reason for referral to our hospital was mass (excluding dermal neurofibromas) in 67 (44%) patients. There were 12 (8%) patients with MPNST. 80 (53%) patients had DNF or PNF, and 31 (20%) had pain.

Conclusion: In this study, orthopaedic surgeons, who are mainly responsible for the diagnosis and treatment of a tumor mass, play a significant role in the NF1 care. Considering malignant potential of deep-seated tumors, routine follow-up and clinical surveillance should be recommended for all individuals with NF1. It is important for the development of our clinical network to facilitate an opportunity for affected family members to have a medical examination. We hope that our multidisciplinary management with in-hospital network may contribute to the improvement of the prognosis and quality of life in patients with NF1.

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Pregnancy Outcomes in Women with NF1: A Danish Population-Based Cohort Study

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Background: The fertility in women with neurofibromatosis type 1(NF1) is thought to be normal, but no studies have investigated pregnancy outcomes in women with NF1. Thus, we conducted a population-based cohort study to assess the probability of given birth to a live born child, stillbirth and abortion in 1,006 women with NF1.

Methods: We included 1,006 women in the fertile age (15−50 years) registered with a diagnosis of NF1 in the Danish National Patient Register or followed at two national Centres for Rare Diseases. All women were matched to 10,020 women in the general Danish population on age. Information on livebirths, stillbirths and abortions was ascertained from the national Medical Birth Register, Register of Induced Abortions and the National Patient Register. The proportion of pregnancies among the women with Nf1 that resulted in a livebirth, a stillbirth or an abortion was compared with the equivalent proportion among the population comparisons in the period 1977−2013. Survival analysis was used to assess recurrent pregnancy outcomes.

Results: During follow-up, women with NF1 had 1,252 pregnancies, of which 785 resulted in a livebirth, 14 in a stillbirth and 459 in an abortion. The proportion of pregnancies that resulted in a live birth and stillbirth was 62.5% and 1.1% , respectively, among women with NF1 and 67.7% and 0.3%, respectively, among the comparisons, resulting in an age-adjusted proportion ratio (PR) of 0.92 (95% confidence interval (CI) 0.88−0.97) and 2.83 (95% CI 1.62−4.91). The proportion of abortions was higher among women with NF1 (36.7%) than among the comparisons (32.1%) with a PR of 1.08 (95% CI 1.00−1.18). The higher proportion of abortions observed in women with NF1 was due to an increased number of spontaneous abortions (NF1: 108 (23.5%), comparisons: 738 (18.4%)), whereas the number of provoked abortions was similar in the two groups (NF1: 286 (62.3%), comparisons: 2.549 (63.7%)). The hazard ratio for a negative event (abortion or stillbirth) was 14% lower among NF1-free women than among women with NF1 (hazard ratio 0.86 (95% CI 0.98−0.98, P=0.03)).

Conclusion: Women with NF1 experience more negative pregnancy outcomes than women in the general population. Close follow-up of pregnant NF1 women is warranted.

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Vascular Changes in Evaluation of Cardiac Function in Pediatric Patients with Neurofibromatosis Type 1

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Background: Cardiovascular complications are a major source of morbidity and mortality in patients with neurofibromatosis type 1. Increased risk of hypertension, stroke and early cardiac death have all been reported, however, the incidence of cardiac abnormality and benefit of screening have not been well established, particularly in the pediatric population.

Methods: We enrolled 25 patients with neurofibromatosis type 1 between the ages of 11 and 18. After informed consent was obtained, each patient had blood and urine biomarkers of cardiac health and inflammation obtained and underwent a non-contrast, non-sedated MRI to assess aortic wall thickness, aortic distensibility, aortic area and pulse wave velocity.

Results: Of the 25 patients consented, 24 underwent study procedures. Of the study participants, the mean age was 15.3 years with 44% female. 20/24 were Caucasian, 8 of those of Hispanic ethnicity. All participants completed biomarker and MRI testing. 41% of patients had detectable BNP levels; no patients demonstrated elevation in troponin 1. 3 (12.5%) patients had elevation of HS-CRP and only one had detectable plasma interleukin-6. On MRI, aortic diameter, wall thickness and distensibility were not significantly different than published controls. Pulse wave velocity across the aortic arch, however, was significantly elevated compared to expected norms.

Conclusions: Cardiac MRI did not demonstrate significant structural cardiac or aortic differences in a pediatric NF1 population, nor did standard blood biomarkers of heart health and inflammation. Pulse wave velocity was significantly higher than expected and may warrant further investigation.

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Monitoring and Supportive Care for Pediatric NF1 Patients on MEK Inhibitors: Recommendations from the CTF Clinical Care Advisory Board

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Early phase clinical trials utilizing oral inhibitors of MEK, the mitogen-activated protein kinase kinase, have demonstrated clear utility for patients with neurofibromatosis type 1 (NF1) associated tumors, particularly progressive low grade gliomas and plexiform neurofibromas. Given the potential benefit of MEK inhibition, use of targeted agents in the NF1 population is likely to increase substantially in the upcoming years. For MEK inhibition to be successful, care should be taken to limit the time off medication as interruptions in treatment have been associated with tumor regrowth. Long-term use of these medications in individual patients may be warranted; therefore appropriate monitoring for toxicity is also critical. For clinicians with limited experience with these medications, concern about toxicity and monitoring may be a barrier to use. The Clinical Care Advisory Board of the Children's Tumor Foundation has suggested monitoring guidelines for potential ophthalmologic, cardiac and dermatologic toxicities as well as basic recommendations for side effect management. These recommendations can serve as a beginning template for NF providers.

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Medical Costs of Surgical Intervention in Hospitalized Patients with Neurofibromatosis 1 in Japan

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Although skin surgeries are often performed to remove neurofibromas (NF) in patients with neurofibromatosis 1 (NF1), the costs of these surgical treatments have not been well investigated. To elucidate the medical costs of patients hospitalized for surgery for NF1, a retrospective study was conducted of 96 patients with NF1 at two major institutions in Japan. All of the patients met the diagnostic criteria by National Institutes of Health for NF1. Mean patient age was 37.5 ± 17.4 years. Preoperatively, 33.3% of patients had fewer than 100 cutaneous NF (cNF) lesions; 14.6% had more than 1,000 cNF lesions. Patients with diffuse NF (dNF) had longer hospitalization (13.8 ± 7.73 days) than those with cNF (7.86 ± 3.83 days; $p < 0.05$) because of several factors, including massive intra- and postoperative bleeding. The hospitalization period showed a positive correlation with total costs ($r = 0.757$). The total medical costs of dNF were higher than those for cNF ($p < 0.05$), but the surgical costs were not significantly different between cNF and dNF ($p = 0.843$). In conclusion, treatment of cNF lesions is desirable for patients with NF1 in their late thirties, irrespective of the number of tumors. We have to be careful with surgical intervention for dNF due to the medical costs related to longer hospitalization.

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Robust Surgical Approach for Cutaneous Neurofibroma in Neurofibromatosis Type 1

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Background: Cutaneous neurofibromas (cNF) are physically disfiguring, irritated, and cause extensive psychologic harm in patients with neurofibromatosis type 1 (NF1). There is currently no effective medical treatment and available surgical procedures are inaccessible to most NF1 patients globally.
Objective: While research is underway to find an effective medical treatment for cNF, there is an urgent need to develop surgical approach that is accessible to all NF1 patients in the world with the skill set and equipment found in most general medical office settings. Here, we present a robust surgical approach to remove cNF that does not require sterile surgical field, utilizes accessible equipment that are readily available in any general medical clinic, and can be performed by any health care providers including family practitioners, and physician assistants.

Methods: In a prospective case-series, patients with NF1 underwent this surgical procedure which removes multiple cutaneous neurofibromas per visit. This surgical technique is based on the biology and anatomy of the cutaneous neurofibroma where a large portion of the tumor is in the dermis. Therefore, a critical component to this procedure is further removal of the mass within the deeper dermis. The Dermatology Life Quality Index was given to subjects before and after the procedure as surrogate for patient satisfaction.

Results: 83 tumors were removed throughout the body from twelve individuals. Examination at follow-up visits revealed well-healed scars without infection or adverse events including aberrant scarring. Patient satisfaction with the procedure was high with significant improvements in symptoms, daily activities, leisure, personal relationships, and treatment experience (p=0.00062).

Conclusion: This study demonstrates a robust surgical approach for management of cutaneous neurofibromas which can be accessed world-wide to individuals with NF1 and performed by a wide-variety of medical providers with high clinical efficacy and patient satisfaction.

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Imaging Comparative Analysis of Pediatric and Adult NF2 Intracranial Meningioma

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Objective: Find the predominant locations of new developed NF2 meningiomas and analyze clinical risk factors of NF2 meningioma annual growth rate (AGR).

Methods: Cranial MRI images of 289 NF2 patients were carefully reviewed with specific attention to the location of meningioma. Location patterns of pediatric and adult NF2 meningiomas were compared with each other. New developed meningiomas were defined as tumors undetectable on the previous imaging during the follow up. Location patterns of new developed NF2 meningiomas during the follow up were also analyzed. 3D Slicer software was used to perform volumetric measurements and AGR analysis. Absolute AGR =

$$\frac{\text{Current volume} - \text{Previous volume}}{\text{Years}}. \quad \text{Relative AGR} = \sqrt[\text{Years}]{\frac{\text{Current volume}}{\text{Previous volume}}} - 1.$$

Results: Among the 289 NF2 patients, 49 pediatric patients had 81 meningiomas and 240 adult patients had 939 meningiomas. Pediatric NF2 patients had significantly more cranial meningiomas than the adult patients in the following regions: cranial fossa (anterior, middle and posterior), lateral ventricle ($p < 0.05$). Adult NF2 patients had significantly more cranial meningiomas than the pediatric patients in the following regions: parasagittal (anterior, middle and posterior), parafalcine (middle and posterior), frontal surface, parietal surface and cerebellar surface ($p < 0.05$). During the follow up, 39 new developed meningiomas in 24 NF2 patients were detected. New developed NF2 meningiomas tend to occur in the locations that the adult patients had more meningiomas than the pediatric patients. The absolute AGRs of NF2 meningiomas ranged from -874.5 to 71561 mm^3 (median 217.8 mm^3). The relative AGRs of NF2 meingiomas ranged from -20.4 to 3542.62% (median 19.3 %). Statistically significant multiple linear regression models of absolute NF2 meningioma AGR ($R^2 = 0.283$, F=4.195, P=0.000) and relative NF2 meningioma AGR (($R^2 = 0.256$, F=3.658, P=0.000) were created. The number of new developed meningioma and petrosal location were associated with absolute and relative AGR ($P < 0.05$). Meningioma volume was associated with absolute AGR ($P < 0.05$). The presence of trigeminal schwannoma was associated with relative AGR ($P < 0.05$).

Conclusions: New developed NF2 meningiomas tend to occur in regions that adult patients have more meningiomas than the pediatric group. The most predominant locations of new developed NF2 meningioams were parasagittal (anterior, middle and posterior), parafalcine (middle and posterior), frontal surface, parietal surface and cerebellar surface. The number of new developed meningioma and petrosal location are the risk factors of both absolute and relative AGRs.

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Awake Craniotomy for Assisting Placement of Auditory Brainstem Implant in NF2 Patients

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Objectives: Auditory brainstem implants (ABIs) may be the only opportunity for patients with neurofibromatosis type 2(NF2) to regain some sense of hearing sensation. However, only a very small number of individuals achieved open-set speech understanding and high sentence scores. Suboptimal placement of the ABI electrode array over the cochlear nucleus may be one of main factors for poor auditory performance. In the current study, we present a method of awake craniotomy to assist with ABI placement.

Methods: Awake surgery and hearing test via the retrosigmoid approach were performed for vestibular schwannoma resections and auditory brainstem implantations in four patients with NF2. Auditory outcomes and complications were assessed postoperatively.

Results: Three of 4 patients who underwent awake craniotomy during ABI surgery received reproducible auditory sensations intraoperatively. Satisfactory numbers of effective electrodes, threshold levels and distinct pitches were achieved in the wake-up hearing test. In addition, relatively few electrodes produced non-auditory percepts. There was no serious complication attributable to the ABI or awake craniotomy.

Conclusions: It is safe and well tolerated for neurofibromatosis type 2 (NF2) patients using awake craniotomy during auditory brainstem implantation. This method can potentially improve the localization accuracy of the cochlear nucleus during surgery.

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Patient Reported Outcomes (PROs) Document Clinical Benefit among Adults with NF1 and Inoperable Plexiform Neurofibromas (PNs) on a Phase II Trial of the MEK 1/2 Inhibitor Selumetinib

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Background: On this study of selumetinib (AZD6244, ARRY-142886) for adults with NF1 related PNs, we examined PROs over time in order to assess clinical benefit and document their relationship to tumor response.

Methods: As of May 2019, 17 patients (35% female, M age = 33.8 yrs, M yrs education = 14.8) completed PROs at baseline, then after 4, 8, and 12 cycles (1 cycle[c] = 28 days). Measures assessed pain intensity (NRS-11), pain interference (Pain Interference Index [PII]), quality of life (PedsQL-NF scale), and global impressions of change (GIC). Patients also rated the severity and visibility of their disease (mild, moderate, severe). Volumetric measurement was conducted on a pre-identified target tumor at each time point, with ≥ 20% reduction required for response.

Results: On the NRS-11, 0-10 pain intensity ratings decreased over time (F = 3.13, $p < .05$) from M = 5.5 + 3.4 at baseline to M = 3.6 + 3.3 at c12. Pain interference (PII) ratings decreased between baseline (M = 2.9 + 1.5) and c12 (M = 1.5 + 1.6; F = 6.1, $p < .001$), with significant improvements occurring after 8 cycles ($p < .05$). Correspondingly, 70.6% (n = 12) of patients were taking pain medication at baseline, while only 52.9% (n = 9) reported using pain medication at c12. Significant improvements were noted by c4 on PedsQL subscales assessing movement (F = 6.5, $p < .05$) and social functioning (F = 5.0, $p < .05$), while treatment anxiety increased from baseline to c4 then declined again by c9 (F = 17.5, $p < .001$). Forty-one percent of patients rated their NF1 as severe at baseline, while this number dropped to 11.8% at c12 ($p < .05$). Further, 11.8% of participants rated their baseline NF1 visibility as severe, while no patients rated their visibility as severe at c12 ($p < .05$). Pain severity and pain interference ratings of patients categorized as having stable disease (53%) were not significantly different than those with a partial response (47%) at c12; similarly, pain changes (NRS, PII) did not correlate with volumetric tumor shrinkage ($ps > .05$). On the GIC, 45% of patients reported early positive changes and 35% of patients reported negative changes from baseline to c5. The most frequent positive changes related to decreased pain and improved function; negative changes related primarily to side effects (e.g., rashes).

Conclusions: Similar to previous results obtained in children on selumetinib, pain intensity, pain interference, and aspects of quality of life significantly improved by 12 cycles of selumetinib, with some positive changes occurring after just 4 cycles. Pain improvements did not correspond systematically to tumor response, suggesting that the effect of selumetinib on pain is likely multifactorial and does not seem to be directly related to the degree of change in PN volume.

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Surgical Resection of Peripheral Nerve Sheath Tumors in Neurofibromatosis Type 2: Clinical Outcomes and Incidence of Hybrid Histologic Phenotype

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Background: Neurofibromatosis type 2 (NF2) is a tumor predisposition syndrome characterized by the development of central and peripheral nervous system tumors. Management of peripheral nerve sheath tumors in NF2 is complicated by their frequent involvement of major peripheral nerves, their multiplicity, and reported concern that they may be plexiform and/or involve multiple fascicles. Additionally, hybrid tumors with histologic features of both schwannoma and neurofibroma have been described in NF2, although their relative incidence is unknown. In this study we sought to better define the outcomes of surgical management for tumors involving major peripheral nerves in NF2, as well as define the rate of hybrid histology in this disease.

Methods: Charts from of patients with a clinical diagnosis of NF2 who underwent surgery for peripheral nerve sheath tumors were reviewed in this study. Functional outcomes of surgery for tumors on major (named) peripheral nerve were recorded. Histopathologic analyses were performed on all available excised tumors.

Results: Nineteen operations were performed in 12 patients with NF2, for resection of 28 peripheral nerve sheath tumors. Among 11 tumors involving major peripheral nerves, 10 involved nerves related to motor function. Presenting symptoms in this group with major nerve tumors included pain (9 tumors) and weakness (4). Median tumor diameter was 3.4 cm (range 2.2 to 10.3 cm). Intracapsular resection with neuromonitoring resulted in gross-total resection in 10 cases and subtotal resection in 1 case (involving small motor fascicle). Motor function was diminished after surgery in this latter case, but was stable or improved in the remaining cases. All tumors were found to involve a single fascicle. There were no other postoperative complications. Histopathologic analyses of 21 tumors available for review revealed pure schwannomas among 19 tumors (90%). In two cases (10%) hybrid histopathologic features consistent with both schwannoma and neurofibroma were identified. Mast cells were identified within regions consistent with neurofibroma.

Conclusions: Peripheral nerve sheath tumors can typically be resected safely in NF2 at experienced centers, even if they involve major peripheral nerves. A small proportion (10%) of these tumors harbor hybrid histologic features. This pathologic finding may explain reports of plexiform or multi-fascicular tumors in NF2.

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Genomics of MPNST (GeM) Consortium: In-Depth Genomic Characterization of NF1-Associated and Sporadic MPNSTs

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Background: Malignant Peripheral Nerve Sheath Tumors (MPNSTs) are aggressive sarcomas affecting 8-13% of individuals with Neurofibromatosis Type 1 (NF1); no effective treatment exists. We hypothesize that genomic characterization of a large tumor set, at high depth, and correlation with annotated clinical data, will identify distinct tumor subsets.

Methods: We established an international consortium to collect 85 fresh frozen (FF) MPNSTs (~70% NF1-related). Whole-genome sequencing (WGS; FF tumor at 80x and matched germline DNA at 30x), RNAseq (referenced to RNAseq data from a normal peripheral nerve tissue subset), and MethylationEPIC 850k microarray data will be processed by uniform protocols.

Results: We anticipate a high degree of genomic complexity based on pilot data. Tumors will be classified into subgroups based on recurrent alterations. Our comprehensive profiling, at high coverage depth, adds to prior efforts by providing increased power to detect subclonal alterations. Tumor subset analysis will be performed on RNAseq and epigenetic datasets.

Conclusions: This MPNST genomics study provides the most comprehensive assessment of this genomically complex tumor type at the genomic, epigenomic and transcriptomic levels. Our ongoing efforts include highly detailed pathology characterization through tissue microarray with incorporation of histologic, genomic, and clinical data to improve clinical diagnosis, prognostication, and development of effective medical treatments. Summary data will be made available through a publicly accessible instance of cBioPortal.

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Cognitive Function in Neurofibromatosis Type 1 Patients Greater than 60 Years of Age

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Background: Neurofibromatosis type 1 (NF1) gives rise to developmental disabilities affecting learning and other cognitive functions in 30 to 50% of individuals. These characteristics persist into adulthood, tending to remain relatively stable. However, there are no systematic studies of cognition in older adults with NF1. Our study addresses this aspect of the natural history of NF1.

Methods: A retrospective chart review of adults with NF1 > 60 years of age was performed to determine the frequency of memory and cognitive complaints. The frequency of coexistent psychiatric and medical disorders, pain complaints, and medication use were noted. Brain MRIs were reviewed when available.

Results: Medical records were reviewed for 21 NF1 patients, mean age 69.3 years (range 61-84), including 13 females and 8 males. Seven (33%) reported memory and cognitive complaints. None had a diagnosis of dementia made by us or by their primary care or referring physicians. None was on medical treatment for memory complaints. Thirteen (66%) had multiple clinic visits totaling 42 patient years of follow up, and none had an observed decline on simple mental status assessment. Nine patients were on medication for depression/anxiety. Eight were on medications for chronic pain management. One was on methylphenidate and one was on modafinil. Brain MRI scans were available for 9 patients (mean age 67 years). Observations include: white matter ischemic changes 7, generalized atrophy 5 (mild in 3, moderate 2), ectatic basilar artery 4, asymmetric ventricles 3, stroke 2, arachnoid cysts 2, tumor 1, empty sella 1. Quantitative regional volumetric analysis was available for one 71-year-old with cognitive complaints and depression. Bilateral hippocampal volumes were at 50th percentile for age and thalamic volumes were >95th percentile.

Conclusions: Cognitive complaints are common in older adults with NF1, however in our small cohort none carried a diagnosis of dementia. Among those with serial observations in our clinic none manifested progressive cognitive decline. Mild brain atrophy was common, but severe atrophy was not observed. Our data suggest that older adults with NF1 are not at an increased risk of dementia. The diagnosis of dementia in NF1 patients may be more difficult given preexisting cognitive problems and coexisting depression. Alternatively, adults with NF1 may be protected from dementia, an interesting possibility that deserves further investigation. Prospective studies with more detailed neurocognitive examination are needed to determine the natural history of cognitive function in adults with NF1.

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A Single Arm, Monocenter Phase II Trial of RAD001 as Monotherapy in the Management of Neurofibromatosis Type 2 Vestibular Schwannoma

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Background: Currently, there is a paucity of medical therapies directed for NF2-associated vestibular schwannomas (VS). Prior pre-clinical studies in NF2 mouse models highlighted the potential of mTORC1 inhibition (Everolimus) in delaying schwannoma progression[1]. Recently, two monocenter phase II clinical trials for this drug did not demonstrate VS tumor shrinkage, but rather exhibited tumor growth delay and stabilization with a tolerable drug side effect profile[2,3]. Furthermore, a follow-up study demonstrated that long-term RAD001 treatment increased the time to tumor progression by fourfold at four years[4].

Methods: Our clinical trial enrolled twelve patients from January 2013 to February 2017 at two separate institutions. Patients who met diagnostic criteria for NF2 (NIH 1988) with progressive VS over the past 12 months, adequate bone marrow, liver and renal function, and WHO performance status $\geq$ 2 were eligible for the trial. We excluded patients who had recent radiation therapy or anti-cancer therapies, uncontrolled medical conditions, any malignancies, and any contraindications to bevacizumab. Outcomes for our trial were: (1) VS tumor response, (2) hearing response, (3) safety and tolerability. Tumor response was determined by volumetric measurements performed by two independent researchers. Hearing outcomes were evaluated using word recognition scores and pure tone averages. Adverse events were documented as per the National Cancer Institute Common Toxicity Criteria (CTCAE) version 3.0.

Results: Seven patients (#1,2,6,7,8,10,11) completed the one-year trial with the remaining discontinuing the drug due to tumor progression. There was no radiographic response ($>$=20% decrease in VS tumor volume). However, six patients (#1,2,7,8,9,11) experienced reduction in the tumor growth rate, which increased following discontinuation of the drug. Overall, three patients (#1, 7,9) had stable non-progressive tumor volumes while on Everolimus. One patient (#9) was restarted on Everolimus following study completion due to continued drug efficacy and rapid progression following drug cessation. Hearing outcomes remained stable for patients while on Everolimus. The most common side effects included: mouth ulcers (n = 9), fatigue (n = 6), acne (n = 6), skin rashes (n = 5), and headache (n = 5), while the most severe side effects included CTCAE grade 3 pneumonia (n = 1) and CTCAE grade 4 hypertriglyceridemia (n = 1).

Conclusions: Our findings corroborate the prior two trials, demonstrating Everolimus's cytostatic effect on tumor growth. Everolimus may serve as a safe treatment modality in the medical management of newly diagnosed NF2 to delay surgical intervention and further complications.

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Elevator Pitches as Targeted Communication Tools for Rare Conditions

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The ERN on Genetic Tumour Risk Syndromes (ERN GENTURIS) aims to improve the identification, genetic diagnostics, prevention of cancer, and treatment of European patients with a genetic predisposition for cancer. The ERN GENTURIS focuses on syndromes such as neurofibromatosis, hereditary breast/ovarian cancer, hereditary colorectal cancer and polyposis, and some more rare familial cancer syndromes. It is expected that through the ERNs, European patients with a rare disease get access to expert care more often and more quickly, and that research and guideline development will be accelerated resulting in improved diagnostics and therapies. The aim of our project was to create tools to improve communication about ERN GENTURIS with different stakeholders to create awareness.

Methods: Key messages on ERN GENTURIS and its conditions were developed by a group of health care experts and patient representatives. Different stakeholder groups were defined. HCP (health care provider) members participating in the ERN GENTURIS annual meeting reviewed the draft (general) elevator pitch which is based on the 'key messages' and adapted the messaging to selected stakeholders.

Results: We identified four stakeholder groups, i.e. i) managing board of a hospital, ii) health insurance, iii) health care experts for one of the ERN GENTURIS conditions but not yet member, and iv) regional professionals who come across ERN GENTURIS. Key messages were refined, highlighting in 1-2 minutes the main messages of ERN GENTURIS relevant for the target groups. An ERN GENTURIS standard slide for each of the main target groups was developed which the HCP Members of the Network will use for presentations at meetings and conferences. The complete set of 'elevator' pitches was distributed among HCP ERN GENTURIS members to use for their communication about ERN GENTURIS and familial cancer syndromes.

Conclusion: Elevator pitches provide a valuable tool in creating awareness by targeted short messages for different audiences. These messages may need to be adapted each year as the narrative around ERN GENTURIS starts to mature.

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Trametinib Leads to Rapid Clinical and Radiographic Response in Adult NF1-Associated Hypothalamic Astrocytoma

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Optic pathway gliomas (OPG) occur in 15% of patients with neurofibromatosis type 1 (NF1), and these tumors are most commonly diagnosed in children. Due to the predominantly indolent behavior of OPGs in NF1, treatment is reserved for tumors that impact vision or neurological function. While carboplatin-based therapies are standard for children with NF1-associated OPG, there is no accepted standard therapy in adults with these tumors. Further, biological differences between adult- and pediatric-onset cases are not understood. There is growing interest in the use of MEK inhibitors for NF1-associated tumors based on recently demonstrated efficacy of selumetinib for pediatric plexiform neurofibromas and trametinib in pediatric low-grade gliomas. Our patient is a 41 year old female with NF1, and a newly diagnosed, biopsy-proven hypothalamic astrocytoma, heralded by headaches and vision loss. After a ventriculoperitoneal shunt was placed, she was observed for seven months but had clinical and radiographic progression during this time. As such, we initiated treatment with trametinib at 0.5mg daily, and titrated up by 0.5mg weekly until she reached 2mg daily. Surveillance magnetic resonance imaging (MRI) was performed every two months, showing near complete resolution of contrast-enhancing tumor and a partial response of non-enhancing tumor. Clinically, she experienced improved best-corrected visual acuity from 20/100 (OD) and 20/25 (OS) to 20/25-2 (OD) and 20/25 (OS), and while a left homonymous hemianopsia remained unchanged, her right eye temporal visual field cut had dramatic improvement. She developed a grade 2 acneiform rash on her face, upper chest, upper back, and extremities which was treated with topical and oral antibiotics and steroid cream. She also developed grade 1 serous chorioretinopathy which resolved spontaneously. Otherwise she had no significant adverse effects. Based on our observation, we conclude that symptomatic, adult onset, NF1-associated OPGs may be highly responsive to MEK inhibitor therapy. This represents a novel treatment consideration for a rare, and otherwise challenging clinical scenario. Further study is warranted.

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The Development of a Histopathological Based Classification System for Cutaneous Neurofibromas

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Background: The most common tumor in people with NF1 is cutaneous neurofibroma (cNF). These tumors are disfiguring, socially debilitating, and impede activities of daily living. There is currently no widely accepted standardized language for describing cNF clinically or on histopathologic examination. Development of standardized language for describing cNF will allow for correlation of histological features with clinical and molecular data, eventual stratification of neurofibromas, and treatment trials in appropriate subtypes.

Methods: De-identified histopathology pictures of neurofibromas involving the skin from patients diagnosed with NF1 were obtained from the French national referral center (NF1 Ile de France). Samples were biopsied or resected between 01 April 2014 to 31 April 2017 and analyzed in the Department of Pathology of Henri Mondor hospital. Tumors were removed by surgery and/or laser. Tumors with morphological artifacts hampering interpretation were excluded from this study. All samples were stained with hematoxylin and eosin. Samples analyzed (N = 28) were from 12 females and 5 males, who had an age range of 15-62 (average age 44) at time of biopsy/resection. The size of the lesions evaluated ranged from 0.2 cm – 3.0 cm (average size 1.2 cm and median size 0.9 cm). The body locations from which samples were resected were scalp, digital, shoulder, arm, cheek, foot, ankle, and back. Individual reviews were conducted by 4 neuropathologists and 2 dermatopathologists, who evaluated each image and entered free text of up to a 200 word description for each case into a REDcap database. Responses were analyzed for the most commonly used terms as shared by at least 4 of 6 reviewers for frequency and agreement.

Results: The top 10 most commonly used terms were identified, in addition to levels of agreement across the reviewers. Agreement was commonly observed for a diagnosis of cNF, tumor involvement in the dermis, the absence of atypical features, and heterologous features, and subcutaneous penetrance. Less agreement was observed for architecture in terms of being diffuse or having fibrous stroma, the involvement/proximity of fat/adipose tissue, and subtype of cNF diagnosed.

Conclusions: A 'baseline level' of understanding has been obtained for the language used by pathologists for the histological classification of cNF, and areas of agreement and disagreement. This represents an important first step towards developing standardized language for describing all neurofibromas clinically and on histopathologic examination, with a longer term goal of improving communication between researchers, the development of therapies, and improving patient care.

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Consultative Problem Solving Intervention for Executive Function Weakness

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Children and adolescents with Neurofibromatosis Type-1 (NF-1) are at risk for under developed executive functions[1] (EF). Executive function weaknesses can result in children with NF-1, and their families, experiencing multiple challenges at home, in school, and in the community.

The **E**xecutive **F**unction **C**onsultation **E**ducation and Skil**L**s Clinic (EXCEL Clinic; https://www.chop.edu/excel) at the Children's Hospital of Philadelphia was developed to help children/adolescents and their families proactively problem-solve self-identified challenges through brief, collaborative consultation. This intervention is informed by social problem-solving therapy, specifically Bright IDEAS Problem-Solving Skills Training[2], which was designated as a research-tested intervention program (RTIP) by the National Cancer Institute in 2010. By providing education about the neurocognitive profile associated with NF-1, identifying current barriers to success, providing general resources, and generating "tips and tricks" to implement new strategies, children/adolescents and their families can be more successful in their daily life. To our knowledge this is the first consultative problem-solving intervention for executive functions within the NF-1 population.

55 patients with NF-1 have been seen in the EXCEL Clinic in the past 3 years. Caregivers complete a brief demographic intake form, the BRIEF-2 Parent Screener, and participate in a clinical interview in order to identify specific EF needs. The remainder of the session focuses on problem solving strategies. The interventions address: 1) education and resource dissemination, 2) cognitive strategies, and 3) behavioral modifications. The session is summarized in a detailed letter provided to families and the care team via the electronic medical record. From a hospital systems perspective, this is a billable service consistent with the 2019 billing codes for psychological and neuropsychological evaluation.

Preliminary acceptability and feasibility data collected anonymously via Redcap Likert scales and free-text responses has indicated that caregivers are receptive to this intervention, and find it helpful. We have been able to offer EXCEL visits as stand-alone appointments, and to incorporate them into a multidisciplinary NF clinic. As a next step in evaluating the effectiveness of the EXCEL program, we plan to prospectively evaluate which aspects of the EXCEL visit are most useful: general information about NF-1, specific resources for navigating educational systems, the tailored problem solving strategy for specific problems, or a combination of these. We will also evaluate a stepped-care approach to determine the level of care required to improve patient functioning. This knowledge can inform implementation of an executive function intervention in settings that care for patients with NF-1.

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Electrosurigcal Ablation for the Management of High Burden Cutaneous Neurofibromas

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Background: Cutaneous neurofibromas (cNF) are the most common tumor of NF1. Electrosurgical ablation is an important treatment option for cNF but with limited utilization due to several factors including lack of awareness of technical aspects, limited availability of providers, perceived cost and limited outcomes data. Herein, we address the technical features of electrosurgical ablation for large volume destruction of cNF.

Methods: Two techniques for electrosurgical ablation are available using two types of electrosurgical units (ESU): hyfrecator and "Bovie"-type ESU. Each of these approaches were compared regarding their applications, feasibility, requirements and tolerability.

Results: The hyfrecator approach uses an electrosurgical generator that is capable of two modes: desiccation and fulguration. Both modes can be used with a single monopolar electrode depending on whether or not there is contact with target tissues. The handpiece allows for easy operator adjustability to increase or decrease energy delivered depending on size of the target lesion. No grounding pad is required, and it may be performed in limited fashion in the office under local anesthesia. However, large volume electrodesiccation (300-900 cNF of up to 4mm in diameter) requires general anesthesia in the operating room. Typical surgical time for each procedure was 2.5 hours, and on average most patients required a minimum of 3 sessions to treat the entire body.

"Bovie"-type monopolar ESU utilizes a microdissection needle electrode resulting in localized effects without producing cardiac effects. A grounding pad is needed for this approach, and the patient is under general anesthesia in an operating room setting. The instrument can be used to cut or coagulate tissues. This allows for maximal flexibility in addressing both superifical and deep cNF of all sizes as it can cut tissue, ablate tumors and coagulate blood vessels in one episode with a single tool. An average of 450-1000 cNF of any size can be treated with this approach in a single session with a surgeon and an assistant operating simultaneously. Larger defects may require suture repair.

Post-operative management of pain and wound care requires antibiotic ointment, and oral analgesics.

Conclusion: There is tremendous clinical need to treat cNF, and electrosurgical ablation represents a highly effective modality for large-volume destruction of NF1-associated cNF. There are important differences in methodology and scope of use for this method which may influence use in clinical practice and affect patient experience; it is important for both care providers and patients to be aware of these differences.

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Motor and Attention Functioning in Early Childhood as Predictors of Working Memory in the School Age Years in Children with Neurofibromatosis Type 1

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Background: Previous work has established difficulties in attention (Templer, Titus, Gutman, 2013), motor (Hyman, Shores, & North, 2005), and working memory (Casnar & Klein-Tasman, 2016) for children with neurofibromatosis type 1 (NF1). However, little is known about the developmental trajectories and interrelations among these variables. We aimed to determine whether motor or attention functioning in the early childhood years were viable predictors of working memory in the school-age years for children with NF1.

Methods: Participants with NF1 ($N=23$) were followed longitudinally between early childhood (3-6 years) and the school age years (9-13 years). Early motor functioning was assessed using the NEPSY-II Visuomotor Precision subtests, the Scales of Independent Behavior-Revised (SIB-R) Motor scale, as well as the Differential Ability Scales-II (DAS-II) Copying subtest. Early attention functioning was assessed using the Conners Parent Rating Scales—Revised Short Form (CPRS-R) Cognitive Problems/Inattention and Hyperactivity scales and the DAS-II Digits Forward. School aged working memory outcome variables included the DAS-II Working Memory Index (WMI) and Digits Backward subtest, Cogstate One and Two Back Tests, and NIH Toolbox List Sort Working Memory Task (LSWM). Overall cognitive functioning was assessed using the DAS-II.

Results: Hierarchical regressions were conducted controlling for overall cognitive functioning. Motor functioning at 3-6 years significantly predicted the DAS-II Digits Backward [$F(4,18)=3.08$, $p=.043$, $R^2=.406$] and WMI [$F(4.18)=9.04$, $p<.001$, $R^2=.668$] in the school age years, as well as Cogstate One Back Accuracy [$F(4,17)=6.62$, $p=.002$, $R^2=.609$]. Attention functioning in early childhood was a significant predictor of school-age DAS-II WMI [$F(4,17)=5.58$, $p=.005$, $R^2=.568$], Cogstate One Back Accuracy scores, [$F(4,16)=7.96$, $p<.001$, $R^2=.665$], and Cogstate Two Back Accuracy scores [$F(4,16)=4.30$, $p=.015$, $R^2=.518$].

Conclusions: Overall, findings suggest that motor and attention functioning in early childhood is are both useful predictors of school age working memory. All three areas of functioning seem to be related in children with NF1 and should be monitored throughout development. Better characterizing these difficulties in early childhood may allow for early intervention to reduce the impact of working memory deficits in the school age years.

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Feasibility of Motor Phenotypes as Biomarker for NF1 Clinical Trials in Children

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Background: Neurofibromatosis (NF1) is one of the most common autosomal dominant disorders causing neurocognitive impairments. Clinically, 50% of children with NF1 are underperforming or failing in school. This frequently leads to decreased educational attainment and fewer opportunities as adults. The high prevalence of behavioral, emotional, and/or cognitive problems in persons with NF1 suggests that neurofibromin may play a role in maintaining the structure and/or healthy, efficient function of the brain. Assessments in children and adults have suggested symptoms of Attention Deficit Hyperactivity Disorder (ADHD), impaired motor function, and/or altered motor physiology may also be characteristic.

Objective: To pilot and demonstrate feasibility of using motor assessment and motor physiology protocols in children with NF1 as possible biomarkers for future clinical trials.

Methods: Motor function and physiology were assessed using Pediatric Assessment of Neurological Subtle Signs (PANESS) and Transcranial Magnetic Stimulation (TMS) measures. PANESS scores, motor thresholds, and paired pulse 3 msec (inhibitory) and 10 msec (facilitatory) measures were compared and utilized for sample size calculations for children ages 8-16 years with NF1 versus both typically developing controls and children with ADHD.

Results: All participants tolerated the procedures well. Preliminary data for NF1 (n=9; mean full scale IQ=94; 5 with ADHD) compared with ADHD (n=52; IQ=102) and typically developing children (n=62; IQ=109) cohorts suggest that reduced short interval cortical inhibition (SICI) (p = 0.001) could distinguish NF1 children from controls but not children with ADHD. Two other TMS measures (resting motor threshold and intracortical facilitation) were not significant in this study. Higher (worse) PANESS scores may differentiate NF1 from both typically developing (p = 0.001) and ADHD (p = 0.01) children.

Conclusions: We identified PANESS and TMS – evoked SICI as promising biomarkers for abnormal motor behavior in NF1 with values as extreme or more extreme than those found in the ADHD cohort. These methods are feasible and may quantify treatment-sensitive differences in motor function and physiology in NF1.

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Sensory Processing in Children with Neurofibromatosis Type 1

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Background: The appropriate pre-attentive filtering of sensory stimuli and its integration with other cognitive processes is critical for healthy brain function. It is common for sensory filtering and integration to be disrupted in autism spectrum disorder (ASD), which can contribute to sensory hypersensitivity, sensory avoidance, diminished responses to sensory stimulation, and/or sensory seeking behaviour. These cardinal features of ASD can have a debilitating impact on social interactions and daily functioning. Importantly, sensory deficits have been shown to be accompanied by neurophysiological sensory processing abnormalities, including an imbalance of neuronal excitation and inhibition and/or impaired sensory gating. Given the frequent overlap with ASD and neurofibromatosis type 1 (NF1), in combination with evidence suggesting deficits in sensory gating in a *NF1* mouse model (*nf1+/-*), further research is needed to examine whether sensory processing abnormalities are a commonly experienced by children with NF1. Accordingly, the aims of the current study are to (1) determine whether children with NF1 show differences in sensory processing compared to a typically developing (TD) control group, and (2) examine the relationship between sensory processing and atypical behaviours, including ASD symptomatology, in NF1.

Methods: This preliminary data set is part of a multi-site, cross-sectional cohort study (Predictors of Autism in Neurofibromatosis type 1: Development to Adolescence), in which children with NF1 and TD children (aged 3-15 years) completed a detailed assessment of their cognition and behaviour. Group differences on The Sensory Profile 2, a parent questionnaire used to assess sensory processing, were analysed. The association between sensory processing outcomes and comorbid ASD symptoms (Social Responsiveness Scale -2; SRS-2) was also examined.

Results: To date, data from 105 children (NF1 =51; TD = 54) have been collected and analysed. Compared to the TD group, children with NF1 demonstrated significantly more difficulties in all four quadrants of the Sensory Profile (Sensory Avoiding, Sensory Sensitivity, Poor Registration, and Sensory Seeking). Deficits in the subsections of Auditory, Touch, Movement, Body Position and Oral, were also evident (all $p < 0.005$). Children with NF1 and elevated ASD symptoms (SRS-2 T score > 60) demonstrated significantly more severe sensory processing problems, across all domains, compared to children with NF1 without ASD symptoms.

Conclusions: Sensory processing difficulties are an under-recognized problem in children with NF1. The extent of these sensory processing difficulties indicates a substantial burden on daily functioning and may explain an important part of behavioural dysfunction associated with children with NF1.

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Photodynamic Therapy for Benign Dermal Neurofibromas

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The primary hypothesis was whether Levulan (aminolevulinic acid, ALA) would accumulate, and be converted to Protoporphyrin IX (PpIX), by tumor tissue more than surrounding normal tissue. Using microneedling and overnight incubation, followed by tissue excision, we were able to demonstrate that the ALA was specifically converted to the active agent PpIX in the tumor region as seen by fluorescence microscopy. Average fluorescence for PpIX positive tumor areas was 318 ± 31 densitometry units/micro m^2 (subject n=2). Fluorescence values in the non-tumor areas were zero. Using a one-sample t-test comparing the absolute value to an alternate expected value of zero, the results are significant with $p = 0.044$.

The secondary hypothesis was that tumors incubated with Levulan would show greater fluorescence than untreated tumors and tumors incubated with vehicle only (placebo application). Control and placebo tumors did indeed show no significant fluorescence,

Red light treatment at a radiant flux of 100 mW/cm^2 and at a dose of 50 J/cm^2 resulted in marked erythema and edema around ALA (n= 24) treated tumors which lasted for 48 hours, but not placebo (n=20) tumors. There was no blister formation, erosion or crusting. Red light treatment induced marked pain and discomfort in ALA tumors, and in one case prompted cessation of treatment due to pain, but not placebo tumors. Due to this pain, MTD will be set at 100 J/cm^2, though only one subject experienced dose limiting toxicity.

Measurement of the tumor size at 2 weeks, 1 and 3 months after treatment did not show significant tumor reduction, likely due to the presence of large amounts of fibrous material not susceptible to PDT treatment. A non-significant reduction in average tumor growth rates when comparing the area of treated and non-treated tumors on the same subject (-0.11 $\pm$ 0.20 mm^2/day post treatment) was associated with PDT treatment.

Light microscopy of ALA but not placebo tumors showed mixed infiltrate consisting of lymphocytes, neutrophils and rare eosinophils, showing an inflammatory response and possible necrosis of tumor material. TUNEL assay showed significant increase in number of apoptotic cells as compared to placebo, further showing cell death in treated tumor tissue.

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Telomere Alterations in Neurofibromatosis Type 1-Associated Solid Tumors

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The presence of Alternative lengthening of telomeres (ALT) and/or ATRX loss, as well as the role of other telomere abnormalities, have not been formally studied across the spectrum of NF1-associated solid tumors. Utilizing a telomere-specific FISH assay, we classified tumors as either ALT-positive or having long (without ALT), short, or normal telomere lengths. A total of 426 tumors from 256 NF1 patients were evaluated, as well as 99 MPNST tumor samples that were sporadic or of unknown NF1 status. In the NF1-glioma dataset, ALT was present in the majority of high-grade gliomas: 14 (of 23; 60%) in contrast to only 9 (of 47; 19%) low-grade gliomas (p=0.0009). In the subset of ALT-negative glioma cases, telomere lengths were estimated and we observed 17 (57%) cases with normal, 12 (40%) cases with abnormally long, and only 1 (3%) case with short telomeres. In the NF1-associated malignant nerve sheath tumor (NF1-MPNST) set (n=75), ALT was present in 9 (12%). In the subset of ALT-negative NF1-MPNST cases, telomeres were short in 9 (38%), normal in 14 (58%) and long in 1 (3%). In the glioma set, overall survival was significantly decreased for patients with ALT-positive tumors (p<0.0001). In the NF1-MPNST group, overall survival was superior for patients with tumors with short telomeres (p=0.003). ALT occurs in a subset of NF1-associated solid tumors and is usually restricted to malignant subsets. In contrast, alterations in telomere lengths are more prevalent than ALT.

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Projected Benefits of Access to MEK Inhibitors for the Management of Neurofibromatosis Type 1 (NF1) Related Plexiform Neurofibromas (PN) in India

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Background: Preliminary results of MEK inhibitor trials in NF1 patients with PNs have demonstrated beneficial responses in size, pain, and quality of life with tolerable side effects. Though this class of drugs is not currently available in India, we conducted a retrospective review of our NF1 patients to determine the proportion of patients who may potentially benefit from treatment in the future based on eligibility criteria from the SPRINT trial (NCT02407405).

Methods: From January 2008 to May 2019, patients meeting diagnostic criteria for NF1 were included in our analysis. Variables abstracted from hospital records included demographics, presence of large or symptomatic PNs (>5cm or based on location), PN-related symptoms (pain, disfigurement, neurologic symptoms), PN-related surgical details (type of surgery, indication, recurrence) and surgical morbidity details (disfigurement, neurologic, orthopedic, and airway complications). Patients with malignant peripheral nerve sheath tumors (MPNST) were excluded from the analysis.

Results: Eighty-eight NF1 patients (57 male, median age 11.9 years, range: 0.3-83.3 years, median office visits: 3) were seen in our clinic during this time period. Thirty-eight NF1 patients (23 male, median age of presentation: 19.8 years) had large or symptomatic PNs which were located in the head (n=8, 21.0%), head and neck (n=13, 34.2%), neck and chest (n=3, 7.9%), trunk (n=4, 10.5%), trunk and extremity (n=7, 18.4%), and extremity (n=3, 7.9%). MPNSTs developed in 7 out of 38 (18.4%) patients with PNs and were excluded from the potential benefit analysis. Of the remaining 31 patients, 27 (87.1%) reported PN-related pain (n=12, 38.7%), PN-related disfigurement (n=12, 38.7%), and/or PN-related neurologic symptoms (n=13, 41.9%). The highest frequency of PN-related pain and neurologic symptoms were reported in head and neck PNs (n=7, 58.3% and n=6, 46.2%, respectively), while PN-related disfigurement was reported most frequently in head-only PNs (n=7, 58.3%). Surgery was attempted in 12 patients with large or symptomatic PNs which resulted in complete resection (n=5, 41.7%), laminectomy with partial resection for paraspinal PNs (n=3, 25.0%), and partial resection (n=4, 33.3%). Recurrence of PNs (n=3, 25.0%), surgically-related disfigurement (n=1, 8.3%) and neurologic complications (n=2, 16.7%) were observed post-operatively.

Conclusions: In our setting, a high proportion of patients with NF1-related PNs experience PN-related symptoms that may benefit from MEK inhibition. Though our data is likely an over-representation due to patients seeking healthcare only when symptomatic, access to MEK inhibitors may offer a better alternative to surgery which can have unintended morbidity and a non-trivial risk of recurrence.

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Coarctation of the Aorta in NF1: Possible Etiology

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Vascular anomalies have been recently recognized as relatively rare but significant complications of NF1. We report a case of a child with NF1 and coarctation of the aorta, with molecular data of tissue showing a potential etiology.

The patient, a 6 yo female with NF1, had been followed for several years in NF clinic, with typical skin manifestation and asymptomatic optic pathway glioma. She had mild intermittent elevations of blood pressure noted. On exam at age 6 years, she was noted to have a blowing holosystolic murmur heard over her back. BP was 118/74 - 137/78. Echocardiogram showed coarctation of the aorta located distal to the left subclavian artery, with a peak gradient of 35 mm Hg. CTA of the chest showed a moderate discrete coarctation, with mild enlargement of the intercostal arteries. Renal US and Doppler showed no stenosis of the renal arteries. She successfully underwent surgical repair, with resection and end-to-end anastomosis via thoracotomy. Three samples of aortic tissue were sent for genetic testing. In two samples, only the known NF1 germline pathogenic variant was found (c.4110+2delT). However, in the third sample, a different NF1 pathogenic variant, c.5571DelT, was found at low level mosaicism, in approximately 3% of alleles. Presence of the variant was confirmed by high resolution melting curve analysis. Unfortunately, histopathologic evaluation of the tissue was not available.

Vasculopathy in NF1 can include renal artery stenosis, pulmonic valve stenosis, cerebral artery stenosis, and other arterial narrowing. Coarctation of the aorta is rare, but has been reported in several other patients. Pathology reports of other stenotic blood vessels has shown excess thickening of the endothelial cell layer, and the patchy distribution of stenosis has suggested that a second hit event may have led to the focal area of narrowing. We have reported an apparent mosaic somatic mutation event in aortic tissue, which strongly suggests that a second genetic hit event occurred in aortic tissue and resulted in the focal area of stenosis. The case highlights the need for thorough workup, including echocardiogram, of young patients with NF1 and elevated blood pressure.

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Selumetinib for Treatment of Unresectable Lesions in Neurofibromatosis-1

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Pediatric central nervous system (CNS) malignancies are the most common malignancies of childhood. The standard treatment plan for most CNS malignancies involves surgery, chemotherapy, radiation, and/or a combination of above therapies. Unresectable symptomatic low grade gliomas, such as pilocytic astrocytomas and gangliogliomas, are slow growing tumors that are typically responsive to a single course of intravenous chemotherapy, but in select patients these WHO grade I or II tumors can recur and be refractory to multiple courses. Molecular diagnostics can offer valuable insight into the tumor microenvironment, where targeted therapies can be offered for specific actionable mutations.

Here we report a case series of 3 pediatric patients, with unique CNS malignancies, currently on targeted therapies for tumor-specific somatic mutations. Patient A is a 5 year old male with unresectable Neurofibromatosis-1 related plexiform neurofibroma of the nasopharyngeal space as well as optic pathway glioma, with a mutation of the MAPK/ERK pathway. Patient B is a 6 year old male with recurrent, refractory pilocytic astrocytoma of the optic pathway and hypothalamus, with progression through several courses of intravenous chemotherapy, noted to have somatic NACC2-NTRK mutation. Patient C is a 17 year old female with unresectable, pontomedullary ganglioglioma, noted to have BRAF-V600E mutation. Patient A is treated with Selumetinib, for about 6 months, with near resolution of nasopharyngeal mass. Patient B is treated with Larotrectinib, for about 3 months, with stability of clinical symptoms. Patient C is treated with Vemurafenib, for about 10 months, with stability of lesion size and patient-reported clinical improvement. No adverse events were noted for any of these patients and all medications were administered orally. Significant improvement in quality of life was reported, as they did not have central lines or bone marrow suppression.

Targeted inhibitors provide a reasonable treatment option for relapsed, refractory CNS malignancies with actionable mutations.

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Surgical Management of Peripheral Nerve Tumors in Neurofibroma Patients

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Introduction: Peripheral nerve tumors are a heterogeneous group of mostly benign tumors that are rare in the general population; however, they are a common entity in patients with Neurofibromatosis (NF). Conventionally, there has been hesitance in excising these peripheral nerve tumors in this population; given the benign nature in the majority, the risks of nerve deficits from surgery and the extent of the disease. There is a paucity of data to guide the surgical management of these tumors, while access to surgeons with expertise in excision of these tumors remains a challenge to patients with NF.

Methods: We present a single surgeon's 10-year experience in the surgical management of peripheral nerve tumors in Neurofibromatosis patients by describing the indications for surgery, the surgical technique, complications and the post-operative outcomes. The authors reviewed the demographics, clinical diagnosis, histologic diagnosis, and the surgical outcomes were analyzed from the patients' electronic medical records, and patient reported outcomes were collected through a quality of life survey. Measures of central tendency and descriptive statistics were used to describe absolute and mean results. Student's t-tests were used to analyze binary data sets. P-values of less than 0.05 were significant.

Results: A total of 129 peripheral nerve tumors were excised in 85 patients. The mean age of patients was 35.7 years (19-43); 49% of the patients were female. A diagnosis of NF type 1 was carried in 65% of patients, NF type 2 in 13% of patients, while Schwannomatosis in 22%. Surgical excision of the tumors from the upper extremities comprised 40% from the data set, from the lower extremities in 33%, from the trunk in 11% and from the head and neck region in 11%. Tumors were histologically diagnosed as nerve sheath tumors in 85% of cases, 12% as Schwannomas, and 3% as mixed tumors. There were four cases of malignant peripheral nerve sheath tumors. We report the following complications: 9.3% reported sensory deficit, 7% reported motor deficit (which was transient), 4.5% developed a post-operative hematoma. The patient-reported overall state of health improved from an average of 2.5 (of 5)(pre-operative) to 3.9 (of 5)(post-operative)(p<0.05), interference of symptoms with daily activities improved from 4.0 (of 5)(pre-operative) to 2.9 (of 5)(post-operative)(p<0.05), 94% of the patients responded that they would undergo surgery again.

Conclusion: We describe the details of the surgical technique and report a large single surgeon's experience on the beneficial role of surgery in the management of peripheral nerve tumors in Neurofibroma patients by presenting data that will help clinicians guide their care and counsel patients on the expected outcomes.

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Use of F-18 Fluorodeoxyglucose (FDG) Positron Emission Tomography/Computed Tomography (PET/CT) Imaging in Distinguishing Benign Neurofibromas from Malignant Peripheral Nerve Sheath Tumours (MPNST) in Patients with Neurofibromatosis Type 1 (NF1)

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Introduction: Patients with NF1 have a lifetime risk of ~15.8% of developing a malignant peripheral nerve sheath tumour (MPNST)[1]. MPNSTs are difficult to diagnose as symptoms of MPNSTs overlap with benign symptomatic plexiform neurofibromas.

Previously, FDG PET CT imaging has been shown to be important in distinguishing between benign neurofibromas and MPNST. We showed a cut-off SUVmaxD (delayed) of >3.5 had a sensitivity of 0.97 and specificity 0.87 in distinguishing benign neurofibromas from MPNST (2004-2008).[3]

This study re-evaluates the clinical utility of FDG PET CT between 2017-2019 in distinguishing benign neurofibromas from MPNST and whether SUVmaxD cut off of >3.5 is still highly sensitive and specific.

Methods: We examined data from NF1 patients with symptomatic neurofibromas who underwent FDG PET CT imaging at 90 and 240 mins in the UK National NF1 service in London between 2017-2019. Patient demographics, site and size of lesion, SUVmaxE (early) and SUVmaxD values and biopsy result were analysed.

Results: 39 patients had FDG PET CT to examine 47 lesions. 20 were male and 19 female. Age range was 6-75 years (mean age 35.6 years, SD 17.5). The commonest site of symptomatic lesion was abdomen/pelvis (n=13), followed by lower limb (n=11) and head and neck (n=8). Lesion size ranged from 1.5-40cm (Mean 9.4 cm, SD 8.4).No significant difference in size was found between benign and malignant lesions. 25 lesions were biopsied, the remaining 18 lesions were deemed clinically benign neurofibromas. Of those biopsied, 13 were histologically benign neurofibromas, 6 were MPNST, 1 was an atypical neurofibroma, 4 were found to be other pathologies (Gastrointestinal stromal tumour, paraganglioma, high grade tumour likely of pelvic origin, paraganglioma, likely colon tumour).

For lesions biopsied and found to be benign neurofibromas (n=13), SUVmaxE levels ranged from 2.3-12.4 (mean 6.1, SD 2.8), SUVmaxD levels ranged from 2.7 – 16.4 (mean 7.2, SD 3.6). Biopsy proven MPNST (n=6) SUVMaxE range was between 3.7-29.7 (mean 10.2, SD 9.8), SUVmaxD ranged between 3.1-38.3 (mean 12.6, SD 13). There was a significant difference between SUVmaxE and SUVmaxD (p=0.002, 2 tail paired t test) but overlap between the values. There was also a significant difference between SUVmaxD between benign and malignant lesions (p=0.001) but overlap in the values.

Conclusion: The previous cut-off level for SUVmax[3] is no longer useful in distinguishing between benign neurofibromas and MPNST.

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Phenotypic Heterogeneity and Clinical Subtypes of Neurofibromatosis Type 1 in Patients from a Large National Registry

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Background: Neurofibromatosis type 1 (NF1) displays considerable individual variability in phenotypic expression and it is currently unclear which patients will develop certain phenotypic manifestations. Understanding the co-association of phenotypes can lead to more personalized treatment of NF1.

Methods: Surveys from 2051 adult NF1 patients were collected from 2012 to 2018 using the large nationwide registry from the Children's Tumor Foundation.

Findings: The cohort mean age was 42·3 years (SD 13·6) and 66·9% were female. Among major characteristics, prevalences were: café au lait macules (98%), skinfold freckling (84%), cutaneous neurofibromas (91%), attention deficit and learning disabilities (70%), scoliosis (45%), plexiform neurofibromas (43%), spinal neurofibromas (35%), fractures (36%), optic gliomas (18%), osteoporosis (14%), malignant peripheral nerve sheet tumor (4%), and sphenoid wing dysplasia (3%). Correlations revealed co-association of traits, including spinal neurofibromas and pain (OR = 5·25 [4·02, 6·85], $p<0·001$), spinal neurofibromas and scoliosis (OR = 3·01 [2·42, 3·74], $p<0·001$), spinal neurofibromas and optic gliomas (OR= 2·00 [1·52, 2·64], $p<0·001$), and optic gliomas and sphenoid wing dysplasia (OR= 6·48 [3·57, 11·75], $p<0·001$). Furthermore, cutaneous findings inform the risk of systemic disease. With increasing numbers of cutaneous neurofibromas (1-10, 11 to 100, and >100), the odds ratio of malignant peripheral nerve sheet tumor increases from 2·88 (0·74, 19·09) to 3.63 (1·01, 23·37) to 4·30 (1·17, 27·93), respectively. Finally, clustering of phenotypes revealed six distinct patient subtypes: mild, freckling predominant, neurofibroma predominant, skeletal predominant, late onset neural severe, and early onset neural severe.

Interpretation: We comprehensively defined the prevalence and co-association of phenotypic traits in 2051 adult NF1 patients. Phenotypic clustering revealed six distinct subtypes of disease: mild, freckling predominant, neurofibroma predominant, skeletal predominant, late onset neural severe, and early onset neural severe. In addition, our study demonstrates the power of large registries to understand rare diseases.

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Quality of Life in Adults with Neurofibromatosis 1 Associated Optic Pathway Glioma

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Introduction: Few studies have assessed the impact of Neurofibromatosis type 1 (NF1) related optic pathway glioma (OPG) on quality of life (QoL). A recent study[1] including both NF1 and non NF1 patients with low grade gliomas suggested bilateral visual impairment had a negative impact on employment and marital status. The aim of this current study was to explore the impact of NF1-OPG on QoL.

Methods: 62 adults with NF1-OPG, attending the London NF1 Center agreed to participate. Demographics, marital status, OPG treatment, driving status and visual acuity were assessed. Visual acuity (in logmar) and visual fields were reported. Sight impairment was defined as Logmar 0.7 or worse and vision insufficient for driving as logmar 0.3 or worse in the best eye; impaired visual fields were taken into consideration. A self-reported, disease specific QoL questionnaire (INF1-QOL) was used to assess Qol.[2] INF1-QOl scores were compared with a further sample of 44 NF1 adults without OPG.

Results: There were 62 adults with NF1-OPG age 16-69 years (median 26), 39 females and 23 males. Prior treatment for NF1 OPG included chemotherapy (n= 11), radiotherapy (n= 6), surgery (n=3). Ten individuals (16.1%) were married or in relationships; 20 (32.2%) were unable to drive due to vision impairment; 23 (37%) were not limited by vision, but were not driving for other reasons. On the INF1-QOl, visual impairment was reported by 14 (22.6%) as causing a moderate to severe impact on performing activities; 15 (24.2%) reported moderate learning problems; 17 (27.4%) reported NF1 as having a moderate to severe effect on role and outlook on life. 16 (25.8%) reported depression or anxiety as causing moderate to severe impact on performing activities. Median INF1-QOL score was 8 in both the treated (n=19) and non-treated OPG groups (n=43). Mean INFI-QOL total score was 9.5 (SD 6.8) in the NF1-OPG adults (n=62) and 8.5 (SD 6.5) in 44 NF1 adults with no OPG (p =ns).

Conclusions: Overall, self-reported QoL was not significantly different between NF1 adults with OPG and without OPG. None of the subitems differed significantly apart from perception of visual impairment on quality of life. Visual impairment precluded driving in 32.2%, but 37% with sufficient vision to drive, were not driving for other reasons. The study suggests that OPG is only one of several factors contributing to impaired Qol in NF1.

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Speech, Language, and Feeding Skills of Children with RASopathies: A Rapid Review

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Background: Children with RASopathies are at-risk for delays in speech, language, and feeding. Early identification and treatment of these disorders is important for facilitating children's social and emotional development. The purpose of this rapid review was to determine current assessment and intervention approaches for disorders related to speech, language, and feeding in the RASopathies, with the purpose of identifying a pathway of care provided by speech-language pathologists.

Methods: Following rapid review protocol, search strings using appropriate vocabulary were developed and used to search electronic databases identified by the investigators. Electronic databases were searched using the following search terms: ("Neurofibromatosis" OR "Costello Syndrome" OR "Cardio-Facio-Cutaneous Syndrome" OR "Noonan Syndrome" OR "Noonan Syndrome with multiple lentigines" Or "Legius Syndrome" Or "RASopathies" Or "RAS" Or "MAPK") AND ("Speech and language" OR "language development" OR "feeding" OR "swallowing" OR "language skill*") AND ("pediatric*" OR "child*"). Databases searched included PubMed, CINAHL and PsycINFO. Handsearching was completed for further identification of relevant publications. Inclusion criteria included all article types published in English after 2003 that described any assessment, intervention, or path of care for concerns related to speech, language, and feeding in individuals with a confirmed diagnosis of Costello syndrome, Noonan syndrome, Noonan syndrome with multiple lentigines, Cardio-Facio-Cutaneous Syndrome, Neurofibromatosis type 1, Neurofibromatosis type 2, or Legius syndrome.

Results: Seventy-three publications were retrieved. Thirty publications met inclusion criteria. Publications described speech, language, and/or feeding profiles of individuals with Neurofibromatosis type 1 (n=16), Noonan syndrome (n=9), and Costello syndrome (n=8). Two publications documented significant language and feeding concerns in children with Cardio-Facio-Cutaneous Syndrome. No papers provided information concerning the speech, language, and feeding skills of children with Legius syndrome or Neurofibromatosis type 2.

Conclusions: Evidence from this rapid review suggests that of the RASopathies evaluated, the greatest number of publications focused on describing the speech and language skills of children with Neurofibromatosis type 1. Moreover, all children with RASopathies are at risk for delays in speech and language. There is a paucity of research and no established clinical practice for the assessment and intervention of speech, language, and feeding concerns of children with RASopathies. Future research should seek to document appropriate assessment and intervention procedures for the identification and remediation of speech, language, and feeding concerns of children with these disorders.

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The Role of Genetic Counselling in Reproductive Decision Making of Young Adults with NF1

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Background: The broad spectrum of severity of the clinical manifestations in NF1 has been shown to be a concern for patients who pursue a family project, making reproductive decisions complex and difficult. Genetic counsellor play an important role in providing information about the condition, genetic risk and reproductive options available, as well as guiding and supporting the patients through their family planning process. Considering the limited available information about family planning needs and reproductive decisions of NF1 patients in the Québec population, we sought to identify the medical, psychosocial, educational, and informational needs of young adults with NF1 in relation to the family project as well as to explore their experience with genetic counselling through reproductive decision-making.

Methods: Patients with NF1 aged 18-40 years old were recruited through the Québec Neurofibromatosis Association (ANFQ) and through the NF1 patient list of the Centre Hospitalier de l'Université de Montréal (CHUM) and the McGill University Health Center (MUHC). Participants were invited to complete a self-report questionnaire available online exploring subjects such as their current medical care management, reproductive decisions, knowledge about NF1, informational needs, and quality of life.

Results: 69 young adults aged between 19-40 years old were included in the study. Our results show heterogeneous reproductive decisions, regardless of the self-assessed quality of life of participants. For young adults with NF1, the possibility of having an affected child is an important concern. However, it does not prevent them from pursuing a family project, with 79% of the participants having or wanting to have children. We also observe important psychosocial and information needs in relations to family planning, with more than half of the participants qualifying their concern level of moderate or high in relations to the variability of the condition (63%) and the long-term care of an affected child (63%). Our results also enlighten an important lack of knowledge about the available reproductive options, which is partially compensated by genetic counselling. More participants who had seen a genetic counsellor were aware of the reproductive options available compared to the others.

Conclusions: The results confirm that family planning is a complex and relevant problem for young adults with NF1, who could benefit from better knowledge of the reproductive options available to them. This highlights the importance of genetic counselling to educate as well as support these patients through their reproductive decision making process.

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Trametinib Induces Neurofibroma Shrinkage and Enables Surgery

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Background: Plexiform neurofibromas occur in 30-50% of NF1 patients, significantly contribute to the burden of disease and may also progress to malignancy. Their treatment still is a major challenge although significant shrinkage of these tumors induced by the MEK inhibitor selumetinib has been reported. While selumetinib is not yet available as medication for neurofibromas, the MEK inhibitor trametinib is routinely used for the treatment of malignant melanomas and has also been applied for NF1 associated neurofibromas.

Case report: We report an 11 year old girl with NF1 and a large plexiform neurofibroma of the neck which had led to a sharp-angled kinking of the cervical spine. Spasticity of the right leg with compromised motor function was indicative of progressive myelopathy. Although surgical stabilization of the cervical vertrebral column was urgently recommended the vertebral column was inaccessible due to extensive tumor growth. In this situation oral treatment with trametinib was started.

Results: Patient follow-up included monthly clinical, ophthalmological, laboratory and cardiac examinations. The only side effects observed were mild facial rash and occasional epistaxis. To monitor tumor size volumetric MRIs were performed before as well as after 3 and 6 months of therapy. After 6 months of trametinib treatment a reduction of tumor volume by 22% was observed which finally enabled further surgical treatment.

Discussion: Our patient was treated with trametinib to prevent imminent paralysis caused by progressive neurofibroma growth. The observed partial response indicates that MEK inhibitors are likely to play an essential role in a multimodal therapeutic approach for NF1- associated plexiform neurofibromas. MEK inhibition might also be a therapeutic option for other manifestations of NF1. Similar issues are on debate for tuberous sclerosis complex (TSC), a neurocutaneous disorder caused by mTOR hyperactivation where the mTOR inhibitor everolimus provides a therapeutic option for several aspects of the disease.

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Preventing Stroke in Paediatric NF1

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Abstract: The association of NF1 with Moya Moya is well known[1]. Linkage between specialist services offers the best opportunities for detection, management and prevention of stroke in Paediatric NF1.

Background: The Highly specialized NF1 service at St. Mary's Genomic Centre for Medicine in Manchester is one of the two national centres for complex NF1. This was established in 2009. The paediatric neurovascular service in Manchester started in 2017 and is rapidly expanding.

Methods: The Complex NF1 service was responsible for the clinical care of 256 children with NF1 (133 of which were complex)-2018 clinical activity. Similarly the paediatric neurovascular service served 91 patients. 31 (34%) of these children have a genetic diagnosis, 7 (8%) children have NF1, all of which have a Moyamoya vasculopathy. The number of children with NF1 vasculopathy (7) exceeded that of children with Down`s syndrome (5) in this service.

The notes and neuroimaging of these children were reviewed.

There were 3 females and 4 males. Age at presentation ranged from 5-14years. (Mean7.4 Median 5) 4 children were asymptomatic at presentation and their Moya Moya was found incidentally on MR of the brain carried out for other reasons (median 8.5, mean 7.5), one of these children also had evidence of an old small infarction with no clinical signs or any relevant past history . 3 were symptomatic (Mean 6 Median 5. One girl of 4 presented with acute intermittent choreathetosis—she had evidence of a very small infarction in the basal ganglia[2], one girl of 5 with an isolated TIA and one boy of 9 with progressive cognitive and behavioural decline.

6 children had the "Ivy sign" on their MR brain and one teenage female did not.[1] Her initial management was in another centre. Of the 6 children with a positive "Ivy Sign" two are still undergoing evaluation, two have just had their revascularization surgery and will have their repeat MR Brain in 6 months, and in two their "IVY sign" has resolved/improved significantly post revascularization surgery[2]. Similarly, a dynamic assessment of cerebral perfusion, ASL (Arterial Spin Labelling) confirmed reduced perfusion in the 6 children who have been evaluated by our centre at presentation. ASL showed improved perfusion on the two children to have had their repeat neuroimaging after the surgery. Two have just had their revascularization surgery and will have their ASL in 6 months and two are presently being evaluated. Neuroradiological evidence of neovascularization after pial synangiosis is present in the three patients for whom post-surgery MRA is available including the teenage girl who had her pial synangiosis in another centre. Clinically of the three children who were symptomatic two have had no further neurological events and one has only just had his surgery. 6 children have unilateral disease. One child may have signs of emergent contralateral involvement. This is being followed up closely. All four children who underwent Pial Synangiosis tolerated the procedure very well and were discharged 4 days after surgery. No complications were reported.

Conclusion: Paediatric MoyaMoya associated with NF1 is the second largest genetic contributor to the Manchester Paediatric Neurovascular service. (The most common contributor was familial cavernomas). This is largely unilateral in nature. Awareness and close linkage between the two specialized services has led to a seamless pathway of care for affected children and offers the best opportunities for detection, management and prevention of stroke and neurodisability.

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Gross Haematuria in a Child on MEK inhibitors

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Abstract: MEK inhibitors in children with NF1 and other conditions are being used in Trials and for children where no other treatment is available on compassionate grounds.[1] With increased use more side effects are likely to be recognised. Many of these children have highly complex disease and it is not always absolutely clear if an observed event is drug related. Sharing of unusual events will enhance experience.

Case: We present a highly complex child of 4 years with NF1, exomphalos, learning difficulties, global developmental delay, autistic spectrum disorder, history of pulmonary hypoplasia (now resolved), history of Nasojejunal feeding due to severe reflux (Now on bolus NG feeds and oral feeds) and a large, very painful, plexiform that was showing rapid infiltrative growth involving the abdomen, pelvis, buttocks, anus and spinal cord.

MEK inhibitors (Trematenib –Novartis) were started on compassionate grounds for the following reasons:
(1) Intractable pain.
(2) Threat to permanent loss of neurological function in the lower part of the body.

The appropriate baseline investigations were carried out and there were no contraindications to the MEK inhibitors. The initial dosage regimen was very well tolerated with none of the even commonly recognised side effects being reported. The child changed from having intractable pain (miserable and crying all the time) to a happy, playful toddler in a couple of months. After three months finally achieved the ability to walk a few steps however there was no observed change in the size of the plexiform either clinically or radiologically.

At four months of treatment, due to an increase in weight, the dose of the MEK inhibitors was increased according to established GOSH dose/weight banding guidelines. (Dose initially 0.63mgs/12.6mls to 0.76mgs/15.2mls)

Mum observed that after a few days there was the development of generalized skin eczema and loose stools. An area of skin covering the previous site of his exophalos started discharging pus and about a week after the increased dose the child woke up in the morning with frank haematuria, with the urine containing also blood clots.

The child presented to A/E. He was very well in himself with stable observations and playing happily. He was apyrexial. He had an area of wet discharging skin at the margin of his exomphalos. Basic bloods were all normal and his inflammatory markers were not raised.

After discussion it was felt that the MEK inhibitors should be stopped, he should be admitted, observed and investigated appropriately.

His haematuria settled down rapidly, An ultrasound of the renal tract did not reveal any new pathology and his kidneys and kidney functions were normal.

He was discharged home three days later with no further episodes of haematuria. Wound swab and MSU subsequently grew proteus mirabilis and he was started on antibiotics orally a week after presentation. With the antibiotics the abdominal discharging wound healed rapidly. His urine MSU had been showing intermittent increased WBC and asymptomatic bacteriuria with Proteus Mirabilis since 2018. There had been and there is no indication for treating this as discussed with renal and urology teams.

Conclusion: MEK inhibitors may be associated with bladder mucositis escalating to haematuria particularly in children with multiple complex additional predisposing factors.

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Clinical Outcomes and Epigenomic Profiling of Malignant Peripheral Nerve Sheath Tumors (MPNSTs)

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Introduction: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive cancers associated with neurofibromatosis type 1 or a remote history of radiation exposure. MPNSTs display a high propensity for local recurrence and metastasis, and overall prognosis is poor. Moreover, due to the rarity of these tumors, definitive clinical trials are lacking. In this study, we retrospectively evaluated patients diagnosed with MPNST and performed methylation array profiling to identify parameters associated with improved outcomes.

Materials/Methods: A total of 38 consecutive patients who underwent surgical resection at UCSF between 1992 and 2015 with tissue available to confirm histopathologic diagnosis of MPNST were retrospectively identified. Demographic, clinical, and outcome data were extracted from the medical record and institutional cancer registry, and we conducted methylation arrays (n=35) on samples with sufficient tissue. Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method. Methylation data was processed in the *minfi* Bioconductor package. Survival analysis, multivariate regression, hierarchical clustering, and statistics were performed in R.

Results: The majority of patients harbored a clinical diagnosis of NF1 (n=24, 63%) while a smaller number had a remote history of radiotherapy (n=5, 13%). All patients underwent surgery, and the majority received postoperative radiation (n=22, 58%) and a minority received adjuvant chemotherapy (n=10, 26%). Five-year OS was 50% (median 2.87 years) and five-year PFS was 39% (median 1.35 years). Patients treated with adjuvant radiation demonstrated significantly improved PFS (median PFS 5.27 years vs. 1.14 years, p=0.04) but not OS (median OS 5.72 years vs. 2.28 years, p=0.3). On univariate Cox regression analysis, gross total resection (p =0.009) and postoperative radiation (p=0.01) were associated with improved PFS while spine location (p=0.01) was associated with worse PFS. Multivariate Cox regression analysis demonstrated postoperative radiotherapy was significantly associated with improved PFS (p = 0.03) as was anatomic location, with improved PFS for patients with non-spine tumors in the head and neck (p =0.003) or extremity (p=0.01), although our sample size limits the generalizability of these results. There were no differences in outcome based on neurofibromatosis type 1 or prior radiotherapy. Unsupervised hierarchical clustering of methylation arrays identified a high methylation and low methylation subgroup enriched for epigenomic cell proliferation and cell differentiation signatures.

Conclusions: Postoperative radiation therapy and non-spine location are associated with improved PFS in patients undergoing surgical resection for MPNST. Epigenomic profiling identifies two subgroups enriched for cell proliferation and cell differentiation.

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Psychometric Properties of Outcome Measures Imperative to Appropriate Selection and Interpretation of Cognitive Outcomes in NF Clinical Trials

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Clinical trials targeting cognition in children with NF1 is growing. The results of the studies targeting cognitive impairments have been mixed and methodological weaknesses are one possible source for inconsistent results. Operationalization of cognitive constructs and choice of assessment methodology differ greatly between trials. This limits comparability of results and the choice of primary outcome variables may be a factor in negative findings. NF specific psychometrics has not been established, which is the goal of this research.

This is a limited-site, multi-institutional prospective research study that aims to establish the reliability and validity of computerized assessment tools (Cogstate and NIH Toolbox) that are under consideration by the REiNS Neurocognitive Committee. We administered Cogstate and NIH Toolbox to children diagnosed with NF1 and age and gender-matched healthy controls. The BRIEF-2 was administered to parents. Neurocognitive assessments occur at two time points – initial (T1) and follow-up (T2) 6-8 weeks after T1. The battery is counter-balanced at the T1 evaluation to account for test order variability.

Four NF1 and 14 TD participants have been enrolled in the study to date with a mean age of 10 years (Range 7-15), 53% male. Wechsler Vocabulary was above average for the HC group (median ss=13, range 10-17) and average for the NF group (median ss=11, range 11-13). Test-retest reliability was variable across performance-based tests in both groups. High correlation coefficients ($r \geq .70$) were found in 3/5 Cogstate tasks in the NF group and 2/5 in the HC group ($r = .73-.93$). The other tasks had weak to moderate correlations ($r = .13-.68$). For the NIH Toolbox, 3/5 tasks and the Cognitive Composite showed strong correlations in the NF group and 2/5 plus Composite in the HC group ($r = .76 - .99$). The remaining tasks showed weak correlations ($r = .34 - .56$). Parent rating on BRIEF was more reliability, with high correlations for all index scores in both groups (HC $r = .71 - .93$; NF $r = .92 - .98$).

These results highlight the importance of examining the psychometric properties of outcome measures for NF clinical trials. Variability is found when examining the reliability of performance on both computerized batteries over a 6-8 week period, and composite scores are likely to be the most appropriate for clinical trials endpoints versus individual subtests. We also found that parent ratings of a child's executive function in everyday life over that period of time was consistently reliable across all indices, supporting use of these tools as well as clinical trials endpoints.

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Amputation in Adults with NF1, a Review of 7 Cases

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In Neurofibromatosis Type 1 (NF1), amputation is a recognised treatment for children with bone dysplasia. Amputation in adulthood has been less well characterised but can be a helpful intervention. We reviewed the case records of 1075 adults seen in our National Neurofibromatosis clinic up to 2018 and identified patients who had undergone amputation in adulthood. We present the indications and outcomes for those patients.

We identified 7 patients with limb amputations in adulthood, age range at time of amputation 26-44, 5 men and 2 women, 1 upper limb and 6 lower limb, 3 below-knee amputations (BKA) and 3 above-knee (AKA). Another patient was referred for amputation of a leg with a large plexiform and non-union of a fracture but declined and died of an unrelated malignancy.

The indications were complications of tibial pseudarthrosis in 2 patients, (recurrent knee dislocation and stress fractures), 1 malignant peripheral nerve sheath tumour, 4 patients had multiple complications of bulky plexiform neurofibromas: recurrent dislocations (knee and shoulder), neurological deficit, fatigue, pain and reduced mobility, 2 had deep venous thrombosis in the affected limb, with increased swelling and discomfort. 2 patients had recurrent severe haemorrhage into a large plexiform, one complicated by sepsis. All patients had tried multiple medical and rehabilitative treatments prior to opting for amputation. Difficulties standing and moving at work were cited by 2 patients as a major factor in the decision to go ahead with amputation.

There were a range of outcomes. 6 patients reported phantom limb pain, severe in 2. 4 of the patients remain under the care of a pain clinic because of significant pain in the stump and other regions. 3 patients, 2 with with tibial pseudarthrosis and BKA, 1 with bulky plexiform and AKA had excellent response, with significant reduction in pain and medication, improvement in mobility with prostheses, and increased ability to participate in work and leisure activities. 2 of the 3 patients with AKA are unable to tolerate prostheses due to stump pain, one of whom did well initially but subsequently fractured the femur with poor healing and pain. The patient with upper limb amputation has ongoing pain due to scoliosis and other plexiform neurofibromas.

This review illustrates the diverse complications and symptoms that may lead to amputation in adults with NF1. Half of our group report an excellent outcome with rapid improvement in mobility, pain, work and social life.

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Hearing Good News in NF2 – A Life Changing Cochlear Implant, 20 Years After Hearing Loss

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We present the case of a man with a life changing benefit from cochlear implant (CI) surgery after 20 years of hearing loss due to Neurofibromatosis type 2 (NF2).

This 48 year old man with NF has a mis-sense mutation in exon 1. His father and both his children also have NF2. He required intracranial meningioma surgery at the age of 10. In 1997 at the age of 31 he had hearing preservation right retro-sigmoid VS surgery. The cochlear nerve remained anatomically intact and he had some hearing post-operatively, but this faded to a dead ear over the next year.

His left sided hearing gradually declined. In 2017, his growing left VS was treated with stereotactic radiosurgery, followed by more abrupt decline in this only hearing ear, with speech discrimination score of 83%. He could no longer use the telephone, became socially isolated, depressed and lost his job.

He requested cochlear implantation in the right ear; there was doubt about the likelihood of success given the 20-year history of deafness in that ear. He underwent promontory stimulation to assess functional integrity of the cochlear nerve, with a positive result. He had a CI funded by the English National NF2 service, with immediate benefit. Within 2 months of implantation and auditory rehabilitation, the implanted ear has become his better hearing ear. His independence has increased, he can hear the door bell and alarms, can use the telephone and earphones and is teaching adults with learning disability. He has begun socialising again and has been able to participate in family activities with a concomitant improvement in his mood and quality of life.

This case highlights the potential benefits of CI in patients with NF2 and we would recommend considering this intervention even many years after hearing loss.

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The NF Family Wellness Retreat: Improving Mental and Physical Wellness in Families Living with Neurofibromatosis

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Background: Neurofibromatosis (NF) can severely impact quality of life, generate stress, and impede family functioning, making these areas critical health intervention targets. The NF Family Wellness Retreat addresses the psychosocial challenges of NF through wellness based programs guided by a compassion-building philosophy, Family Systems Theory, and a mindfulness model. Program activities include mindfulness and meditation exercises, talking circles, educational sessions, outdoor activities, and plant based eating to achieve three primary goals: strengthen the family unit, promote healthy lifestyles, and address NF manifestations.

Methods: To assess whether these primary objectives were met, researchers conducted quantitative surveys with 20 teen and adult participants and qualitative interviews, analyzed by two independent coders, with 12 adult participants after the 2018 Retreat. Surveys were analyzed using one-sample t-tests and tracked levels of mental distress, measured by depression and anxiety level scales, the Self-Esteem Scale, and the Perceived Stress Scale, as well as attitudes toward nutrition and health to determine the Retreat's impact on mental and physical wellness. The Communication Abilities Scale and Family Interaction Scale measured the Retreat's effects on family functioning. Taken together, these measures evaluated how the Retreat addressed related NF manifestations.

Results: Participants reported significantly low levels of anxiety on the State-Trait Anxiety Inventory ($t = -7.60$, $p < .01$) and the Zung Self-Rating Anxiety Scale ($t = -4.64$, $p < .01$), depression on the Depression Scale ($t = -9.50$, $p < .01$), and stress ($t = -4.50$, $p < .01$) compared to the average for each measure. High levels of self-esteem ($t = 10.44$, $p < .01$) and positive attitudes toward family interaction ($t = 11.87$, $p < .01$), nutrition, and health ($t = 10.13$, $p < .01$) were also reported. Qualitative interview findings corroborated the survey data, as connection, emotional safety, and improved family functioning emerged as the primary themes. The interview data indicate that the Retreat helped participants create structures of social support, cope with stress, and improve communication within their families.

Conclusions: We conclude that the NF Family Wellness Retreat effectively strengthens families, promotes healthy lifestyles, and addresses NF manifestations, leading participants to report improved family interactions, low mental and emotional distress, feeling supported and safe, and positive attitudes toward nutrition and health. These findings align with previous data from the Retreat. Having found that the measures were well received by participants, the same measures will be used in pre and post test sampling along with qualitative interviews in 2019.

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Business Case for an NF Network Clinic at The Medical University of South Carolina (MUSC) - A Model for Underserved and Less Populated States

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Purpose: The purpose of the study was to develop a business case for the formation of an NF Network Clinic in the state of South Carolina, in response to the results of a previous study, "Access to Care for the Neurofibromatosis Type 1 Pediatric Population of South Carolina" which indicated a critical lack of access to care due to both the lack of provider awareness and absence of a South Carolina NF Network Clinic.

Methods: Potential partners within the Medical University Hospital Authority (MUHA) were identified and a proposal was made to pediatric neurosurgery to participate. An initial business case development meeting was held which also included Pediatric and Adult Neuro-oncology and Pediatric Hematology/Oncology. Using "A Business Plan Model for Funding Your Clinic and How a Database might help" by Kaleb Yohay, MD, a template pro forma was developed. A list of relevant CPT codes associated with the ICD-9/ICD-10 codes for NF Type 1 and Type 2, and Schwannomatosis was developed and vetted. Three years of de-identified statewide claims data using these codes from Blue Cross Blue Shield (BCBS) of South Carolina was solicited and obtained. Support from the Medical Director of SC Medicaid was also obtained given the high percentage of citizens with Medicaid and the inability of SC Medicaid to provide services within the state. MUSC leadership also acknowledged interest and provided access to internal analytic resources to complete the business case. Mapping of the claims data and related MUSC charges was performed to determine the potential statewide census and revenue. In addition, statewide Medicaid charges were obtained. These two revenues were combined and the projected internal costs were determined.

Results: Based upon a phased 3 year roll-out, a positive ROI was shown in years 1 – 3 of $65,769, $212,007, and $272,476 respectively. Additional prospective patients (those with other payers, seeking care out of state, and receiving suboptimal care with local clinicians) are not included in this business case. Thus, these are conservative estimates.

Conclusions: Through internal and external partnerships, the business case was validated. The CMO of the Children's Hospital and Pediatrics Department Chair authorized resources to complete final approval prior to implementation.

Summary: Clinicians in states such as South Carolina, who have no NF Network Clinic, suboptimal access to care, and smaller populations, can partner with the largest payers to develop a business model which supports the establishment of an NF Network Clinic.

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LigaSure™ Vessel Sealing System Reduces Intraoperative Blood Loss During Diffuse Plexiform Neurofibroma Surgeries
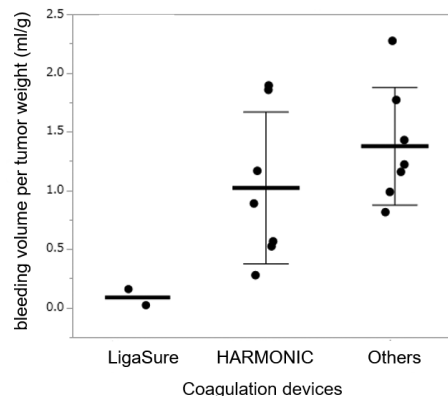
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Background: Diffuse plexiform neurofibromas(DPNs) can develop into intratumoral hemorrhages, malignant transformations, functional impairment, and disfigurement. However, removal of DPNs is challenging due to the risk of massive bleeding during or hematoma formation after surgery.

Methods: Medical records for three patients with Neurofibromatosis type 1, who have undergone resection of DPNs during a 9 year period were reviewed. Surgeries were categorized into three groups based on the coagulation device used intraoperatively. Group 1 was LigaSure™ (Meddtronic, Dublin, Ireland); Group 2, "HARMONIC® (Johnson & Johnson, New Jersey, U.S.A.)"; And Group 3, used other devices. Differences in intraoperative blood loss, tumor weight, operation time, length of hospitalization, blood transfusion and postoperative complications were evaluated.

Results: Three patients underwent 16 surgeries. Among these surgeries we categorized 2 surgeries as group one, 7 surgeries as group two, and 7 surgeries as group three. Means of blood loss per tumor weight were 0.10 ml/g, 1.02 ml/g, and 1.38 ml/g in groups one, two, and three respectively. Means of operation time per tumor weight were 0.22 minutes/g, 0.39 minutes/g, and 0.65 minutes/g in groups one, two, and three, respectively. Means of hospitalization days were 22, 25, and 40 in groups one, two, and three, respectively. No surgeries developed postoperative hematomas or other bleeding complications in group one and group two, while 1 of 7 (14%) surgeries developed bleeding complications in group three. No surgeries required blood transfusion in group one, while 6 of 7 surgeries (86%) and 4 of 7 surgeries (57%) required blood transfusions in groups two and three, respectively.

Conclusions: LigaSure™-assisted DPN surgery could allow shorter operation time and fewer hemorrhage complications, resulting in shorter hospitalization.

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Emotional and Behavioral Functioning Among Patients Diagnosed with Neurofibromatosis – Type 1

Melissa Young, *Kaleida Health*

Patients diagnosed with Neurofibromatosis – Type 1 (NF1), an autosomal dominant disorder, are at an increased risk for experiencing significant emotional and behavioral challenges that can impact developmental, academic, and interpersonal functioning. Our goal was to identify and quantify emotional and behavioral challenges amongst patients with NF1 receiving treatment within our NF multi-disciplinary clinic. Emotional and behavioral functioning were assessed using the Child Behavior Checklist (CBCL)/Adult Self-Report (ASR), standardized and psychometrically validated assessments, for use with children, adolescents, and adults. Fifty-two primary caregivers of pediatric patients and 14 patients over the age of 18 with NF1 have been recruited (n = 66). Mean age was 12.9 years. Forty-one patients (62%) scored in the borderline range and 33 patients (50%) scored in clinical range on at least one CBCL subscale. Twenty-nine patients (43.9%) had at least one borderline and one clinical subscale elevation across multiple domains. When examined more closely, pediatric patients between the ages of 6 and 18 were reported to experience significantly more challenges, as evidenced by borderline (n = 32; 72%) and clinical (n = 32; 81%) elevations on the Activities, Social, School, Attention Problems, Internalizing Problems, and Anxiety Problem CBCL subscales. These results indicate that a significant portion of our patient population are experiencing sub-clinical and/or clinically significant challenges in at least one domain of emotional and/or behavioral functioning. These data suggest that pediatric and adult patients with NF1 experience challenges that may negatively impact their functioning in daily life without appropriate psychosocial intervention. Administration of the CBCL/ASR within the NF multi-disciplinary clinic has led to early identification of challenges and focused intervention, which we anticipate will lead to improvement of both medical and psychosocial outcomes amongst our patient population.

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Utilizing the Psychosocial Assessment Tool to Identify the Need for Psychosocial Intervention among Pediatric Neurofibromatosis Patients within a Multi-Disciplinary Clinic

Melissa Young, *Kaleida Health*

Pediatric patients with Neurofibromatosis (NF) have a complex clinical presentation. Patients have a lifetime high risk of cancers, developmental delay, academic underachievement, and attention deficit disorder, which inevitably impacts the entire family system. Despite this, there is a paucity of psychometrically sound psychosocial assessments for use with pediatric NF patients and their families. The Psychosocial Assessment Tool (PAT) is an easily administered self-report instrument that has been validated for use with pediatric patients diagnosed with a multitude of other chronic illnesses, with extensive research demonstrating clinical utility and sound psychometric properties. To date, there are no empirical studies that have utilized the PAT with pediatric NF patients and their families. Data yielded from the PAT affords clinicians the opportunity to classify family's level of psychosocial risk into one of three categories (i.e., universal, targeted, and clinical), to target psychosocial interventions based on level of need. The purpose of our study is to utilize the PAT to identify the need for psychosocial intervention amongst pediatric NF patients and their families. Our goal is to identify and target these families shortly after diagnosis in order to identify psychosocial need and thus provide expedited, appropriate services. Caregivers of pediatric patients with NF from birth through 18 years of age are eligible and continue to be recruited during their child's new patient or follow-up appointment at a monthly pediatric NF clinic. To date, 36 caregivers of 35 patients with NF1 (97.2%) and 1 patient with NF2 (2.8%) have completed the PAT with 2 caregivers (5.6%) scoring in the clinical range, 9 (25%) scoring within the targeted range, and 25 (69.4%) of caregivers scoring within the universal range, which is consistent with estimates obtained from pediatric oncology populations. These results indicate that all pediatric patients with NF and their families require psychosocial intervention regardless of risk level, with intensive psychosocial intervention being required for approximately 31% of patients and families. The PAT is a simple, inexpensive, and standardized screening tool, that can be implemented within multi-disciplinary NF clinics to identify need and provide immediate access to personalized psychosocial services.

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Comprehensive Biopsychosocial Care within a Pediatric Neurofibromatosis Clinic

Melissa Young, *Kaleida Health*

Pediatric patients with neurofibromatosis (NF) often experience life-long medical and psychosocial challenges. NF, an autosomal dominant disorder, has a complex clinical presentation, and patients struggle with a lifetime high risk of cancers, developmental delay, academic underachievement, and symptoms of attention-deficit hyperactivity disorder and autism spectrum disorder. Due to a myriad of factors, pediatric patients with NF are often misunderstood within primary care settings. Rather these patients benefit from receiving treatment within a comprehensive multi-disciplinary program with pediatric subspecialists who are experienced in recognizing the complications associated with NF. Within our newly developed program, in which we provide specialized multi-disciplinary care to pediatric patients with NF, we quickly recognized that close to 100% of our patients were not receiving the long-term psychosocial and developmental care that they require for success when accessing outside services for developmental, academic, and behavioral health challenges. Difficulties to accessing appropriate care often include under-recognition of developmental and behavioral health co-morbidities, that lead to a lack of appropriate school services and interventions, as well as a paucity of programs to help transition patients from childhood to adulthood. In order to address the multidimensional needs of pediatric patients with NF, a neurosurgeon, neuro oncologist, neurologist, endocrinologist, resident psychologist supervised by both a licensed psychologist and a board-certified pediatric neuropsychologist, social worker, program coordinator, registered nurse, & health home care manager, provide specialized multi-disciplinary care to these complex patients with the goal of improving medical and psychosocial outcomes. Through this effort, we have begun to develop standards of care for the ongoing assessment and management of medical and psychosocial concerns of pediatric NF patients. The primary purpose of our clinical work is to develop comprehensive standards to guide providers in addressing the medical and psychosocial needs of pediatric NF patients that can be utilized and implemented within primary care, academic, and behavioral health settings.

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Surgical Treatment of Large Vestibular Schwannomas in Patients with Neurofibromatosis Type 2: Outcomes on Facial Nerve Function and Hearing Preservation

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Background: Surgical treatment of vestibular schwannoma (VS) in patients with neurofibromatosis type 2 (NF2) along with functional preservation of cranial nerves is challenging. The aim of this study was to analyze the outcomes of hearing and facial nerve function in patients with NF2 who underwent large-size VS (> 2 cm) surgery.

Methods: From 2006 to 2016, one hundred and forty NF2 patients were included with 149 large-size VS resections using retrosigmoid approach. Hearing function was classified according to the American Academy of Otolaryngology–Head and Neck Surgery (AAO–HNS) criteria. Preoperative and one-year postoperative facial nerve function were both assessed using the House-Brackmann (H-B) grading scale. A multivariate logistic regression was performed to identify preoperative predictors for facial function outcomes.

Results: No operative death we noted. Total tumor removal was achieved in 82.6% of the operated VSs. The anatomical integrity of the facial nerve was preserved in 67.8% of surgeries. Good facial nerve function (H–B Grades I–III) was maintained in 49.6% of patients at 12 months after surgery. Tumor size larger than 3 cm and preoperative facial weakness related with worse outcome of facial nerve function (P < 0.001; for both). Hearing preservation surgeries were attempted in 31 ears. Class B or C hearing according to the AAO-HNS criteria was maintained in 7 ears (22.5%), and measurable hearing was maintained 11 ears (35.5%).

Conclusions: It is challenging to maintain hearing and facial nerve function in NF2 patients with large VSs. Early surgical intervention is an appropriate choice to decrease the risk of neurological functions deficit.

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Shrinking Cutaneous Neurofibromas in NF1 with Selumetinib

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There is a strong unmet need for the development of effective medical therapies for cutaneous neurofibromas (cNFs). Selumetinib (AZD6244) is a specific inhibitor of MEK 1, which may mediate anti-tumor effects in NF1 related tumors by inhibition of downstream signaling of Ras. In an NCI CTEP sponsored phase I/II trial of selumetinib for children and young adults with NF1 and inoperable plexiform neurofibromas (pNF) we have observed pNF volume decrease in ≥71% of patients enrolled. We hypothesize that selumetinib will result in shrinkage of existing cNFs in individuals with NF1 and may prevent or delay the development of new cNFs. We are actively enrolling for a limited institution (UAB and NCI) open label pilot study (NCT002839720) in which patients ≥18 years old with NF1 and cNFs receive oral selumetinib for 24 cycles (1 cycle = 28 days). For the primary response evaluation, the average volume of target cutaneous neurofibromas in 3 different body regions are manually measured with calipers. Preliminary data is now available from the first four study participants. The average cNF volume decrease was 25% after 4 cycles and 35% after 8 cycles. Furthermore, the decrease in cNF volume was visibly apparent. No significant change in cNF number has been observed to date. The Skindex-29 quality-of-life results do not correlate with cNF volume response, suggesting that this patient reported outcome measure may lack the sensitivity needed to detect cNF treatment responses. In conclusion, preliminary data demonstrates the feasibility of manual caliper measurement of cNF volume for clinical trials and that selumetinib decreases cNF volume but additional study participant data is needed.

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