

2016 **NF** CONFERENCE



“YOU’VE GOT
THE POWER!”

CHILDREN’S
TUMOR
FOUNDATION
NF CONFERENCE

June 18-21, 2016
JW Marriott
Austin, TX

Dear NF Conference Attendees:

Welcome to Austin—the capital of Texas, the live music capital of the world, and for these next three and a half days, the capital of NF research! We are thrilled to be with you all in such a thriving city, an ideal location for the greatest minds in NF research to congregate, collaborate, and make strides towards ending NF.

This year's 2016 NF Conference, brief neighbors of the NF Patient Forum, is based on the larger premise that you, along with the incredibly brave NF patients and their families, have the power to end NF. There are three core principles of the NF Conference that drive this premise: creating new networks and friendships for attendees; bringing patients, researchers and clinicians face-to-face, and ensuring that we at the Foundation continue to use this event as a platform to stimulate collaborative NF research, and to showcase our incredible progress. Moreover, at this Conference, there will be the first open data release in NF. It is only as a team that we can succeed!

It is you, our friends and colleagues, and your dedication to NF research, who have made this progress and promise a reality. You represent the expansive, and often unpredictable spectrum that encompasses science, and within the singularly difficult realm of NF research. It is you who have made all of the accomplishments in NF since last year's Conference possible, and it is you who will foster further accomplishments this year and beyond. These accomplishments have been powerful beyond words.

In the past year, and after seeing the tremendous success of the NF Therapeutic Consortium (NFTC) and Synodos for NF2, the Foundation kicked off three Synodos for NF1 projects and officially launched the call for Synodos for Schwannomatosis. This means that more of you are seeing the value of collaborative science, and are becoming a part of the Synodos network! You will hear more about Synodos and all of our team science during the Conference. We are proud to show the world that in our community team science is not a new concept; we started team science in 2008 and have shown repeatedly that it delivers.

We have also formed the Business Advisory Council, so we can look towards the future and pave the way for new partnerships to add to our incredible network of researchers, doctors, clinicians, donors, and patients.

And once again, by combining the Conference and the Forum we offer you the chance to show your work to a community who so deeply needs and appreciates it. No matter how many failed experiments you encounter or how many hypotheses disappoint you, I can promise you that interacting with the patients will give you the motivation you need to accelerate the pace of progress to bring the best drugs to clinical trials, and in time, to those patients.

And last, but not least, I want to offer my sincere thanks to our dedicated Conference co-chairs, Michael Fisher and Eduard Serra, who brought their sheer enthusiasm, innovation, and pure smarts to the task of creating an NF Conference for the books! I don't want to forget my dear teammate: Patrice Pancza for whom nothing was too difficult, too complicated, or too late!

Thank you all!

I truly do hope this year's NF Conference sparks creative ideas for each and every one of you, and leaves you feeling like you have the power to change the lives of those with NF. Together, I know we can!

Cheers,



ANNETTE BAKKER

President and Chief Scientific Officer

CONTENTS

Table of Contents

INTRODUCTION

The Friedrich von Recklinghausen Award: NF Tradition and Progress	5
2016 Excellence in Team Science.....	6
Foundation Staff	7
National Programs.....	8

INFORMATION

Schedule At-A-Glance	9
Important Notes To Chairs, Speakers & Poster Presenters.....	10
Agenda	11
Speaker Bios – Conference Chairs / Keynote Speakers	18

SPEAKER ABSTRACTS

Abstracts	21
-----------------	----

POSTER ABSTRACTS

Basic Research	47
Clinical.....	73

ANCILLARY MEETINGS

NF Clinics Breakout Session	101
International Neurofibromatosis Autism Consortium Team (INFACT).....	102

APPENDIX

Participants.....	105
Floor Plan	111



The Friedrich von Recklinghausen Award: Neurofibromatosis Tradition and Progress

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

2016 Friedrich von Recklinghausen Award Recipient



It is with great pleasure to announce the recipient of the 2016 Friedrich von Recklinghausen Award, Dr. David H. Viskochil. For three decades, with the tremendous commitment and dedication, Dr. Viskochil has contributed invaluable to every aspect of the neurofibromatoses, from the lab, to the clinic, to academia. His research accomplishments, including helping to identify the NF1 gene, his mentoring skills, his administration of national and regional programs, and most important, as a caring and unwavering advocate of the NF patient – all these qualities define him as most worthy of this prestigious award. Dr. Viskochil has been involved in the activities of the Children's Tumor Foundation for many years, and serves currently on the Board of Directors, the Foundation's Medical Advisory Committee, and is chair of the CTF Clinical Care Advisory Board.

One very notable contribution and much deserving of attention, was Dr. Viskochil's idea of, and ongoing support for, the Children's Tumor Foundation's NF Camp for children and teens in the canyons of Utah. For 20 years now, countless young NF patients have spent a glorious week of fun and friendship at the NF Camp in an atmosphere of great support and encouragement. A highlight of their week is a visit to the camp by Dr. Dave for a lively session of Q&A, which is a thing to behold. It cannot be understated that countless young lives have been transformed by this experience. For this and so many other contributions to the entire NF community, please join us in congratulating Dr. Dave Viskochil for this much-deserved award.

The following are the most recent recipients of the Award:



2008
Vincent 'Vic' Riccardi, MD,
The Neurofibromatosis Institute



2009
Luis Parada, PhD,
University of Texas Southwestern



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



2012
David Gutmann, MD, PhD,
Washington University



2013
Brigitte Widemann, MD
National Cancer Institute



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



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



2016 Excellence in Team Science Award

In recognition of the value and impact of the Children's Tumor Foundation's growing portfolio of team science initiatives, we are launching for the first time the Excellence in Team Science Award.

In 2008, the Foundation initiated its first consortia-based program, the NF Preclinical Consortium, and though it ended in 2013 the **NF Therapeutic Consortium** (NFTC, 2013-2016) was created as a continuation of the NFPC, was co-funded by NTAP and was completely built on existing infrastructure and collaborations from NFPC.

These preclinical consortia have shown the impact for improvement of NF treatment by facilitating new clinical trials that are based on mechanistic scientific data. This has undoubtedly increased over time through routine interactions between the Consortium and clinical investigators. The existence of the NFPC/ NFTC has changed the NF clinical trials landscape by promoting trials that hone scientific rationale thereby increasing the chance of optimizing care improvements. In addition, their work has not only led to Selumetinib, the first ever NF drug that is in registration trial, it also lead to a \$12 million, 5-year NCI SPORE grant. This grant is a multi-institutional research program spearheaded by the NFTC PIs, Dr. Wade Clapp (IU), and Dr. Kevin Shannon (UCSF), with the goal of developing better treatments for tumors in patients with NF1. CTF's investment was instrumental in providing initial funding and a research group infrastructure to develop key preliminary data and to demonstrate an effective collaboration model.

We are pleased to announce The 2016 Excellence in Team Science Award goes to the NF Therapeutic Consortium and each of the labs that makes up the NFTC.

Karen Cichowski Lab
D. Wade Clapp Lab
Kevin Shannon Lab
Nancy Ratner Lab
Benjamin Braun Lab

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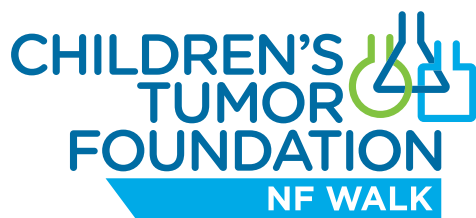
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NATIONAL PROGRAMS

Children's Tumor Foundation National Programs



NF Walk Program (www.nfwalk.org)

The Children's Tumor Foundation NF Walk Program was established in 2009 as a national fundraising effort to support neurofibromatosis research, raise awareness, and provide support for individuals with NF and their families. A key feature of the Walk program is that it is a community-based event organized by local volunteers, which offers the opportunity for individuals, their families, friends, neighbors, and organizations to join together, through a truly enjoyable event, in the fight against NF. **Every Step Makes a Difference.**



NF Endurance Program (www.nfendurance.org)

NF Endurance offers the opportunity for individuals to participate in marathons, triathlons, bike races, and other high endurance sporting events to raise money for research, promote awareness, and provide a network of caring support for those living with NF and their families. **NF Endurance: iNFinite possibilities.**



Racing4Research (www.racing4research.org)

Racing4Research (R4R) utilizes competitive, professional auto racing as a vehicle to increase awareness of neurofibromatosis and raise funds for research through corporate sponsorship, personal donations, and individual fundraising by NF Heroes: children and adults from around the country who live with neurofibromatosis. The program offers children and families living with the disorder a uniquely empowering weekend, and has raised over \$2 million dollars since its inception five years ago. **Fuel the Cure.**



Community Building (www.ctf.org/communityrelations)

The Community Building and Patient Engagement program encompasses the Foundation's efforts to support and empower people with NF, NF caregivers and NF volunteers. We believe that patients (and their families) should continue to become more informed and sophisticated about their role in their care and the NF research system. We promote and help to facilitate the active partnering of NF patients with physicians, clinicians, researchers and others involved in NF care and research. And through our Volunteer Leadership Council, we provide a forum for volunteers to share best practices and gain new skills in a broad spectrum of areas and activities.

SCHEDULE

Schedule At-A-Glance

	TIME		EVENT	LOCATION
FRIDAY JUNE 17	4:00 PM	6:00 PM	Registration	Lone Star South Foyer
	7:00 AM	5:00 PM	Registration	Lone Star South Foyer
	8:00 AM	12:15 PM	Satellite Educational Symposium	Lone Star E
	12:00 PM	12:45 PM	Box Lunch Served	Grand Foyer & Lone Star South Foyer
	12:45 PM	1:00 PM	NF CONFERENCE KICK-OFF	Lone Star E
SATURDAY JUNE 18	1:00 PM	2:00 PM	KEYNOTE 1: Entering a World of New Data, New Roles and New Ways to Care and Treat	Lone Star E
	2:00 PM	2:05 PM	Poster Advertisements - #1	Lone Star E
	2:05 PM	4:05 PM	SESSION 1: Next Generation Genetic Diagnostics, Gene Discovery and Genotype-Phenotype Correlations for the NF's	Lone Star E
	4:05 PM	4:20 PM	Coffee Break	Lone Star South Foyer
	4:20 PM	4:25 PM	Poster Advertisements - #2	Lone Star E
	4:25 PM	5:25 PM	Clinical Mystery Session	Lone Star E
	6:30 PM	7:30 PM	Cocktail Reception and Special Poster Session with NF Forum Attendees	Lone Star F-H
	7:30 PM	9:30 PM	Welcome Dinner, von Recklinghausen and Team Science Awards Presentations	JW Grand Ballroom
	6:30 AM	2:00 PM	Registration	Lone Star South Foyer
	6:30 AM	8:00 AM	Breakfast	Lone Star South Foyer
SUNDAY JUNE 19	7:00 AM	8:00 AM	OPTIONAL SUNRISE SESSION: Basic Science Mentoring	303/304
	8:00 AM	8:05 AM	Poster Advertisements - #3	Lone Star E
	8:05 AM	10:05 AM	SESSION 2: Plexiform Neurofibroma to Malignant Peripheral Nerve Sheath Tumor	Lone Star E
	10:05 AM	10:20 AM	Coffee Break	Lone Star South Foyer
	10:20 AM	11:20 AM	PANEL: The Role of Surgery in the Neurofibromatoses	Lone Star E
	11:25 AM	11:30 AM	Poster Advertisements - #4	Lone Star E
	11:30 AM	12:30 PM	SESSION 3: Select Platform Presentations	Lone Star E
	12:30 PM	1:45 PM	Lunch with the Experts	Lone Star East Foyer
	1:45 PM	2:45 PM	KEYNOTE 2: Immuno-Oncology: A New Paradigm for Cancer Therapy	Lone Star E
	2:45 PM	3:45 PM	Consortia Updates 1	Lone Star E
	3:45 PM	4:00 PM	Coffee Break	Lone Star South Foyer
	4:00 PM	5:20 PM	SESSION 4 CONCURRENT SESSIONS	Lone Star E & 303/304
	5:20 PM	6:50 PM	Poster Session: Basic Science	Lone Star F-H
			Dinner On Your Own	
	8:00 PM	11:00 PM	Networking for Pre-and Post Doc's	Congress Avenue Deck
MONDAY JUNE 20	7:00 AM	12:00 PM	Registration	Lone Star South Foyer
	7:00 AM	8:30 AM	Breakfast	Lone Star South Foyer
	7:30 AM	8:30 AM	OPTIONAL SUNRISE SESSION: Clinical Science Mentoring	303/304
	8:30 AM	10:30 AM	SESSION 5: CNS Tumors in the Neurofibromatoses	Lone Star E
	10:30 AM	11:10 AM	Coffee Break	Lone Star South Foyer
	11:10 AM	12:30 PM	SESSION 6: Present and Future Impact of Novel Imaging in the Management of NF	Lone Star E
	12:30 PM	1:30 PM	Lunch	Lone Star South Foyer
	1:30 PM	2:30 PM	KEYNOTE 3: Defining the Actionable Genome	Lone Star E
	2:35 PM	3:55 PM	SESSION 7 CONCURRENT SESSIONS	Lone Star E & 303/304
	3:55 PM	4:15 PM	Coffee Break	Lone Star South Foyer
	4:15 PM	5:15 PM	KEYNOTE 4: Using Pluripotent Stem Cells to Understand Human Neural Crest Development and Disease	Lone Star E
	5:15 PM	6:45 PM	Poster Session: Clinical Science	Lone Star F-H
			Dinner On Your Own	
	7:00 AM	8:00 AM	Breakfast	Lone Star South Foyer
	8:00 AM	9:00 AM	KEYNOTE 5: New Ways of Targeting RAS	Lone Star E
TUESDAY JUNE 21	9:00 AM	10:00 AM	Top Poster Oral Presentations	Lone Star E
	10:00 AM	10:20 AM	Coffee Break	Lone Star South Foyer
	10:20 AM	11:40 AM	SESSION 8: New Preclinical Models in NF	Lone Star E
	11:40 AM	12:30 PM	Consortia Updates 2	Lone Star E
	12:30 PM	12:45 PM	2016 NF Conference Wrap-Up	Lone Star E

IMPORTANT NOTES

Important Notes to Chairs, Speakers & Poster Presenters

Important Notes to Speakers, Chairs & Poster Presenters

NOTE TO SPEAKERS

- Bring your slides to the meeting on a flash drive. We prefer that you not use your own laptop. You will be notified by Foundation staff at registration as to when to bring your slides to the a/v technicians.
 - 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.
-

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 are “Perspectives” speakers, invited or platform speakers. If they are CTF awardees (indicated on the agenda), please mention so in the introduction.
- 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 THE CO-CHAIRS BY THE END OF JULY.
-

NOTE TO POSTER PRESENTERS

- Posters will be on display throughout the Conference, June 18th – 21st in LoneStar F – H.
- Posters can be set up starting Friday, Jun 17th after 4pm; your poster should be on display for the duration of the Conference.
- There will be a special poster session for the patients and families attending the NF Forum on Saturday at 6:30pm – 7:30pm prior to the dinner. There will be a selection of posters deemed “family and patient-friendly” and those presenters will be asked to stand by their posters to answer any questions the patients and families may have. All poster presenters and attendees are encouraged to attend this session/reception.
- The Basic Science poster session (these posters will carry odd numbers) will be held on Sunday, June 18th, from 5:20pm to 6:50pm. Please stand by your poster during this time. Wine, beer and cheese will be served.
- The Clinical Science poster session (these posters will carry even numbers) will be held on Monday, the 20th, from 5:15pm – 6:45pm. Please stand by your poster during this time. Wine, beer and cheese will be served.
- A jury will be judging posters during each session and will select the top 3 posters. The winners will present for 10 minutes including 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”.

Questions?

Please contact a Foundation staff member!

AGENDA

Friday · June 17, 2016

4:00 PM

6:00 PM

Registration

Lone Star South Foyer

Saturday · June 18, 2016

7:00 AM

5:00 PM

Registration

Lone Star South Foyer

8:00 AM

12:15 PM

SATELLITE EDUCATIONAL SYMPOSIUM

Lone Star E

(breakfast provided for symposium attendees)

8:00 AM

9:00 AM

Best Practices for the Management of NF1 in Adults: What Does the Evidence Say?

Chair: Douglas Stewart, MD, *National Cancer Institute*

8:00 AM

8:05 AM

Introduction

Douglas Stewart, MD, *National Cancer Institute*

8:05 AM

8:20 AM

Breast Cancer

8:05 AM

8:10 AM

Breast Cancer Screening for NF Patients under 50

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

8:10 AM

8:20 AM

Breast Cancer and NF1: a New(ish) Association with an Old Disease

Douglas Stewart, MD, *National Cancer Institute*

8:20 AM

8:35 AM

Hypertension, Vascular Anomalies and Pheochromocytoma

Kaleb Yohay, MD, *NYU Langone Medical Center*

8:35 AM

9:00 AM

Panel Discussion on Proposed Clinical Care Guidance in Adult NF1

Moderator: Douglas Stewart, MD, *National Cancer Institute*

Discussants: Bruce Korf, MD, PhD, *University of Alabama at Birmingham*; Kaleb Yohay, MD, *NYU Langone Medical Center*; David Stevenson, MD, *Stanford University*

9:00 AM

10:30 AM

Low Grade Glioma

Chair: Robert Avery, D.O., *Children's Hospital of Philadelphia*

9:00 AM

9:20 AM

Clinical Management of Optic Pathway Gliomas in NF1

Peter deBlank, MD, *Rainbow and Babies Children's Hospital*

9:20 AM

9:30 AM

Visual Assessment and Biomarkers of Optic Pathway Gliomas in NF1

Robert Avery, D.O., *Children's Hospital of Philadelphia*

9:30 AM

9:50 AM

Clinical Management of LGG Outside of the Visual Pathway of NF1

Roger Packer, MD, *Children's National Medical Center*

9:50 AM

10:10 AM

Next Generation of Therapeutics for LGG in NF1

Mark Kieran, MD, PhD, *Dana-Farber Cancer Institute*

10:10 AM

10:30 AM

Q&A

All speakers

10:30 AM

10:45 AM

Coffee Break

10:45 AM

12:15 PM

Meningioma in NF2

Chair: Scott Plotkin, MD, PhD, *Harvard University/Massachusetts General Hospital*

10:45 AM

11:05 AM

Clinical Features of Meningioma in NF2

Justin Jordan, MD, *Massachusetts General Hospital*

11:05 AM

11:25 AM

Surgical Decision Making for NF2: The Challenge of Skull Base and Multiple Meningiomas

Michel Kalamarides, MD, PhD, *Hopital Pitie - Salpetriere, Universite de Paris*

11:25 AM

11:55 AM

Germline and Somatic Mutations in Meningiomas: the Path to Target Therapies

Miriam Smith, PhD, *University of Manchester, England*

11:55 AM

12:15 PM

Tumor Board (3 Cases)

Moderator: Scott Plotkin, MD, PhD, *Harvard/MGH*

Discussants: Jaishri Blakeley, MD, *Johns Hopkins University*; Michel Kalamarides, MD, PhD, *Hopital Salpetriere, Universite de Paris*; Justin Jordan, MD, *Massachusetts General Hospital*

AGENDA

Saturday · June 18, 2016

12:00 PM	12:45 PM	Lunch	Grand Foyer (pick-up) & Lone Star South Foyer (additional seating)
12:45 PM	1:00 PM	NF CONFERENCE KICK-OFF	Lone Star E
Annette Bakker, PhD, President, Chief Scientific Officer, <i>Children's Tumor Foundation</i>			
2016 NF Conference Co-chairs: Michael Fisher, MD, <i>Children's Hospital of Philadelphia (CHOP)</i> ; Eduard Serra, PhD, <i>Institute of Predictive and Personalized Medicine of Cancer (IPPMC)</i> , Barcelona, Spain			
1:00 PM	2:00 PM	KEYNOTE 1: Entering a World of New Data, New Roles and New Ways to Care and Treat	Lone Star E
Stephen H. Friend, MD, PhD, <i>President, Co-Founder and Director of Sage Bionetworks</i>			
2:00 PM	2:05 PM	Poster Advertisements #1	Lone Star E
2:05 PM	4:05 PM	SESSION 1: Next Generation Genetic Diagnostics, Gene Discovery and Genotype-Phenotype Correlations for the NF's	Lone Star E
Chairs: Conxi Lazaro, PhD, <i>Catalan Institute of Oncology, Barcelona, Spain</i> ; Eric Pasmant, PhD, <i>Universite Paris Descartes, France</i>			
2:05 PM	2:45 PM	Perspectives: Changing the Landscape of NF Genetics: The Next Step	
Ludwine Messiaen, PhD, <i>University of Alabama at Birmingham</i>			
2:45 PM	3:05 PM	NGS for Diagnosis of NF1 and NF2: An Update	
Beatrice Parfait, PhD, <i>Hopitaux Universitaires Paris Central - Hopital Cochin, France</i>			
3:05 PM	3:25 PM	Platform: DNA Damage Repair and NF1 Disease Severity	
Kristine Vogel, PhD, <i>UTHSCSA</i>			
3:25 PM	3:45 PM	Platform: Novel Methods for Genotype-Phenotype Correlation in Schwannomatosis	
Justin T. Jordan, MD, <i>Massachusetts General Hospital</i>			
3:45 PM	4:05 PM	SPRED1, the Gene Responsible for Legius Syndrome, Suppresses Ras Activation by Interacting with the GAP-Related Domain of Neurofibromin	
Akihiko Yoshimura, PhD, <i>Keio University, Japan</i>			
4:05 PM	4:20 PM	Coffee Break	Lone Star South Foyer
4:20 PM	4:25 PM	Poster Advertisements #2	Lone Star E
4:25 PM	5:25 PM	Clinical Mystery Session	Lone Star E
Rosalie Ferner, MD, <i>Guy's and St. Thomas' Hospital, London, UK</i> ; Robert Listernick, MD, <i>Ann and Robert H. Lurie Children's Hospital</i>			
"A Fun and Educational Session" - drinks included			
6:30 PM	7:30 PM	Special Poster Session with NF Forum attendees	Lone Star F-H
Cocktail reception and special poster session for attendees of NF Patient and Family Forum			
7:30 PM	9:30 PM	Award Dinner and Presentation of the 2016 Friedrich von Recklinghausen Award	JW Grand Ballroom
The NF Conference and NF Forum attendees will convene in celebration of the largest gathering ever of the entire NF community.			

Sunday · June 19, 2016

6:30 AM	1:00 PM	Registration	Lone Star South Foyer
6:30 AM	8:00 AM	Breakfast	Lone Star South Foyer

AGENDA

Sunday · June 19, 2016

7:00 AM	8:00 AM	Optional Sunrise Session: Basic Science Mentoring	303/304
8:00 AM	8:05 AM	Poster Advertisements #3	Lone Star E
8:05 AM	10:05 AM	SESSION 2: Plexiform Neurofibroma to Malignant Peripheral Nerve Sheath Tumor Chairs: Lu Le, MD, PhD, <i>UT Southwestern</i> ; Aerang Kim, MD, PhD, <i>Children's National Medical Center</i>	Lone Star E
8:05 AM	8:45 AM	Perspectives: Strategies to Prevent the Development of MPNST in NF1: Identification and Management Brigitte Widemann, MD, <i>National Cancer Institute</i>	
8:45 AM	9:05 AM	Transposon-Based Forward Genetics and Targeted Nucleases to Define Factors Causing Progression from Neurofibroma to Malignant Peripheral Nerve Sheath Tumor Development David Largaespada, PhD, <i>University of Minnesota</i>	
9:05 AM	9:25 AM	PRC2 loss in malignant transformation of MPNST Ping Chi, MD, PhD, <i>Memorial Sloan-Kettering Cancer Center</i>	
9:25 AM	9:45 AM	Platform: Comparative Genomic Analysis of NF1-Associated Atypical Neurofibromas (ANF) and Malignant Peripheral Nerve Sheath Tumors (MPNST) Alexander Pervov, PhD, <i>National Cancer Institute, National Institutes of Health</i>	
9:45 AM	10:05 AM	Platform: Atypical Neurofibromas in NF1: Clinical, Imaging and Pathology Characteristics Christine Higham, MD, <i>National Cancer Institute, National Institutes of Health</i>	
10:05 AM	10:20 AM	Coffee Break	Lone Star South Foyer
10:20 AM	11:20 AM	PANEL: The Role of Surgery in the Neurofibromatoses Chairs: David Viskochil, MD, PhD, <i>University of Utah</i> ; Allan Belzberg, MD, <i>Johns Hopkins University Hospital</i> Panel Discussion Moderator: David Viskochil, MD, PhD, <i>University of Utah</i> Discussants: Carol Diane Morris, MD, MS, <i>Johns Hopkins University Hospital</i> ; Allan Belzberg, MD, <i>Johns Hopkins University Hospital</i> ; R. Lor Randall, MD, FACS, <i>Huntsman Cancer Institute, University of Utah</i>	Lone Star E
11:25 AM	11:30 AM	Poster Advertisements #4	Lone Star E
11:30 AM	12:30 PM	SESSION 3: Select Platform Presentations Session Chair: Nancy Ratner, PhD, <i>Cincinnati Children's Hospital</i>	Lone Star E
11:30 AM	11:45 AM	Platform: Tumor Volume Predicts Axonal Loss in Children with Optic Pathway Gliomas Secondary to Neurofibromatosis Type 1 Robert Avery, DO, MSCE, <i>Children's Hospital of Philadelphia</i>	
11:45 AM	12:00 PM	Platform: The Neurofibromin Recruitment Factor Spred1 Directly Interacts with the Neurofibromin GAP Domain without Interfering with Ras Inactivation Theresia Dunzendorfer-Matt, PhD, <i>Innsbruck Medical University, Austria</i>	
12:00 PM	12:15 PM	Platform: Exploiting Proteotoxic and Oxidative Stress in MPNSTs to Drive Tumor Regression Clare F. Malone, PhD (Young Investigator Awardee), <i>Brigham and Women's Hospital/Harvard Medical School</i>	
12:15 PM	12:30 PM	Platform: Clostridium Novyi-NT Can Cause Tumor Regressions in Malignant Peripheral Nerve Sheath Tumors Across Species Verena Staedtke, MD, PhD, <i>Johns Hopkins University Hospital</i>	
12:30 PM	1:45 PM	Lunch with the Experts (Sign up at registration)	Lone Star East Foyer

AGENDA

Sunday · June 19, 2016

1:45 PM	2:45 PM	KEYNOTE 2: Immuno-Oncology: A New Paradigm for Cancer Therapy <i>Crystal L. Mackall, MD, Professor of Pediatrics and Medicine, Stanford University</i>	Lone Star E
2:45 PM	3:45 PM	CONSORTIA UPDATES 1 <i>Chairs: Brigitte Widemann, MD, National Cancer Institute, NIH; Brian Weiss, MD, Cincinnati Children's Hospital</i>	Lone Star E
2:45 PM	3:00 PM	Synodos for NF2	
3:00 PM	3:15 PM	Progress Report – Synodos for NF1, Drug Discovery <i>Christopher Moertel, MD, University of Minnesota</i>	
3:15 PM	3:30 PM	Synodos for NF 1 – Preclinical Acceleration B <i>Jill Weimer, PhD, Sanford Health Center</i>	
3:30 PM	3:45 PM	NF1 LGG Synodos <i>David Gutmann, MD, Ph. D, Washington University St. Louis</i>	
3:45 PM	4:00 PM	Coffee Break	Lone Star South Foyer
4:00 PM	5:20 PM	SESSION 4A: Musculoskeletal Abnormalities in NF1 <i>Chairs: Elizabeth Schorry, MD, Cincinnati Children's Hospital; Florent Elefteriou, PhD, Baylor College of Medicine</i>	Lone Star E
4:00 PM	4:20 PM	Mechanisms of Muscle Weakness in NF1 <i>Matthew Summers, PhD Candidate, University of Sydney</i>	
4:20 PM	4:40 PM	Tibial Bowing: Pathogenesis, Prevention and Outcome Measures <i>David Stevenson, MD, Stanford University</i>	
4:40 PM	5:00 PM	NF1 Pseudarthrosis: Cell of Origin, Targets, Potential Treatments and Needs <i>Florent Elefteriou, PhD, Baylor College of Medicine</i>	
5:00 PM	5:20 PM	Platform: Neurofibromatosis Type 1-Related Pseudarthrosis: Beyond the Pseudarthrosis Site <i>Carlijn Brekelmans, PhD Candidate, KU Leuven, Belgium</i>	
4:00 PM	5:20 PM	SESSION 4B: Assessing Therapeutics in NF2 and Schwannomatosis through Translational Research <i>Chairs: Filippo Giancotti, MD, PhD, Memorial Sloan Kettering Cancer Center; Michel Kalamarides, MD, PhD, Hopital Pitie - Salpetriere, Universite de Paris</i>	303/304
4:00 PM	4:20 PM	Crizotinib suppresses NF2 deficient schwannoma through inhibition of Focal Adhesion Kinase 1 <i>Joseph Kissil, PhD, Scripps Research Institute</i>	
4:20 PM	4:40 PM	A Path toward Combination Therapy for NF2 <i>Vijaya Ramesh, PhD, Harvard University</i>	
4:40 PM	5:00 PM	Platform: Effects of Lapatinib on Meningiomas in Adults with Neurofibromatosis Type 2 <i>Matthias Karajannis, MD, MS, NYU Langone Medical Center, NYC</i>	
5:00 PM	5:20 PM	Platform: Ponatinib Causes Cell Cycle Arrest at G1 of Merlin-null Human Schwann Cells <i>Alejandra Petrilli, PhD, Burnett School of Biomedical Sciences, University of Central Florida</i>	
5:20 PM	6:50 PM	Poster Session - Basic Science <i>Wine, beer and cheese served.</i>	Lone Star F-H
DINNER ON YOUR OWN			
8:00 PM	11:00 PM	NETWORKING FOR PRE- AND POST DOC'S	Congress Avenue Deck

AGENDA

Monday · June 20, 2016

7:00 AM	12:00 PM	Registration	Lone Star South Foyer
7:00 AM	8:30 AM	Breakfast	Lone Star South Foyer
7:30 AM	8:30 AM	Optional Sunrise Session: Clinical Science Mentoring	303/304
8:30 AM	10:30 AM	SESSION 5: CNS Tumors in the Neurofibromatoses Chairs: Yuan Zhu, PhD, <i>Children's National Medical Center</i> ; Matthias Karajannis, MD, <i>NYU Langone Medical Center</i>	Lone Star E
8:30 AM	9:10 AM	Perspectives: Defining the Factors that Underlie the Pathogenesis of NF1 Optic Glioma David Gutmann, MD, PhD, <i>Washington University, St. Louis</i>	
9:10 AM	9:30 AM	Developmental Origin and Therapeutic Window for NF1-Associated Optic Pathway Glioma Yuan Zhu, PhD, <i>Children's National Medical Center</i>	
9:30 AM	9:50 AM	Spinal Ependymomas in NF2 Michel Kalamarides, MD, PhD, <i>Hopital Pitie - Salpetriere, Universite de Paris</i>	
9:50 AM	10:10 AM	Platform: Characterization of Brain Tumors and Spongiotic Change using Magnetic Resonance Fingerprinting: Initial Experience Peter deBlank, MD, <i>Rainbow and Babies Children's Hospital</i>	
10:10 AM	10:30 AM	Platform: Similarities and Differences in Tumor Characteristics and Treatment Response in NF2-Associated Vestibular Schwannomas and Meningiomas Sarah S. Burns, BA, <i>Nationwide Children's Hospital and The Ohio State University</i>	
10:30 AM	11:10 AM	Coffee Break	Lone Star South Foyer
11:10 AM	12:30 PM	SESSION 6: Present and Future Impact of Novel Imaging in the Management of NF Chairs: Peter de Blank, MD, <i>Rainbow and Babies' Children's Hospital, Case Western Reserve University</i> ; Gordon Harris, PhD, <i>Harvard University</i>	Lone Star E
11:10 AM	11:30 AM	Imaging White Matter in Children with Brain Tumors: Lessons for Late Effects, Plasticity and Repair Donald Mabbott, PhD, <i>Hospital for Sick Children, Toronto, CAN</i>	
11:30 AM	11:50 AM	Building Quantitative MRI: Implications for NF1 Vikas Gulani, MD, PhD, <i>University Hospitals of Cleveland</i>	
11:50 AM	12:10 PM	Platform: Computerized Working Memory Training for Children with Neurofibromatosis Type 1: a Pilot Resting-State Study of Changes in Intrinsic Functional Connectivity Maria Acosta, MD, <i>The Jennifer and Daniel Gilbert Neurofibromatosis Institute, Children's National Medical Center</i>	
12:10 PM	12:30 PM	MEG as Part of Multimodal Investigation of ASD: Towards Biomarkers for Diagnosis, Prognosis, Stratification and Response Timothy Roberts, PhD, <i>Children's Hospital of Philadelphia</i>	
12:30 PM	1:30 PM	Lunch	Lone Star South Foyer
1:30 PM	2:30 PM	KEYNOTE 3: Defining the Actionable Genome David Solit, MD, <i>Geoffrey Beene Chair in Cancer Research, Director, Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center</i>	Lone Star E
2:35 PM	3:55 PM	SESSION 7A: Beyond the Medical Model: Exploring Psychological, Social and Biological Factors in NF Chairs: Staci Martin, PhD, <i>NIH</i> ; Nicole Ullrich, MD, PhD, <i>Boston Children's Hospital/Harvard University</i>	303/304
2:35 PM	2:55 PM	Relating the NF Cognitive Phenotype to Environmental and Family Variables Jennifer Janusz, PsyD, ABPP-Cn, <i>University of Colorado School of Medicine/ Children's Hospital Colorado</i>	

AGENDA

Monday · June 20, 2016

2:55 PM	3:15 PM	Mind-Body Therapy for Individuals with NF1, NF2 and Schwannomatosis: Class II Evidence for Improvements in Quality of Life <i>Ana-Maria Vranceanu, PhD, Harvard University</i>	
3:15 PM	3:35 PM	Platform: Parental Coping with Child with NF1 <i>Taylor Smith, PhD, CalPoly</i>	
3:35 PM	3:55 PM	Dedicated Social Media for Adolescents and Parents of Adolescents with Neurofibromatosis Type 1 <i>Nicole Ullrich, MD, PhD, Boston Children's Hospital/Harvard University</i>	
2:35 PM	3:55 PM	SESSION 7B: Cellular Pathophysiology in Neurofibromatosis Type 2 and Schwannomatosis <i>Chairs: Wei Li, PhD, Penn State Milton S. Hershey Medical Center; Helen Morrison, PhD, Leibniz Research Institute for Aging</i>	Lone Star E
2:35 PM	2:55 PM	Factors Secreted by SMARCB1 Mutant Schwann Cells Contribute to Schwannomatosis Pain <i>Lawrence Sherman, PhD, Oregon Health & Science University</i>	
2:55 PM	3:15 PM	Merlin Controls the Proliferation and Repair Capacity of Schwann Cells Following Injury By Regulating Hippo/YAP Activity <i>David Parkinson, PhD, Plymouth University, UK</i>	
3:15 PM	3:35 PM	Platform: Angiomotin Phosphorylation Regulates Scaffolding Function and Localization of YAP at the Plasma Membrane <i>Susana Moleirinho, PhD (Young Investigator Awardee), Scripps Institute</i>	
3:35 PM	3:55 PM	Platform: Global Proteomic Analysis of the Merlin Interactome by Proximity Biotinylation <i>Robert Hennigan, PhD, Cincinnati Children's Hospital</i>	
3:55 PM	4:15 PM	Coffee Break	Lone Star South Foyer
4:15 PM	5:15 PM	KEYNOTE 4: Using Pluripotent Stem Cells to Understand Human Neural Crest Development and Disease <i>Stephen Dalton, PhD, Professor and GRA Eminent Scholar of Molecular Cell Biology, University of Georgia</i>	Lone Star E
5:15 PM	6:45 PM	Poster Session - Clinical Science <i>Wine, beer and cheese served.</i>	Lone Star F-H

DINNER ON YOUR OWN

Tuesday · June 21, 2016

7:00 AM	8:00 AM	Breakfast	Lone Star South Foyer
8:00 AM	9:00 AM	KEYNOTE 5: New Ways of Targeting RAS <i>Frank McCormick, PhD, UCSF Helen Diller Family Comprehensive Cancer Center and the Frederick National Lab for Cancer Research</i>	Lone Star E
9:00 AM	10:00 AM	Top Poster Oral Presentations	Lone Star E
10:00 AM	10:20 AM	Coffee Break	Lone Star South Foyer
10:20 AM	11:40 AM	SESSION 8: New Preclinical Models in NF <i>Chairs: Wade Clapp, MD, Indiana University; Thomas deRaedt, PhD, Harvard University/Brigham and Women's Hospital</i>	Lone Star E
10:20 AM	10:40 AM	The Use of New Mouse Models for Preclinical Development <i>Thomas deRaedt, PhD, Harvard University/Brigham and Women's Hospital</i>	
10:40 AM	11:00 AM	Mouse Model of Human Schwannomatosis <i>Marco Giovannini, MD, PhD, UCLA</i>	
11:00 AM	11:20 AM	Molecular Mechanisms of Autism Spectrum Disorder Phenotype in NF1 <i>Anantha Shekhar, MD, PhD, Indiana University</i>	

AGENDA

Tuesday · June 21, 2016

11:20 AM	11:40 AM	Platform: Modeling the Tumor Microenvironment in MPNSTs: The Impact on Tumor Biology and Chemotherapeutic Response Rebecca Dodd, PhD, <i>Duke University Medical Center</i>	
11:40 AM	12:30 PM	CONSORTIA UPDATES 2 Session Co-Chairs: Brigitte Widemann, MD, <i>NCI</i> ; Brian Weiss, MD, <i>Cincinnati Children's Hospital</i>	Lone Star E
11:40 AM	11:55 AM	NF Therapeutic Consortium (NFTC) Ophelia Maertens, PhD, <i>Brigham and Women's Hospital/Harvard University</i>	
11:55 AM	12:10 PM	NF Clinical Trials Consortium (NFCTC) Roger Packer, MD, <i>Children's National Medical Center</i>	
12:10 PM	12:25 PM	Response Evaluation in Neurofibromatosis and Schwannomatosis (REINS) Scott Plotkin, MD, PhD, <i>Massachusetts General Hospital/Harvard University</i>	
12:25 PM	12:40 PM	Specialized Programs of Research Excellence (SPORE) D. Wade Clapp, MD, <i>Indiana University</i>	
12:40 PM	12:55 PM	Specimens and Standards: Banking on Gold for Biomarker Development in Neurofibromatosis Carolyn Compton, MD, PhD, <i>Arizona State University</i>	
12:55 PM	1:10 PM	2016 NF CONFERENCE WRAP-UP NF Conference Co-Chairs, Michael Fisher, MD and Eduard Serra, PhD Annette Bakker, PhD, President and CSO, Children's Tumor Foundation	Lone Star E



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

Dr. Michael Fisher is an Associate Professor of Pediatrics in the Division of Oncology at The Children's Hospital of Philadelphia (CHOP) and the Director of the NF Program at CHOP. His research focuses on identifying new treatments and novel biomarkers (particularly using new imaging modalities) and exploring functional outcomes for children with tumors associated with NF1. Dr. Fisher is Deputy Chair of the Steering Committee, Chair of the Neurofibroma Committee, and PI of the CHOP-Penn site of the Department of Defense NF Clinical Trials Consortium. He is Chair of the Visual Outcomes Committee and member of the Steering Committee for REiNS (Response Evaluation in Neurofibromatosis and Schwannomatosis), an international effort to develop standardized outcome measures for clinical trials.



Eduard Serra, PhD, *Institute of Predictive and Personalized Medicine of Cancer (IPPMC)*

Dr. Eduard Serra is a researcher at the Institute of Predictive and Personalized Medicine of Cancer (IMPPC), at the Can Ruti Biomedical Campus, Badalona (Barcelona). He is the scientific coordinator at IMPPC of the Genetic Diagnostics of Neurofibromatosis and RASopathies, performed together with the Catalan Institute of Oncology. In addition he is the PI of the Genetic Variation and Cancer Lab. His research focuses on the genomics and integrative biology analysis of NF-associated tumors; on the generation and use of patient-derived induced pluripotent stem cells (iPSC) to model Neurofibromatosis; on the development and implementation of genomic techniques for the genetic diagnostics of NFs and related syndromes; and on the study of the molecular bases of NF pathogenesis. Dr. Serra is member of the Center of Reference on Neurofibromatosis in

Spain and a close collaborator of the different local NF Associations.

2016 NF Conference Keynote Speakers



Stephen H. Friend, MD, PhD, *President, Co-Founder and Director of Sage Bionetworks*

Dr. Friend is the President of Sage Bionetworks, a non-profit organization that provides the tools and environment to conduct dynamic, large-scale collaborative biomedical research. He is an authority in the field of cancer biology and a pioneer in the field of the genetics of gene expression, integrating system biology approaches to complex diseases. Dr. Friend believes that successful biomedical research requires the active participation from all stakeholders. He is reimagining the role of citizens in the research process and is building tools to empower them to contribute both their data and expertise as they see fit. He also believes in the importance of iteratively generating and testing novel hypotheses transparently and collaboratively.

Under his leadership, Sage Bionetworks has developed an open-source technology platform, called Synapse, for data-intensive analysis, sharing and reuse, enabling researchers to perform cutting edge computational biology and research. Dr. Friend is engaging the community to crowd-source solutions to complex biomedical questions through targeted DREAM challenges.

Previously Dr. Friend was Senior Vice President and Franchise Head for Oncology Research at Merck & Co., Inc. where he led Merck's Basic Cancer Research efforts. Formerly Dr. Friend along with Dr. Hartwell founded and co-led the Fred Hutchinson Cancer Research Center's "Seattle Project", an advanced institute for drug discovery and later they co-founded Rosetta Inpharmatics with Dr. Leroy Hood. Dr. Friend also held faculty positions at Harvard Medical School from 1987 to 1995 and at Massachusetts General Hospital from 1990 to 1995. He received his M.D/Ph.D. from Indiana University. Dr. Friend was named an Ashoka Fellow for his work at Sage Bionetworks.



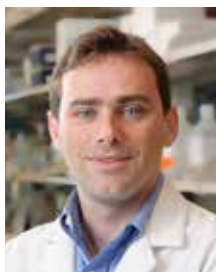
Crystal L. Mackall, *Associate Director of the Stanford Cancer Institute, Professor of Pediatrics and Medicine, Stanford University*

From 1998-2016, Dr. Mackall served as Head of the Immunology Section and subsequently as Chief of the Pediatric Oncology Branch, NCI she built an internationally recognized translational research program spanning basic studies of T cell homeostasis and tumor immunology, and clinical trials of immune based therapies for cancer. Her work is credited with identifying the essential role of the thymus in human T cell regeneration and discovering IL-7 as the master regulator of T cell homeostasis. She has led numerous cutting edge and first-in-human and first-in-child clinical trials spanning dendritic cell vaccines, cytokines, and adoptive immunotherapy using NK cells and genetically modified T cells.

Her clinical trials are distinguished by incorporation of robust biologic endpoints that further our understanding of the biological effects of the agents under study and the basis for success or failure. Her group was one of the first to demonstrate impressive activity of CD19-CAR in pediatric leukemia (Lee et al, Lancet 2015), the first to identify T cell exhaustion as a fundamental barrier to effectiveness for chimeric antigen receptor based therapies, and the first to identify a relationship between T cell costimulation of susceptibility to exhaustion (Long et al, Nat Med 2015).

Since January 2016, as Professor of Pediatrics and Medicine at Stanford University she leads the Cancer Immunology and Immunotherapy Program and serves as Associate Director of the Stanford Cancer Institute. She also serves in numerous national leadership roles, including Co-Leader of the StandUp2Cancer/St.Baldrick's Pediatric Cancer Dream Team, Site Director for the Parker Institute for Cancer Immunotherapy, and Chair-Elect of the Pediatric Cancer Working Group for the American Association for Cancer Research.

2016 NF Conference Keynote Speakers



David Solit, MD, *Geoffrey Beene Chair in Cancer Research, Director, Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center*

David Solit, MD is the Elizabeth and Felix Rohatyn Chair at Memorial Sloan-Kettering Cancer Center. He is a practicing Medical Oncologist and laboratory investigator with a joint appointment in the Department of Medicine and the Human Oncology and Pathogenesis Program. The goal of his research is the development of cancer therapies that target pathways responsible for tumor initiation and progression. He is particularly interested in the study of cancers in which the growth of the tumor depends upon alterations in kinase and steroid receptor signaling. The underlying hypothesis is that the consequences of inhibiting an oncogenic pathway will vary as a function of cell lineage and the complement of mutations

within the tumor. Therefore, in order to design rational therapeutic studies, one must understand not only which genetic changes are commonly found within particular tumor types but the mechanisms whereby these genetic alterations support tumor growth, survival, metastasis or other hallmarks of the cancer phenotype. Dr. Solit's recent laboratory work has focused on the identification of mutational events that co-occur with and cooperate with mutant BRAF in melanomagenesis and abrogate BRAF-addiction and thus response to selective RAF inhibitors. His research group has also been active in the development of novel methods to genetically profile formalin fixed, paraffin embedded tissues for somatic mutations and copy number alterations.



Stephen Dalton, PhD, *University of Georgia*

Stephen Dalton has been Professor and GRA Chair in Molecular Cell Biology in the Department of Biochemistry and Molecular Biology at the University of Georgia since 2003. In 2012 he was appointed founding Director of the Center for Molecular Medicine, a translational research center dedicated to the development of cures for human disease. Dr. Dalton is a Georgia Cancer Coalition Distinguished Scholar and has a research focus on stem cell biology and regenerative medicine.



Frank McCormick, PhD, *UCSF Helen Diller Family Comprehensive Cancer Center*

Frank McCormick, PhD, FRS, is Professor Emeritus of the UCSF Helen Diller Family Comprehensive Cancer Center. Prior to joining the UCSF faculty, Dr. McCormick pursued cancer-related work with several Bay Area biotechnology firms and held positions with Cetus Corporation (Director of Molecular Biology, 1981-1990; Vice President of Research, 1990-1991) and Chiron Corporation, where he was Vice President of Research from 1991 to 1992. In 1992 he founded Onyx Pharmaceuticals, a company dedicated to developing new cancer therapies, and served as its Chief Scientific Officer until 1996. At Onyx Pharmaceuticals, he initiated and led drug discovery efforts that led to the approval of Sorafenib in 2005 for treatment of renal cell cancer, and for liver cancer in 2007, and the approval of ONYX-015 in 2006 in China for treatment of nasopharyngeal

cancer. Sorafenib is being tested in multiple indications worldwide. In addition, Dr. McCormick's group led to the identification of the CDK4 kinase inhibitor, palbociclib, approved for treating advanced breast cancer. Dr. McCormick's current research interests center on the fundamental differences between normal and cancer cells that can allow the discovery of novel therapeutic strategies.

Dr. McCormick holds the David A. Wood Chair of Tumor Biology and Cancer Research at UCSF. Dr. McCormick is the author of over 285 scientific publications and holds 20 issued patents. He also served as President, 2012-2013 for the American Association for Cancer Research (AACR). More recently, he has taken a leadership role at the Frederick National Lab for Cancer Research, overseeing an NCI supported national effort to develop therapies against Ras-driven cancers. These cancers include most pancreatic cancers, and many colorectal and lung cancers, and are amongst the most difficult cancers to treat.

SESSION 1: Next Generation Genetic Diagnostics, Gene Discovery and Genotype-Phenotype Correlations for the NF's

Chairs: Conxi Lazaro, PhD, *Catalan Institute of Oncology, Barcelona, Spain*; Eric Pasmant, PhD, *Universite Paris Descartes, France*

Perspectives: Changing the Landscape of NF Genetics: The Next Step

Session 1: Saturday, June 18, 2:05pm – 2:45pm

Ludwine Messiaen, PhD, University of Alabama at Birmingham

In this session, I will review how the development of specialized techniques and approaches for genetic testing has enhanced the understanding of the genetics and genomics of the neurofibromatoses (NF1, NF2 and schwannomatosis), providing insights in the mechanisms of splicing, revealing deep intronic regions involved in exonisation if mutated, resulting in the definition of the mutational spectrum and mechanisms leading to the intragenic copy number changes / microdeletions, recognizing the importance of mosaicism and cells of origins of disease-associated lesions and its implications for genetic testing and counseling. The use of a standardized phenotypic information form has facilitated the detection of specific *distinct* genotype-phenotype correlations, and recently spurred a renewed interest in this field. Along the way, the existence of (limited yet very relevant) genetic heterogeneity in NF1, formerly believed to be a fully penetrant genetically homogeneous disorder, became clear. Besides *SPRED1*, more recently, patients initially diagnosed clinically with NF1 have been shown to carry a *PTPN11* mutation, further demonstrating the clinical overlap in the rasopathies. Similar strides and successes were obtained for patients affected by schwannomatosis.

I will discuss some areas that may need further collaborative efforts. I also will provide pros and cons on moving forward with genetic testing using new “next” generation based approaches.

NGS for Diagnosis of NF1 and NF2: An Update

Session 1: Saturday, June 18, 2:45pm – 3:05pm

Beatrice Parfait, PhD, Hopitaux Universitaires Paris Central - Hopital Cochin, France

NF1 gene mutations can be found in most of neurofibromatosis type 1 cases. However, mutations in the *SPRED1* gene were identified in patients fulfilling the US NIH criteria for NF1, underlining a genetic heterogeneity for NF1 phenotype. Legius syndrome (caused by *SPRED1* mutations) resembles a mild NF1 phenotype. Molecular diagnosis is essential because distinguishing between NF1 and Legius syndrome is important for prognosis and clinical management. Moreover, molecular diagnosis in NF1 is helpful to confirm the clinical diagnosis, notably in patients with paucisymptomatic, pediatric, or segmental presentations. Similarly, clinical overlaps observed between neurofibromatosis type 2 and other tumor predisposition syndromes such as schwannomatosis and meningiomatosis can make clinical diagnosis difficult. Molecular identification is now a support in case of uncertain clinical diagnosis. In NF1 and NF2, mutation identification in both familial and sporadic cases is also a challenge for genetic counseling. Because early diagnosis improves clinical care, it is important to provide a presymptomatic genetic testing for the offsprings and relatives of NF index cases. Moreover, prenatal and pre-implantation diagnoses can be proposed if the constitutional *NF1* or *NF2* alteration is identified in index cases. In order to perform molecular diagnosis of NF1, NF2 and overlapping syndromes, we have developed two designs for molecular study of *NF1-SPRED1* and *NF2-SMARCB1-LZTR1-SMARCE1-SUFU* genes with a targeted next-generation sequencing (NGS) using the Ion Torrent technology (Life Technologies). This accurate and fast targeted NGS approach also provides quantitative information based on sequencing depth, allowing identification of single and multiple exons deletion or duplication. The strategy can be applied to tumor analysis. In our experience, targeted NGS also allows the detection of mutant alleles present at a low frequency in leukocytes of *NF1* and *NF2* mosaic patients.

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Platform: DNA Damage Repair and NF1 Disease Severity

Session 1: Saturday, June 18, 3:05pm – 3:25pm

Kristine Vogel, PhD, UTHSCSA

Mutations in the *NF1* tumor suppressor gene, responsible for the autosomal dominant disorder neurofibromatosis type 1, are diverse in nature, and exhibit little or no evidence of being limited to hotspots. For almost all NF1 disease manifestations examined, there is considerable clinical variability, even among individuals with the same germline mutation. This variable expressivity has led to searches for environmental and genetic modifiers of NF1 disease severity, and in particular for those factors that might influence somatic mutation rates, and thus the frequency of second hits that can influence tumorigenesis. Characterizations of *NF1* loss-of-heterozygosity and somatic mutation spectra in cutaneous neurofibromas confirmed tumor suppressor behavior for this gene, and raised the possibility that genetic differences in DNA damage repair capacity might act as modifiers of disease severity.

Our central hypothesis is that genes responsible for DNA damage sensitivity and repair capacity act as modifiers that influence somatic mutation rates, and thus affect the frequency and number of cutaneous neurofibromas, café-au-lait spots, malignant neoplasms, and other clinical features of NF1 that are dependent on “second hits.” Four adults with NF1 (age range 40-69 years) were enrolled in our pilot study. Following informed consent, 4-6 cutaneous neurofibromas were removed from each subject, and S100+ Schwann cell cultures were established from these samples, according to UTHSCSA IRB-approved protocols. To characterize DNA damage sensitivity, Schwann cells were exposed to doxorubicin, and DNA damage foci were quantitated using p53 binding protein 1 (53BP1) immunocytochemistry. To measure DNA repair efficiency, Schwann cells were treated with doxorubicin, and repair was quantitated using the comet assay, at time points from 15 to 150 minutes. Retrospective chart review, including both quantitative and binary manifestations, was performed to assess NF1 disease severity. We found significant differences in Schwann cell sensitivity to doxorubicin-induced DNA damage, between all 4 subjects. Moreover, our comet assay data indicate a slow rate of DNA repair in Schwann cells from all 4 subjects, as well as a correlation between DNA repair efficiency and neurofibroma burden, or possibly subject age. Our results are consistent with DNA damage repair characteristics acting as modifiers of NF1 disease severity, and as potential contributors to increasing neurofibroma burden with age in individuals with NF1.

Author List: Kristine Vogel, PhD; Vineet Mishra, MD; Diane Solomon, MD; Alyssa Kosturakis, MS2; Jordan Buckley, MS2 UTHSCSA School of Medicine (all)
Funding: Texas Neurofibromatosis Foundation

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Platform: Novel Methods for Genotype-Phenotype Correlation in Schwannomatosis

Session 1: Saturday, June 18, 3:25pm – 3:45pm

Justin T. Jordan, MD, Massachusetts General Hospital

Purpose: Schwannomatosis has been linked to mutations in both the *SMARCB1* and *LZTR1* genes, but many cases have no known genetic association. Here, we used a novel gene capturing technique with next-generation sequencing (NGS) and whole body MRI (WBMRI) to search for genotype-phenotype correlation within a cohort of patients with schwannomatosis.

Methods: WBMRI and three-dimensional computerized volumetry were used to determine the number and volume of internal nerve sheath tumors in patients with schwannomatosis. Custom biotinylated RNA baits were used to capture the entire coding regions of *SMARCB1*, *LZTR1*, and *NF2* genes from patient blood samples, and next generation sequencing was performed. Genotype-phenotype correlation was evaluated using Chi Square, Wilcoxon, and Spearman testing in SAS 9.4.

Results: Thirty-seven patients with clinically diagnosed schwannomatosis were included in the study. Gene capture was excellent (approximately 70% efficient), and NGS median coverage was 878-fold. An *LZTR1* mutation was identified in 8 patients (median age 39.5, 3 females, 0 familial), *SMARCB1* mutation in 17 patients (median age 42.5, 11 females, 6 familial), and no mutation in 12 patients (median age 40, 5 females, 1 familial). No pathogenic NF2 mutations were identified in the cohort. Peripheral schwannomas were identified on WBMRI in 6 *LZTR1* patients (75%) and 11 *SMARCB1* patients (65%). Among those with peripheral tumors, median tumor number was 4 in the *LZTR1* group (median total body tumor volume 47.2cc) and 10 in the *SMARCB1* group (median volume 61.8cc), ($p=0.3269$ for tumor number and $p=0.5881$ for volume). None had vestibular schwannomas or meningiomas. Median pain score was 4.1 in the *LZTR1* group and 0.5 in the *SMARCB1* group ($p=0.0205$). Correlation coefficient between total body tumor volume and pain score was 0.348 for all patients ($p=0.0349$).

Conclusions: We used a highly efficient method for deep, targeted germline sequencing in patients with schwannomatosis. While numbers were small, pain was significantly higher in *LZTR1*-mutant patients than *SMARCB1*-mutant patients, which should be considered for development of disease models and targeted therapies. The anatomic limitations of WBMRI may be addressed in future studies by the addition of dedicated brain and spine imaging. Larger studies of schwannomatosis patients are needed to refine these preliminary genotype-phenotype correlations.

Author List: Vanessa Merker, BS: MGH; Wenli Cai, PhD: MGH; Gordon Harris, PhD: MGH; Miriam Bredella, MD: MGH; James Gusella, PhD: MGH; Scott Plotkin, MD, PhD: MGH

Funding: Philanthropy

All from the Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester Academic Health Sciences Centre (MAHSC), University of Manchester, Manchester, UK

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SPRED1, the Gene Responsible for Legius Syndrome, Suppresses Ras Activation by Interacting with the GAP-Related Domain of Neurofibromin

Session 1: Saturday, June 18, 3:45pm – 4:05pm

Akihiro Yoshimura, PhD, Keio University, Japan

SPRED (Sprouty-related protein with an EVH1 domain) family proteins, initially discovered as c-kit- and c-fms-binding proteins, have been shown to suppress the Ras-ERK pathway. SPREDs form a subfamily of the Sprouty/Spred family, which is characterized by the Sprouty-related C-terminal cysteine-rich (SPR) domain. SPREDs also contain N-terminal EVH1 domain and central c-kit binding domain (KBD). Constitutional heterozygous loss-of-function mutations in the *SPRED1* gene cause a phenotype known as Legius syndrome, which consists of symptoms of multiple café-au-lait macules, axillary freckling, learning disabilities and macrocephaly. Legius syndrome resembles a mild neurofibromatosis type 1 (NF1) phenotype. It has been demonstrated that SPRED1 functions as a negative regulator of the RAS-ERK pathway and interacts with neurofibromin, the *NF1* gene product. However, the molecular details of this interaction and the effects of the mutations identified in Legius syndrome and NF1 on this interaction have not yet been investigated. In this study, using a yeast two-hybrid system and an immunoprecipitation assay in HEK293 cells, we found that the SPRED1 EVH1 domain interacts with the N-terminal 16 amino acids (aa) and the C-terminal 20 aa of the GTPase-Activating Protein (GAP)-related domain (GRD) of neurofibromin, which form two crossing α -helix coils outside the GAP domain. These regions have been shown to be dispensable for GAP activity and are not present in p120GAP. Several mutations in these N- and C-terminal regions of the GRD in NF1 patients and pathogenic missense mutations in the EVH1 domain of *SPRED1* in Legius syndrome reduced the binding affinity between the EVH1 domain and the GRD. EVH1 domain mutations with reduced binding to the GRD also disrupted the ERK suppression activity of SPRED1. These data clearly demonstrate that SPRED1 inhibits the Ras-ERK pathway by recruiting neurofibromin to Ras through the EVH1-GRD interaction, and this study also provides molecular basis for the pathogenic mutations of NF1 and Legius syndrome. KDB and KBD of Spreds have been shown to be necessary for anchoring the protein to the membrane raft microdomain and binding to c-kit tyrosine kinase domain, respectively. We also characterized several mutations in these domains in the *SPRED1* gene.

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SESSION 2: Plexiform Neurofibroma to Malignant Peripheral Nerve Sheath Tumor

Chairs: Lu Le, MD, PhD, *UT Southwestern*; Aerang Kim, MD, PhD, *Children's National Medical Center*

Perspectives: Strategies to Prevent the Development of MPNST in NF1: Identification and Management

Session 2: Sunday, June 19, 8:05am – 8:45am

Brigitte Widemann, MD, National Cancer Institute

A MPNST consensus manuscript in 2002 reviewed the current knowledge, provided guidance for the diagnosis and management of MPNST, and identified research priorities.

Since 2002 the outcome of MPNST remains unchanged and extremely poor unless complete surgical resection can be achieved. However, substantial progress has been made in several areas. This includes an improved understanding of the natural history and pathogenesis of plexiform neurofibromas, atypical neurofibromas, and MPNST. This knowledge combined with the availability of relevant preclinical models and concerted clinical trials efforts can be utilized to accelerate the development of MPNST prevention and treatment strategies.

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Transposon-Based Forward Genetics and Targeted Nucleases to Define Factors Causing Progression from Neurofibroma to Malignant Peripheral Nerve Sheath Tumor Development

Session 2: Sunday, June 19, 8:45am – 9:05am

David Largaespada, PhD, University of Minnesota

Objective: To define cancer driver and maintenance genes associated with plexiform neurofibroma development and progression to malignant peripheral nerve sheath tumors (MPNST) using *Sleeping Beauty* (SB) transposon-based forward primary genetic screens in mice and CRISPR/Cas9-based secondary genetic screens in human Schwann cells.

Background: Plexiform neurofibromas are likely to have evolved from an *NF1*-deficient progenitor via secondary epigenetic or genetic changes and ill-defined environmental effects. In turn, MPNST are thought to develop by the step-wise acquisition of secondary changes including gene copy number changes, epigenetic changes, and recurrent single nucleotide mutations in specific genes (e.g. *SUZ12*). These targets may be useful for the treatment of MPNST associated with NF1 syndrome. We have carried out *Sleeping Beauty* (SB) transposon-based screens for neurofibroma and MPNST genes (Rahrmann et al., *Nature Genetics*, 2013; Wu et al., *Cell Reports*, 2016). Our studies, and ongoing human genomic studies, have led to the identification of candidate genetic changes that cooperate with loss of *NF1* in neurofibroma initiation and progression to MPNST. These studies demand reliable methods for functional validation of the genes and pathways using human Schwann cells.

Methods: We've adapted targeted nucleases and a *TERT* plus activated *CDK4* immortalized human Schwann cell precursor cell line (called HSC1λ) for functional validation studies (Rahrmann et al., *Nature Genetics*, 2013). We have implemented the CRISPR/Cas9 nuclease system to perform a medium throughput screen of the candidate tumor suppressor genes (TSGs) that we identified in the SB transposon screen. In addition, we have created wild type, *NF1* heterozygous, and biallelic *NF1* mutant clones derived from HSC1λ cells for drug/small molecule library screens, genetic synthetic lethal screens, and functional studies on specific genes/pathways.

Results: We have functionally validated cooperation between *Crebbp* or *Pten* loss and *Nf1* mutation in mice. Using human cells, we've targeted 31 candidate TSGs and showed that mutation of 18 candidates resulted in an increase in HSC1λ anchorage independent growth. These 18 candidates were tested for their ability to induce *in vivo* tumor formation as xenografts, when targeted. Three genes, when targeted, induced xenograft tumors, including *NF1*, *GOSR1*, and *PTCH1*. Additional studies on *GOSR1* and *PTCH1* tumor suppression in MPNST are underway. HSC1λ with *NF1* mutation show biochemical defects and transformed phenotypes. The use of these mutants to study neurofibroma to MPNST progression will be discussed.

Conclusions: These results show that CRISPR/Cas9 system can be used in immortalized human Schwann cells to model neurofibroma to MPNST progression and to validate novel TSGs that may be involved in MPNST development or progression. *NF1* gene mutations are centrally involved in MPNST development, while *GOSR1* encodes a Golgi SNARE complex member and is an entirely novel TSG candidate. *PTCH1* loss activates sonic hedgehog signaling and these results suggest that SHH pathway alterations may contribute to MPNST development or progression. Both *GOSR1* and *PTCH1* are mutated in at least some human MPNSTs. Current work is aimed at identifying alterations that are synthetic lethal with *NF1* loss, cooperate with *NF1* loss, or can cause metastasis.

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PRC2 Loss in Malignant Transformation of MPNST

Session 2: Sunday, June 19, 9:05am – 9:25am

Ping Chi, MD, PhD, Memorial Sloan-Kettering Cancer Center

Malignant peripheral nerve sheath tumors (MPNSTs) represent a group of highly aggressive soft-tissue sarcomas that may occur sporadically, in association with neurofibromatosis type I (NF1 associated) or after radiotherapy. Using comprehensive genomic approaches, we identified loss-of-function somatic alterations of the Polycomb repressive complex 2 (PRC2) components (*EED* or *SUZ12*) in 92% of sporadic, 70% of NF1-associated and 90% of radiotherapy-associated MPNSTs. In contrast, PRC2 was intact in all benign neurofibromas, indicating that PRC2 loss is likely involved in malignant transformation from benign neurofibroma to MPNST. MPNSTs with PRC2 loss showed complete loss of trimethylation at lysine 27 of histone H3 (H3K27me3) and aberrant transcriptional activation of multiple PRC2-repressed homeobox master regulators and their regulated developmental pathways. Introduction of the lost PRC2 component in a PRC2-deficient MPNST cell line restored H3K27me3 levels and decreased cell growth. Additionally, there are multiple signaling pathways were perturbed with PRC2 loss. These data indicate that the plethora context dependent effects caused by PRC2 loss collectively contribute to MPNST pathogenesis.

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Platform: Comparative Genomic Analysis of NF1-Associated Atypical Neurofibromas (ANF) and Malignant Peripheral Nerve Sheath Tumors (MPNST)

Session 2: Sunday, June 19, 9:25am – 9:45am

Alexander Pemov, PhD, National Cancer Institute, National Institutes of Health

Background: NF1-associated atypical ANF are tumors characterized by atypical pathological features that often arise within plexiform neurofibromas (PNF) in NF1 and can transform into MPNST, thus representing an intermediate step in the malignant transformation. We have recently shown that *NF1* second copy-inactivation is the only recurrent genetic event in PNF. Currently, it is not clear what causes PNF transformation into ANF and further into MPNST, however several studies identified deletion of the *CDKN2A/2B* locus as the most frequent genetic event distinctive from PNF in ANF. Sequencing studies of MPNST have identified that in addition to *NF1* and *CDKN2A/2B* inactivation, mutations in PRC2 complex genes occur with high frequency. In this study, we performed genomic analysis of 16 ANF and 4 MPNST (Whole Exome Sequencing (WES) data for 3 tumor/normal pairs) tumors matched with germline DNA obtained from 14 and 4 unrelated NF1 patients, respectively.

Methods: We performed WES and copy-number analyses by using Illumina Hi-Seq 2500 platform (96 Mb SeqCap EZ Exome + UTR Library, NimbleGen) and SNP-arrays (Illumina HumanOmniExpressExome-8, v1.2). We performed deep sequencing of *NF1* and validation of select mutations on IonTorrent platform.

Results: 1) ANF. We identified *NF1* germline mutations in 15/16 (94%) and somatic mutations in 13/16 (81%) samples, and heterozygous *CDKN2A/2B* locus deletion in 12/16 (75%) samples. Besides *NF1* and *CDKN2A/2B*, there were 13 somatic mutations in 9 tumors (median 1, range 0-4; 7 samples could not be validated due to lack of DNA). Out of 13 mutations, 5 were potentially deleterious. All non-*NF1* and non-*CDKN2A/2B* mutations were found only once. *NF1* and *CDKN2A/2B* were the only genes that were inactivated in multiple samples. We observed multiple copy-number changes in the tumors. **2) MPNST.** We identified *NF1* germline and somatic mutations in 4/4 (100%) samples, somatic *CDKN2A/2B* locus deletion in 4/4 (100%) samples (in 3/4 samples the deletion was homozygous) and inactivating mutations in *EED* (c.A721C, p.S241R, novel) and *SUZ12* (c.1236_1240del, p.T412fs, novel). We did not identify PRC2 mutations in the remaining MPNST, however we observed two deleterious mutations in a histone H4-specific acetyltransferase gene in this tumor. Outside of the *NF1* and *CDKN2A/2B* loci, there were 69 somatic mutations in 3 tumors (23 mean, 18-31 range). Of these mutations, 54 were potentially deleterious. Non-*NF1*, non-*CDKN2A/2B* and non-PRC2 mutations were found only once. *NF1*, *CDKN2A/2B* and PRC2 complex genes were the only ones that were inactivated in multiple samples. We observed highly re-arranged chromosomal architecture in all tumors.

Conclusions: To our knowledge, this is the first WES study of ANF. It appears that on the genetic level the PNF-to-ANF transition is predominantly driven by heterozygous deletion of the *CDKN2A/2B* locus. We did not identify any PRC2 mutations in ANF. The somatic mutation burden in the ANF is relatively low and comparable to that in PNF, however the level of genomic instability is elevated in the ANF. We identified two novel somatic mutations in PRC2 genes in two MPNST and a potential novel driver gene in the third MPNST that lacked mutations in PRC2, but harbored two deleterious mutations in a histone H4-specific acetyltransferase gene.

Author List: Nancy F. Hansen PhD, NHGRI; James C. Mullikin PhD, NHGRI; Joseph F. Boland PhD, NCI; Javed Khan MD, NCI; Margaret Wallace PhD, University of Florida; Eric Legius MD, University of Leuven; Brigitte Widemann MD, NCI; Douglas R. Stewart MD, NCI

Funding: The Intramural Research Program of NCI and NHGRI at the NIH

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Platform: Atypical Neurofibromas in NF1: Clinical, Imaging and Pathology Characteristics

Session 2: Sunday, June 19, 9:45am – 10:05am

Christine Higham, MD, *National Cancer Institute, National Institutes of Health*

BACKGROUND: Neurofibromatosis 1 (NF1) is a tumor predisposition syndrome leading to the development of benign and malignant peripheral nerve sheath tumors (MPNST). MPNST frequently develop in pre-existing benign plexiform neurofibromas (PN) and have poor prognosis. Atypical neurofibromas (ANF) were recently described as precursor lesions for MPNST, making early detection and management of ANF a possible strategy to prevent MPNST. However, little is known about the presentation and natural history of ANF. Our aim was to characterize ANF and identify approaches for management of these lesions.

METHODS: Patients with NF1 are followed at the NCI, KU Leuven, and Guy's Hospital with clinical evaluations, MRI and fluorodeoxy-glucose PET (FDG-PET) if malignant transformation of a lesion is suspected. Biopsies or resections are performed for concerning lesions and diagnosis of ANF is made based on pathology review. We retrospectively analyzed the clinical presentation, diagnostic evaluation, management, and pathology of all patients with ANF using standardized data collection.

RESULTS: 73 ANF (2 head, 11 neck, 13 chest, 19 abdomen/pelvis, 28 extremity) were diagnosed in 63 patients (32M/31F; median age 27.2 years, range 7-60 years). On MRI most ANF appeared as distinct nodular lesions (i.e. well demarcated lesions >3cm lacking the central dot sign) and were FDG-avid with median SUV_{max} of 5.6 (range 0-22.3). 43 ANF were associated with pain, 18 with weakness, 43 were palpable or visible, and 13 had no clinical signs. Median time from detection to pathologic confirmation was 1.0 year (0-15 years). With an average follow-up time of 4.0 years (0-14.9 years), completely resected ANF (N=54) have not recurred, but 2 partially resected ANF demonstrated re-growth. 19 patients had additional lesions (median 1, range 1-10) suspicious for ANF that have not been biopsied. 4 ANF transformed into high grade MPNST. 10 patients had a MPNST in a different location prior to ANF diagnosis, and 6 developed a MPNST in a different location after ANF diagnosis.

CONCLUSIONS: Growth of nodular lesions, pain, and PET avidity, should raise concern for ANF/MPNST. Patients with ANF are at greater risk for development of MPNST. Complete resection of ANF may prevent transformation to MPNST. Management of patients with multiple ANF or lesions in deep locations is challenging. Longitudinal monitoring will allow for more complete characterization of ANF and development of more sensitive diagnostic techniques and effective therapies or prevention of malignant transformation.

Author List: Christine Higham MD, NCI; Eric Legius MD, KU Leuven; Nicole Ullrich MD PhD, Boston Children's Hospital; Sucharita Bhaumik MD, NCI; Aljosja Rogers MD, KU Leuven; Eva Dombi MD, NCI; Steve Connor MD, Guy's and St. Thomas' NHS Foundation Trust; Markku Miettinen MD, NCI; Raf Sciort MD, PhD, KU Leuven; Roberto Tirabosco MD, Royal National Orthopaedic Hospital NHS Foundation Trust; Andrea Baldwin PNP, NCI; Brigitte Widemann MD, NCI; Rosalie Ferner MD, Guy's and St. Thomas' NHS Foundation Trust

SESSION 3: Select Platform Presentations

Chair: Nancy Ratner, PhD, *Cincinnati Children's Hospital*

Platform: Tumor Volume Predicts Axonal Loss in Children with Optic Pathway Gliomas Secondary to Neurofibromatosis type 1

Session 3: Sunday, June 19, 11:30am – 11:45am

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

Background: Tumor location (i.e., optic tract involvement) has been proposed as a risk factor for vision loss in children with OPGs secondary to NF1. However, no studies have examined whether OPG size influences visual outcomes. MRI segmentation algorithms now permit volumetric measurement of the entire anterior visual pathway (AVP; optic nerve, chiasm and tract). We hypothesized that children with a larger AVP are more likely to demonstrate decreased retinal nerve fiber layer (RNFL) thickness, a measure of axonal loss and an established biomarker of visual impairment.

Methods: A convenience sample of children with NF1-OPGs involving the optic nerve with or without posterior involvement (i.e., chiasm or tracts) were included in this cross-sectional analysis. Subjects with both high resolution MRI sufficient for segmentation and optical coherence tomography (OCT) measurements of RNFL thickness were eligible. Linear regression models evaluated the relationship between RNFL thickness and total AVP volume while considering the influence of tumor location and total brain volume.

Results: Thirty-eight subjects contributed 55 study eyes. OPGs were either isolated to the optic nerve ($N = 26$), involved the optic nerve and chiasm ($N = 17$), or included the optic nerve, chiasm and tracts ($N = 12$). In unadjusted and adjusted regression models, RNFL thickness had a significant negative relationship to total AVP volume and total brain volume ($p < 0.05$, all comparisons). For every 1 ml increase in AVP volume, RNFL thickness declined by approximately 5 microns. A greater volume in subjects with OPGs of the optic nerve and chiasm, but not the tracts, was independently associated with a lower RNFL thickness ($p < 0.05$). All subjects with an optic chiasm volume > 1.3 ml demonstrated axonal damage (i.e., RNFL thickness < 80 microns).

Conclusions: Greater volume of the AVP predicts axonal loss, a biomarker of vision loss, in children with NF1-OPGs. MRI volumetric measures may help stratify the risk of visual loss from NF1-OPGs.

Author List: Robert Avery, DO, MSCE¹; Awais Monsoor, PhD²; Rabia Idrees, BS²; Carmelina Trimboli-Heidler, C.D.O.S¹; Roger J. Packer, MD²; Marius G. Linguraru DPhil², ¹Children's Hospital of Philadelphia, ²Children's National Health System.

Funding: NIH grant K23EY022673 and The Gilbert Family Neurofibromatosis Institute.

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Platform: The Neurofibromin Recruitment Factor Spred1 Directly Interacts with the Neurofibromin GAP Domain Without Interfering with Ras Inactivation

Session 3: Sunday, June 19, 11:45am – 12:00pm

Theresia Dunzendorfer-Matt, PhD, Innsbruck Medical University, Austria

Patients suffering from neurofibromatosis type 1 display a variety of clinical symptoms which partially overlap with the much milder Legius Syndrome. Both diseases are caused by mutations in proteins negatively regulating the Ras MAP kinase pathway, namely the Ras specific GTPase activating protein neurofibromin and Spred1, a member of the Sprouty protein family, respectively.

Recently, Spred1 has been demonstrated to interact with neurofibromin via its N terminal EVH1 domain and to mediate membrane translocation of its target dependent on its C-terminal Sprouty domain. However, the region of neurofibromin required for the interaction with Spred has remained unclear. We have mapped the Spred binding site to the central GAP related domain of the 320 kDa neurofibromin and have analyzed whether interaction of the Spred EVH1 domain with the neurofibromin GRD affects its properties to bind or inactivate Ras. Using a Biacore approach we have quantified the affinities between neurofibromin and Spred domains as well as activated Ras. Detailed analysis of recombinant protein domains will identify key residues for the complex formation. We have also studied the effect of patient derived nontruncating missense mutation on formation of the neurofibromin Spred complex and on membrane localization of neurofibromin in mammalian cells. Collectively, our studies not only reveal a new role for a subdomain of NF1 GAP, but also identifies functional effects of pathogenic mutations in neurofibromin and Spred.

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Funding: FWF - Austrian Science Fund and Children's Tumor Foundation

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Platform: Exploiting Proteotoxic and Oxidative Stress in MPNSTs to Drive Tumor Regression

Session 3: Sunday, June 19, 12:00pm – 12:15pm

Clare F. Malone, PhD (Young Investigator Awardee), Brigham and Women's Hospital, Harvard Medical School

NF1 patients have an 8-13% lifetime risk of developing a malignant peripheral nerve sheath tumor (MPNST). When MPNSTs cannot be completely removed surgically they are lethal, and current treatment options do not prolong overall survival. MPNSTs remain the leading cause of death in NF1 patients and new therapies are desperately needed. We have shown that elevated proteotoxic stress in MPNSTs represents a vulnerability that can be exploited therapeutically using the combination of ER stress-inducing HSP90 inhibitors and mTOR inhibitors. As a result, this combination is currently in clinical trials in MPNSTs. However, to date, there are no clinically approved HSP90 inhibitors. Therefore, we were interested in identifying a novel combination of FDA-approved agents that might be able to exploit these vulnerabilities and promote MPNST regression.

Here we show that the clinically approved histone deacetylase inhibitor vorinostat induces proteotoxic and oxidative stress in MPNST cells. Furthermore, we demonstrate that the combination of vorinostat and rapamycin, two FDA-approved agents, promotes MPNST cell death *in vitro* and tumor regression in a genetically engineered mouse model of MPNSTs.

Importantly, we find that the efficacy of the combination is dependent on activation of the unfolded protein response, the cellular response to ER stress, as well as induction of reactive oxygen species (ROS).

Interestingly, we identified *TXNIP*, an inhibitor of a key cellular ROS scavenger, thioredoxin, as one of the genes most significantly upregulated by combination treatment. Moreover, ablation of *TXNIP* partially protects MPNST cells from combination induced cell death. *TXNIP* can drive activation of apoptosis signaling kinase 1 (ASK1), a key mediator of stress-induced apoptosis. Importantly, knockdown of ASK1 completely protects MPNSTs from cell death after combined treatment, indicating that ASK1 is necessary for the therapeutic effects.

Together, these findings identify a novel combination of two FDA-approved agents that is effective in MPNSTs, for which there are currently no effective therapies. Additionally, this work reinforces the concept that proteotoxic and oxidative stress represent vulnerabilities in cancer cells that can be effectively exploited therapeutically.

This work was supported by a CTF Young Investigator Award to C.F. Malone

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Platform: *Clostridium novyi*-NT can Cause Tumor Regressions in Malignant Peripheral Nerve Sheath Tumors Across Species

Session 3: Sunday, June 19, 12:15pm – 12:30pm

Verena Staedtke, MD, PhD, Department of Neurology, John Hopkins University of Medicine

Background: Neurofibromatosis type 1 (NF1) is a RASopathy that represents a major risk for the development of malignancies, particularly malignant peripheral nerve sheath tumors (MPNST). To date, surgery is the only treatment modality proven to have survival benefit for MPNSTs and even when maximal surgery is feasible, these tumors are almost never curable. Like other aggressive cancers, MPNSTs develop large areas hypoxia, which limits the efficacy of traditional antineoplastic agents. Although this fact has made MPNST a highly-treatment resistant tumor, it also offers the perfect niche for the growth of the anaerobic “oncolytic” spore-forming bacterium *Clostridium novyi*-NT (*C. novyi*-NT).

Methods: Athymic mice were injected subcutaneously with human-derived NF1 mutated MPNST cells NF90.8. Once tumors reached between 400-800 mm³, 1.5×10^7 *C. novyi*-NT spores were injected into the tumors and the response was monitored by tumor volumes. Furthermore, we investigated the safety and efficacy of intratumoral injection of *C. novyi*-NT in a therapeutic study of 8 dogs with histologically confirmed spontaneous MPNSTs. Each dog received at least one cycle of *C. novyi*-NT spore treatment, defined as a single intratumoral injection of 1×10^8 *C. novyi*-NT spores into the target tumor and were followed for at least 90 days. These data provided a rationale for a phase 1 clinical trial in humans with treatment refractory cancer (NCT01924689).

Results: 87.5% of the xenograft mice carrying NF90.8 MPNSTs responded to *C. novyi*-NT therapy with a dramatic tumor reductions and 2 out of 16 (12.5%) mice had no measurable tumor or recurrence. In dogs with spontaneous MPNSTs, we found that intratumoral injection of *C. novyi*-NT spores was well tolerated, with the most common toxicities being expected symptoms associated with bacterial infections such as tumor inflammation (7 of 8; 87.5%), tumor abscess (3 of 8; 37.5%), tumor pain (2 of 8; 25%), and tumor discharge (3 of 8; 37.5%). Objective treatment responses were seen in 71.5% of dogs of which two dogs had complete responses (28.5%), two dogs had partial responses (28.5%), one dog had stable disease (14.25%), and two dogs had progressive disease (28.5%). On the basis of these encouraging results, 15 human patients with advanced cancers have enrolled into the trial with substantial tumor reductions in some cases.

Conclusions: *C. novyi*-NT can eradicate MPNSTs and other cancers in a safe and efficient way across different species. These results support the development of intratumoral injections of *C. novyi*-NT spores as a therapeutic for NF1 patients with MPNSTs.

Arthur List: Verena Staedtke^{1*}, Ren-Yuan Bai^{2*}, Nicholas J. Roberts³, Bert Vogelstein³, and Shubin Zhou³
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KEYNOTE 2: Immuno-Oncology: A New Paradigm for Cancer Therapy

Saturday, June 18, 1:45pm – 2:45pm

Crystal L. Mackall, MD, Associate Director of the Stanford Cancer Institute, Professor of Pediatrics and Medicine, Stanford University

For more than a century, scientists and clinicians have sought to harness the immune system as a therapeutic against cancer. The fine specificity of immune cells for molecular targets, combined with their capacity for rapid expansion, long-term memory and powerful but controlled killing provide a compelling arsenal for antitumor activity. Consistent, incremental scientific advances and steady acceleration in technology development during the last 50 years have provided a backdrop upon which dramatic acceleration in the efficacy of antitumor immunotherapy emerged beginning around 2010. Since that time, a continuing wave of clinical successes have led to a paradigm shift in cancer treatment, with immunotherapy emerging as a fourth pillar. The most significant impact has come from treatment with blocking monoclonal antibodies that “inhibit inhibitors” of T cell function, so-called checkpoint inhibitors. Checkpoint inhibitors induce durable anti-tumor responses in a wide array of histologies spanning hematologic malignancies, neural crest derived tumors and carcinomas. Current concepts hold that tumors with higher mutational burdens are most responsive to this class of agents, but many questions remain and clear predictors of response in individual patients remain elusive. An alternative approach uses synthetic biology to engineer receptors that drive T cell activation in response to non-mutated or mutated targets overexpressed on tumors compared to normal, vital tissues. Although the breadth of cancers tested with this approach has been more narrow, very high response rates have been observed for hematologic malignancies and work remains underway to extend these successes to solid tumors. In addition, dozens of other approaches, spanning bi-specific antibodies, oncolytic viruses and activators of NK cells and other innate immune cells to name a few, hold promise for single agent activity or for activity in combination regimens. The portfolio of immunotherapies for cancer is broad and deep, yet many indications, such as low-grade malignancies and premalignant conditions, such as occur in the setting of NF-1, have not yet been adequately studied. The next decade will no doubt reveal an increasing role for immuno-oncology across the spectrum of human malignancy.

CONSORTIA UPDATES 1

Chairs: Brigitte Widemann, MD, *National Cancer Institute, NIH*; Brian Weiss, MD, *Cincinnati Children's Hospital*

Progress Report – Synodos for NF1, Drug Discovery

Sunday, June 19, 3:00pm – 3:15pm

Christopher L. Moertel, MD, *University of Minnesota* and **David A. Largaespada**, PhD, *Recombinetics, Inc. and Department of Pediatrics, Masonic Cancer Center, University of Minnesota*

This report updates the NF community on the progress of the NF1 Synodos group that involves collaboration between the University of Minnesota, Cincinnati Children's Hospital Medical Center, Recombinetics Inc., and the National Cancer Institute. The overall goal of this project is to quickly and efficiently identify new drugs that specifically target NF1-/- Schwann cells, demonstrate efficacy of these novel drugs in several mouse models, and perform preclinical pharmacology and toxicology in a large animal model of NF1 syndrome that will allow these new drugs to enter clinical trials quickly and safely. The aims of the project are as follows:

Specific Aim 1: High throughput screen of 11,000+ FDA approved drugs, kinase inhibitors and other drugs, using isogenic human immortalized Schwann cells that are wild-type, heterozygous or homozygous null for NF1. At the time of this report, we have completed generation and detailed analysis of NF1-proficient and isogenic NF1-deficient Schwann cell clones. Initial drug screening has identified at least 75 "hits" to date with about one-third of the compounds screened.

Specific Aim 2: Establish efficacy of hits from the high throughput screen (HTS) in two rapid and well-established xenograft mouse models of human MPNSTs and two genetically engineered mouse models of peripheral nerve sheath tumors. This will begin when final analysis of Aim 1 is complete.

Specific Aim 3: Develop, phenotypically characterize and perform preclinical toxicology and pharmacology in a swine model of NF1. Recombinetics effectively produced multiple viable F0 NF1 mutant boars that have had initial phenotypic characterization. So far it is clear that NF1 +/- swine develop café-au-lait spots, and may also show other manifestations of NF1 syndrome. Sperm has been harvested from three of the boars and F1 offspring (NF1 mutant and sibling controls) are expected to farrow in August and September. Likewise, initial pharmacokinetic modeling has been done in preparation for pharmacokinetic and toxicity analysis using this swine model.

The investigators taking part in this project appreciate the support of CTF and the donors, especially the Moffett Family, and are working hard to find new answers for people living with NF1.

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Synodos for NF1, (Preclinical Acceleration B)

Sunday, June 19, 3:15pm – 3:30pm

Jill Weimer, PhD, *Sanford Health Systems*

As efforts accelerate in the NF1 research community towards development of novel therapies and technologies for early detection of tumor growth, a reliable animal model that fully mimics the human disease is becoming more and more critical. Based on our past successes in creating genetically accurate models of human disease, including those for cystic fibrosis, atherosclerosis, and ataxia telangiectasia, our collaborative team has recently created a porcine model of NF1 that mirrors the human mutation in a Yucatan miniature pig. In this proposal, we provide the infrastructure to bring together top-notch researchers from around the US into a collective with the primary mission of utilizing this novel porcine model to accelerate NF1 research toward clinical applications and treatments. By bringing together clinicians and basic scientists with expertise in genetics, neuroscience, pathology, medicinal chemistry, molecular biology and biomedical engineering, we have synergized our efforts to develop a research program that is able to seamlessly tackle correlative, hypothesis driven, and retrospective studies from our human NF1 data. Moreover, by working with the CTF and the other Synodos for NF1 teams, our group will continue to identify new and exciting projects which align with our mission. This talk will introduce the NF community to our synodos program and provide an update on the status of this work.

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NF1 LGG Synodos

Sunday, June 19, 3:15pm – 3:30pm

David Gutmann, MD, PhD, Washington University St. Louis, Michael J Fisher

Low grade gliomas (LGG) develop in ~20% of children with NF1. While the majority of these tumors are indolent and do not necessitate medical intervention, 30-50% cause symptoms. The identification of prognostic factors to identify those tumors likely to require intervention is crucial to minimizing the morbidity of these tumors as well as to limit treatment to only at-risk tumors. In addition, the present therapies for symptomatic NF1-LGG use genotoxic drugs that can also damage dividing cells in germinal zones critical for normal brain development in children. Thus, there is a pressing need to identify molecular risk factors and treatments targeted to the cellular and molecular properties unique to NF1-LGG. Progress in this area has been hampered by the paucity of human tumors available for analysis because of the relative infrequency of biopsy/surgical resection. In order to accelerate progress aimed at identifying the cell-intrinsic and cell-extrinsic molecular changes that dictate NF1-LGG biology and evaluate their functional significance, we are performing a comprehensive multi-platform analysis (genomic, transcriptomal, and epigenetic) of human NF1-LGG samples as well as tumor-associated monocytes to identify the spectrum of molecular changes in NF1-LGG and targetable stromal growth factors/cytokines for further study. The identified targets will be evaluated in overlapping in vitro and in vivo models for their impact on cell growth and signaling. Collectively, these approaches will elucidate new targets for the treatment of NF1-LGG that incorporate cooperating genetic changes, stromal influences, and tumor escape mechanisms.

SESSION 4A: Musculoskeletal Abnormalities in NF1

Chairs: Elizabeth Schorry, MD, *Cincinnati Children's Hospital*; Florent Elefteriou, PhD, *Baylor College of Medicine*

Mechanisms of Muscle Weakness in NF1

Session 4A: Sunday, June 19, 4:00pm – 4:20pm

Matthew Summers, PhD Candidate, University of Sydney

Neurofibromatosis Type 1 (NF1) can have profound effects on the musculoskeletal system, however to date the clinical and research focus has been on skeletal dysplasias and osteopenia. However, children with NF1 also exhibit reduced muscle size, global muscle weakness, and impaired motor control. Historically, deficits in strength and co-ordination have been attributed to central nervous system dysfunction but there is growing evidence for an underlying primary metabolic deficit in *NF1*-deficient muscle.

We have generated a muscle-specific *Nf1* knockout mouse (*Nf1_{muscle}^{-/-}*) that has profound effects on neonatal growth and survival. Muscle from these mice shows normal sarcomeric structure, but electron microscopy and histochemical staining showed intramyocellular lipid droplet accumulation. This is consistent with a metabolic myopathy. Analysis of patient NF1 muscle tissues using Oil Red-O and BODIPY staining has demonstrated comparable increases in intramyocellular lipid accumulation in humans. Lipidomics analysis using mass spectrometry (LCMS & GCMS) revealed accumulated long-chain fatty acids and esterified cholesterol in muscle. Notably, several cholesterol ester sub-species (CE18:1, CE20:2, CE22:4) were upregulated up to 20-fold in *Nf1* knockout samples.

In addition, this mouse model has been effective for testing therapeutic interventions. Treatment of pregnant females with MEK inhibitor PD0325901 (5mg/kg/day) led to a rescue of the lipid droplet phenotype in neonatal *Nf1_{muscle}^{-/-}* muscle. We are now proposing to screen with other agents and dietary interventions to more directly target cholesterol ester accumulation.

Arthur List: Aaron Schindeler¹, Matthew A. Summers¹, Thusi Rupasinghe², Ute Roessner², David G Little¹

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Tibial Bowing: Pathogenesis, Prevention and Outcome Measures

Session 4A: Sunday, June 19, 4:20pm – 4:40pm

David Stevenson, MD, Stanford University

Neurofibromatosis type 1 (NF1) is a common autosomal dominant genetic disorder with distinct skeletal manifestations. In particular, tibial bowing and pseudarthrosis is a difficult-to-treat morbid manifestation. The natural history typically presents with unilateral anterolateral bowing of the tibia with cortical thickening and medullary canal narrowing radiographically, with subsequent fracture and non-union (ie. pseudarthrosis). Most therapeutic and surgical investigations target the patient after fracture. In infancy, physiologic bowing of the lower leg can sometimes be confused with pathologic tibial dysplasia in NF1. Most current orthopedic therapeutic and surgical investigations target the patient after fracture. At this time, bracing is the only intervention for tibial bowing and efficacy has not been systematically established. Discussion on the underlying pathogenesis of tibial bowing will be discussed in addition to evaluation of the architecture of the tibia. Data will be presented on results of quantitative ultrasound (QUS) comparing non-fractured bowed tibia to the contralateral side. QUS measures speed of sound, avoids radiation, and is reported to be predictive of clinical fracture in the general population. QUS may be a useful outcome measure as new therapies develop aimed at preventing fracture rather than treating pseudarthrosis. Discussion of other potential outcome measures will be discussed.

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NF1 Pseudarthrosis: Cell of Origin, Targets, Potential Treatments and Needs

Session 4A: Sunday, June 19, 4:40pm – 5:00pm

Florent Elefteriou, PhD, Baylor College of Medicine

Recalcitrant bone healing (pseudarthrosis) in children with NF1 is a difficult condition to manage, still often leading to tibia amputation. We and others have developed several conditional mouse models of NF1 skeletal maladies, with the goal of identifying the cell of origin leading to NF1 skeletal dysplasia and pseudarthrosis. These studies revealed that the NF1 bone dysplastic defects are likely the result from loss of *Nf1* function in osteochondroprogenitor cells. Two major abnormalities characterize these cells: they generate high levels of pyrophosphate (PPI), a strong inhibitor of matrix mineralization, which might contribute to the bowing and weaker mechanical properties of the dysplastic tibia (and perhaps vertebrae) of children with NF1 who will progress to fracture or dystrophic scoliosis. The success of enzyme therapy by a recombinant form of alkaline phosphatase, used to hydrolyze excess PPI, to improve bone mechanical properties in models of NF1 skeletal dysplasia supports the use of this drug to complement bracing and to prevent tibia bowing and fracture in NF1. It is now critical to develop methods to predict which children who will progress to these conditions for inclusion into a clinical trial, and to know if this drug could also promote bone healing. *Nf1*-deficient osteoprogenitors also have a proliferative advantage and a reduced ability to differentiate into osteoblasts, which may also contribute to the accumulation of fibrotic tissue at the bone fracture site and impaired healing. Although the use of BMP2 in combination with MEK inhibition successfully promoted bone healing in mouse models of NF1 pseudarthrosis, a clinical trial with BMP2 alone is still required before assessing the efficacy of targeted combined treatments.

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Platform: Neurofibromatosis Type 1-Related Pseudarthrosis: Beyond the Pseudarthrosis Site

Session 4A: Sunday, June 19, 5:00pm – 5:20pm

Carlijn Brekelmans, PhD Candidate, KU Leuven, Belgium

Approximately 5% of NF1 children present with congenital bowing of a long bone which often results in a fracture that can develop into a non-union or pseudarthrosis. Current treatment methods for NF1-related pseudarthrosis are unsatisfactory and if union remains elusive the affected limb is amputated (Stevenson *et al.* 2013). Recently an additional somatic NF1 mutation was identified in pseudarthrosis tissue (Paria *et al.* 2014; Sant *et al.* 2015). The affected cells and the pathogenic mechanism are however still unknown.

We performed *NF1* mutation analysis on cultured cells from the first surgery at the pseudarthrosis site of eight NF1 individuals. Two of these patients were sampled more extensively, including multiple samples at the pseudarthrosis site and periosteum sampling surrounding the pseudarthrosis region and at the osteotomy site. Periosteal-derived cells (PDCs) from outside the pseudarthrosis region and cells from the pseudarthrosis site were cultured and the somatic mutation was analyzed using next generation sequencing.

We found bi-allelic *NF1* inactivation in cultured cells of primary pseudarthrosis tissue in 8/8 NF1 patients. In the two patients sampled more extensively we found a range of *NF1*^{+/-} and *NF1*^{-/-} cells in the pseudarthrosis site. Periosteum outside the pseudarthrosis region contained ±20% (proximal) to almost 100% (distal) *NF1*^{-/-} PDCs. Additionally, one patient even had 30% *NF1*^{-/-} PDCs in the proximal 1/3rd of the tibia close to the knee, far above the pseudarthrosis. The somatic *NF1* mutation identified in this patient was caused by mitotic recombination. These results show that the *NF1*^{-/-} cells are found in the pseudarthrosis region AND in periosteum from proximal and distal regions of the same tibia. We hypothesize that second hits in the *NF1* gene in periosteal cells occur at random at different positions of the developing skeleton. The observation that the pseudarthrosis is nearly always localized in the same region of the tibia suggests an involvement of additional local factors in the development and persistence of NF1-related pseudarthrosis.

Author List: Carlijn Brekelmans, KU Leuven; Silke Hollants, UZ Leuven; Caroline De Groote, UZ Leuven; Marijke Spaepen, PhD, UZ Leuven; Natalie Sohier, UZ Leuven; Marina Maréchal, PhD, KU Leuven; Frank Luyten, MD, PhD, KU Leuven; Johan Lammens, MD, PhD, UZ Leuven; Eric Legius, MD, PhD, KU Leuven; Hilde Brems, PhD, KU Leuven.

Funded by Research Foundation-Flanders (FWO) and agency for Innovation by Science and Technology (IWT).

SESSION 4B: Assessing Therapeutics in NF2 and Schwannomatosis through Translational Research

Chairs: Filippo Giancotti, MD, PhD, *Memorial Sloan Kettering Cancer Center*; Michel Kalamarides, MD, PhD, *Hopital Pitie - Salpetriere, Universite de Paris*

Crizotinib Suppresses NF2 Deficient Schwannoma through Inhibition of Focal Adhesion Kinase 1

Session 4B: Sunday, June 19, 4:00pm – 4:20pm

Joseph Kissil, PhD, Scripps Research Institute

Neurofibromatosis type 2 (NF2) is a dominantly inherited autosomal disease characterized by schwannomas of the 8th cranial nerve. The *NF2* tumor suppressor gene encodes for Merlin, a protein implicated as a suppressor of multiple cellular signaling pathways. To identify potential drug targets in NF2-associated malignancies we assessed the consequences of inhibiting the tyrosine kinase receptor MET. We identified crizotinib, a MET and ALK inhibitor, as a potent inhibitor of *NF2*-null Schwann cell proliferation *in vitro* and tumor growth *in vivo*. To identify the target/s of crizotinib we employed activity-based protein profiling (ABPP), leading to identification of FAK1 (PTK2) as the relevant target of crizotinib inhibition in *NF2*-null schwannoma cells. Subsequent studies confirm that inhibition of FAK1 is sufficient to suppress tumorigenesis in animal models of NF2 and that crizotinib-resistant forms of FAK1 can rescue the effects of treatment. These studies identify a FDA approved drug as a potential treatment for NF2 and delineate the mechanism of action in *NF2*-null Schwann cells.

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A Path toward Combination Therapy for NF2

Session 4B: Sunday, June 19, 4:20pm – 4:40pm

Vijaya Ramesh, PhD, Harvard University

Our earlier work established the aberrant activation of mTORC1 signaling in NF2-deficient meningiomas, which led to clinical trials with rapamycin analog everolimus for NF2-associated tumors. Our more recent work demonstrated distinct activation of SGK1/NDRG1 downstream of mTORC2 in NF2-null human arachnoidal and meningioma cells. Further we showed that the dual mTORC1/mTORC2 inhibitor AZD2014 was more effective than rapamycin in blocking proliferation of meningioma cells. In our continuing efforts, we have undertaken studies to define the adaptive kinome response in isogenic NF2-expressing and NF2-null human arachnoidal cells treated with either rapamycin or AZD2014. We have observed kinases preferentially induced by AZD2014 or rapamycin. More interestingly, we have observed consistent activation of EPH receptor family members in both arachnoidal and meningioma cells with NF2 loss. These observations led us to initiate *in vitro* combination treatment with AZD2014 and dasatinib, a potent inhibitor of many of the EPH family members, and we have observed synergy between these two drugs. Defining adaptive kinome response has the potential to reveal effective combination therapies and will have direct impact on NF2 research as well as patient care.

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Platform: Effects of Lapatinib on Meningiomas in Adults with Neurofibromatosis Type 2

Session 4B: Sunday, June 19, 4:40pm – 5:00pm

Matthias Karajannis, MD, MS, NYU Langone Medical Center, New York, NY

Background: Epidermal growth factor receptor (EGFR) and erb-b2 receptor tyrosine kinase 2 (ErbB2) are overexpressed schwannomas and meningiomas. Preclinical and clinical data indicate that lapatinib, an EGFR/ErbB2 inhibitor, has antitumor activity against schwannomas.

Methods: We conducted a single institution, retrospective review of patients with neurofibromatosis type 2 (NF2) and progressive vestibular schwannomas treated on a Phase II clinical trial with lapatinib (ClinicalTrials.gov identifier NCT00973739). We included patients with at least one volumetrically measurable meningioma (>0.5 cc) who received at least five 28-day courses of therapy. Lapatinib was administered in continuous 4-week courses receiving standard adult doses (1,500 mg once daily). Meningioma response was assessed using 3-dimensional MRI volumetrics. Progressive meningioma growth and response were defined as +20%/-20%-change from baseline in tumor volume, respectively. Off-treatment defined as any period >5 months without lapatinib.

Results: Eight patients (ages: 20-58 years) who met criteria had 17 evaluable meningiomas with a combined volume of 61.35cc baseline, 61.17 cc during treatment, and 108.86cc (+77.44%-change) off-treatment. Tumor volume differences were significant ($p=0.0033$) using a two-sided Wilcoxon-Signed-Rank test. Median time on-therapy: 15 months and off-therapy: 14 months (both ranged 5–29 months). We calculated a median and mean annual growth rate off-treatment: 10.42% and 20.05%, respectively. The best response to therapy was -26.1% after 23 months on lapatinib. Two of 17 tumors grew >20% on-treatment while 9 of 17 grew >20% off-treatment.

Conclusions: These data suggest that lapatinib has modest growth-inhibitory effects on meningiomas in NF2 patients. Prospective studies of lapatinib for NF2 patients with progressive meningiomas may be warranted.

Author List: Diana Osorio [1,2], Jessica Hu [1], Joseph Stanek [2], Mari Hagiwara[1] and Matthias Karajannis [1]. [1] NYU Langone Medical Center, New York, NY; [2] Nationwide Children's Hospital, Columbus, OH.

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Platform: Ponatinib Causes Cell Cycle Arrest at G1 of Merlin-null Human Schwann Cells

Session 4B: Sunday, June 19, 5:00pm – 5:20pm

Alejandra Petrilli, PhD, *Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida*

Bilateral vestibular schwannomas are the hallmark of Neurofibromatosis Type 2 (NF2), a non-malignant tumor-forming disease of the nervous system caused by mutations in the *NF2* gene. The tumor suppressor encoded by the *NF2* gene, merlin, is a scaffold protein responsible for modulating several signaling pathways. Despite increasing knowledge of merlin function, there are still no drug therapies for NF2. In 2012, the FDA approved the use of ponatinib hydrochloride, a tyrosine kinase inhibitor, for a specific form of leukemia. Ponatinib targets key proliferation and survival pathways overactive in schwannomas. Work by us and others have shown that NF2 schwannomas and schwannoma cells have higher levels of activated Src compared to normal human Schwann cells and nerve. We tested the ability of ponatinib to inhibit proliferation/survival of merlin-null human Schwann cells (HSC). We found that ponatinib reduced the viability of merlin-null HSC in a dose dependent manner by inhibiting activity of Src kinase and the Platelet-Derived Growth Factor Receptors -alpha and -beta. Flow cytometry studies revealed that ponatinib caused a G1 cell cycle arrest of the merlin-null HSC. We mapped the downstream signaling cascades associated with a dose-dependent reduction in CyclinD1 levels. Studies are underway to determine the efficacy of ponatinib in an orthotopic xenograft model.

Author List: Jeanine Garcia, University of Central Florida, Marga Bott M.S., University of Central Florida, Cristina Fernández-Valle Ph.D., University of Central Florida.

This work was supported by Department of Defense (NF140044) and by Children's Tumor Foundation Drug Discovery Award to CFV.

SESSION 5: CNS Tumors in the Neurofibromatoses

Chairs: Yuan Zhu, PhD, *Children's National Medical Center*; Matthias Karajannis, MD, *NYU Langone Medical Center*

Perspectives: Defining the Factors that Underlie the Pathogenesis of NF1 Optic Glioma

Session 5: Monday, June 20, 8:30am – 9:10am

David Gutmann, MD, PhD, *Washington University, St. Louis*

Children with neurofibromatosis type 1 (NF1) are genetically predisposed to develop tumors of the central and peripheral nervous systems. One of the most common central nervous system tumors is the optic pathway glioma, encountered in 15-20% of children with NF1. While these brain tumors arise in similar locations (optic pathway) and age populations (mean age = 4 years), they actually comprise a heterogeneous collection of tumors whose clinical behavior is dictated by numerous factors. Understanding the factors that underlie this clinical heterogeneity provides unique opportunities for risk assessment and personalized treatment strategies. This talk will focus on the use of genetically-engineered mouse models to define the factors that underlie the pathogenesis and progression of NF1 optic glioma.

Developmental Origin and a Therapeutic Window for NF1-Associated Optic Pathway Glioma

Session 5: Monday, June 20, 9:10am – 9:30am

Yuan Zhu, PhD, *The Gilbert Family Neurofibromatosis Institute, Center for Cancer and Immunology Research, the Children's National Medical Center*

Approximately 15-20% of individuals with neurofibromatosis type 1 (NF1) develop low-grade glioma, predominantly along the optic pathway, also known as optic pathway glioma (OPG). NF1-associated OPG often extensively grow along and infiltrate throughout the visual pathway and thus the major morbidity associated with this tumor is visual loss. Consequently, surgery is not a therapeutic option or is required for diagnosis. Due to the paucity of clinical specimen, little is known about the mechanism underlying NF1-associated OPG with the exception of loss of the remaining NF1 wild-type allele – a process also known as “second-hit” in OPGs. One unique clinical feature of NF1-associated OPG is that almost all arise from children younger than 7 years of age. These clinical observations suggest that a “second-hit” event must occur during the development of the nervous system, leading to a complete loss of NF1 in neural stem and/or progenitor cells. Genetically engineered mouse (GEM) models and cell culture studies suggest that neural stem cells from the third ventricular zone (III-VZ) are more sensitive to Nf1 loss, suggesting a potential cell-of-origin of NF1-associated OPG. However, a GEM OPG model with a dominant developmental defect has not been previously described. Here we characterized a GEM model that inactivates Nf1 in glial restricted progenitor (GRP) cells in the ventral brain, but develops OPGs with 100% penetrance. Robust glial defects were observed in the developing optic nerve of this GEM model, demonstrating that developing GRPs can serve as a cell-of-origin of NF1-associated OPG. We have previously established a “MEK inhibitor (MEKi)-in-Milk” protocol in which a MEKi is delivered into neonatal pups via milk of lactating females. This “MEKi-in-Milk” protocol has successfully rescued the developmental structural defects in the forebrain (enlarged corpus callosum) and cerebellum (Wang et al., Cell 2012 and Kim et al., Elife 2014). We are currently investigating the possibility of using this “MEKi-in-Milk” protocol to rescue glial defects in the developing Nf1-deficient optic nerve and subsequently prevent OPG formation.

Author List: Emmanuelle Jecrois^{1,2}, BS, Miriam Bornhorst¹, MD, Yuan Wang¹, PhD, The Children's National Medical Center¹, University of Michigan².

Supported by the Gilbert Family Foundation Fund to Y.Z., NSF to E.J., FSC Scholar Program of the NTAP to M.B.

Spinal Ependymomas in NF2

Session 5: Monday, June 20, 9:30am – 9:50am

Michel Kalamarides, MD, PhD, *Hopital Pitie - Salpetriere, Universite de Paris*

Object. The aim of this study was to compare the outcome of conservative treatment of spinal ependymomas in Neurofibromatosis Type 2 with surgical treatment.

Methods. The authors retrospectively reviewed the clinical records, including imaging, of patients with NF2 at two large centres-Manchester, UK and France. In Manchester patients were almost invariably treated conservatively, in Paris surgery was an actively employed treatment option. Inclusion in the study was based on tumour length of greater than 1.5cm at some point during the study period. The two primary measures assessed were tumour size and growth over time, and neurological deficit due to the tumour, as measured by the Modified McCormick Outcome Score.

Results. 23 patients with tumour length greater than 1.5 cm from Manchester and 46 patients from Paris had full clinical data available. From the Manchester series 26% of ependymomas greater than 1.5 cm in overall length deteriorated during the course of the study. This effectively represents the natural history of ependymomas in NF2. In the Paris series, 0% of conservatively managed ependymomas deteriorated. Overall, 36% of surgically managed ependymomas deteriorated but 11% of those operated on in the NF2 therein rather than elsewhere in France. Whilst there was no statistical significance between the Manchester and overall surgical series from France ($P=0.19$, CH12 tests) there was significance between Manchester and the French NF2 center-operated patients with improved outcome with surgical intervention ($P=0.02$, CH12 tests).

Conclusions. Spinal ependymomas can produce morbidity, and surgery can prevent or improve morbidity in selected cases. In contrast surgery can produce morbidity. Surgery should be considered in growing/symptomatic ependymomas, particularly in the absence of overwhelming tumour load, where Bevacizumab is likely to be the preferred option.

Author List: Kalamarides M¹, Essayed W¹, Lejeune JP², Aboukai R², Sterkers O¹, Bernardeschi D¹, Lloyd SK³, Freeman S³, Hammerbeck-Ward C³, Kellet M³, Rutherford SA³, Evans DG³, Pathmanaban O³, King AT³
NF2 Clinic, Paris¹ and Lille², France & NF2 Clinic, Manchester, UK³

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Platform: Characterization of Brain Tumors and Spongiotic Change using Magnetic Resonance Fingerprinting: Initial Experience

Session 5: Monday, June 20, 9:50am – 10:10am

Peter M.K. de Blank, MD, MSCE, Rainbow Babies & Children's Hospital

Introduction: Traditional MRI relies on qualitative comparisons of relative signal intensity. Magnetic resonance fingerprinting (MRF) allows rapid, absolute quantitation of relaxation times (T1 and T2)[1] and may be an important diagnostic tool to measure tumor characteristics and distinguish tumor from mimics such as unidentified bright objects (UBOs). We used MRF to investigate tissue diagnosis and tumor grade in children with and without NF1.

Methods: Subjects were scanned using MRF to determine whether T1 and T2 values can distinguish differences in pathology. A pediatric neuroradiologist defined areas of tumor (enhancement or T2 hyperintensity with mass effect excluding cysts), UBO (areas of T2 enhancement without mass effect in children with NF1), contralateral white matter and normal-appearing non-UBO tissue (normal-appearing matching tissue contralateral to UBO). Nonparametric Wilcoxon and Mann-Whitney tests were used to compare T1 and T2 values between groups.

Results: 15 subjects [12 with brain tumors, 6 with NF1, 9 male, 0-34 years] underwent MRF of the brain. Tumors included 9 low-grade gliomas and 3 high-grade tumors (PNET, AT/RT, anaplastic astrocytoma). 22 UBOs and 4 contralateral non-UBO structures were measured. Among 12 subjects with tumors, T1 and T2 values differed significantly between tumor and contralateral white matter ($p=0.004$, $p=0.010$, respectively). MRF-derived T1 and T2 values were significantly higher in high-grade tumors compared to low-grade tumors ($p=0.013$, $p=0.021$, respectively). There was no significant difference in T1 or T2 values between NF1 and non-NF1 low-grade gliomas. Among subjects with UBO, T1 differed significantly between UBO and contralateral white matter ($p<0.001$), and between UBO and low-grade glioma ($p=0.037$). Among 4 subjects with UBO and matched non-UBO, a trend was observed toward a difference in T1 or T2 values between tissues ($p=0.068$ for both).

Discussion: In this first use of MRF among pediatric brain tumors and children with NF1, MRF-derived relaxation times distinguished tumor from healthy white matter and differentiated tumor grade. In children with NF1, MRF-derived T1 values distinguished UBO from normal white matter and from tumor tissue. MRF may be a useful tool to measure tissue characteristics and distinguish tissue type and tumor grade in children with NF1.

Author List: Dan Ma, Case Western Reserve University; Chaitra Badve, Case Western Reserve University; Deborah Rukin Gold, Rainbow Babies & Children's Hospital; Vikas Gulani, Case Western Reserve University; Mark Griswold, Case Western Reserve University.

Acknowledgement: Neurofibromatosis Therapeutic Acceleration Program [1] Ma, D. (2013) Nature

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Platform: Similarities and Differences in Tumor Characteristics and Treatment Response in NF2-Associated Vestibular Schwannomas and Meningiomas

Session 5: Monday, June 20, 10:10am – 10:30am

Sarah S. Burns, BA, Nationwide Children's Hospital and The Ohio State University

Neurofibromatosis type 2 (NF2) is characterized by the development of multiple nervous system tumors, including vestibular schwannomas (VS) and meningiomas. These tumors cause considerable morbidities, including profound deafness, tinnitus, facial nerve paralysis, ataxia, and brainstem compression; however, an effective medical therapy is presently not available. Previously, we showed that the histone deacetylase inhibitor (HDACi) AR-42 causes tumor regression and inhibits tumor growth in animal models of NF2-deficient meningioma and schwannoma, respectively. Similarly, AR-42 reduced tumor size in meningiomas while slowing the growth of VS in an NF2 patient. To investigate this difference in treatment response, we screened for genetic mutations in 405 cancer-related genes in VS and meningiomas from two NF2 patients treated with AR-42. In addition to mutations in NF2, VS from both patients harbored mutations in NUP98, which is important for nuclear transport, mitotic checkpoint, and immunity. Also, we detected a duplication of exon 2-3 of the MYC gene in one of these patients. Analysis of blood samples from these patients and their parents confirmed that these mutations were present in the germline. Intriguingly, in a patient with multiple tumors, we observed the same genetic changes in both VS and meningiomas, implicating additional factors in treatment response. Interestingly, we detected a significant number of CD163+ macrophages in a majority of meningiomas, whereas little or no macrophages were present in the 20 VS examined. The MYC protein has been shown to regulate tumor microenvironment, which can be affected by HDACi's. Importantly, strong nuclear MYC expression was detected in the majority of VS specimens but not in meningiomas. Experiments are in progress to evaluate MYC inhibitors for their ability to inhibit tumor growth and to enhance sensitivity to AR-42. Our results suggest that targeting tumor microenvironment may enhance therapeutic response in NF2-associated tumors.

Author List: Sarah S. Burns, BA^{1,2}, Elena M. Akhmeteyeva, MD, PhD^{1,2}, Jaishri Blakeley, MD⁴, D. Bradley Welling, MD, PhD⁵, and Long-Sheng Chang, PhD^{1,2,3}

¹Center for Childhood Cancer and Blood Diseases, Nationwide Children's Hospital and Departments of ²Pediatrics and ³Otolaryngology, The Ohio State University, ⁴Departments of Neurology, Neurosurgery, and Oncology, Johns Hopkins University, and ⁵Department of Otolaryngology, Harvard Medical School and Massachusetts General Hospital

Funding: The Galloway Family, Advocure NF2, Meningioma Mommas, CTF, and the Department of Defense

SESSION 6: Present and Future Impact of Novel Imaging in the Management of NF

Chairs: Peter de Blank, MD, *Rainbow and Babies' Children's Hospital, Case Western Reserve University*; Gordon Harris, PhD, *Harvard University*

Imaging White Matter in Children with Brain Tumors: Lessons for Late Effects, Plasticity and Repair

Session 6: Monday, June 20, 11:10am – 11:30am

Donald Mabbott, PhD, *Hospital for Sick Children, Toronto, CAN*

Quantitative neuro-imaging has allowed us to measure the impact of disease and injury on tissue structure and ultimately relate this to thinking and learning. I will discuss how different MRI techniques can be applied to measure white matter structure in normal and perturbed development - focusing on children treated for a brain tumor. I will consider how the tissue properties of white matter structure may affect cognition. Finally, I will present new research regarding white matter plasticity in children and mice and how such plasticity can be harnessed for brain repair.

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Building Quantitative MRI: Implications for NF1

Session 6: Monday, June 20, 11:30am – 11:50am

Vikas Gulani, MD, PhD, *University Hospitals of Cleveland*

The accepted paradigm in medical imaging, including MRI, is to provide anatomical maps of tissue to identify abnormal anatomy. This paradigm has yielded great results, to the point that a 2001 survey of physicians placed MRI and CT technology as the most important medical advances of the past 50 years. However, there are severe limitations. Image interpretation is a subjective and descriptive process, and in the case of MRI, image collection is slow and inefficient. In this talk, I will seek to identify new ways of approaching MRI that leverage fast imaging methods coupled with new ways of obtaining quantitative maps of property. The result is an approaching new era of fast and efficient quantitative imaging with emerging technology (for example Magnetic Resonance Fingerprinting and free breathing through-time GRAPPA from our group) that might allow vastly improved rapid, objective, and non-invasive assessment of disease. I will also discuss the implications of this technology for neurofibromatosis type 1 (NF1).

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Platform: Computerized Working Memory Training for Children with Neurofibromatosis Type 1: a Pilot Resting-State Study of Changes in Intrinsic Functional Connectivity

Session 6: Monday, June 20, 11:50am – 12:10pm

Maria Acosta, MD, *The Jennifer and Daniel Gilbert Neurofibromatosis Institute, Children's National Medical Center*

Objectives: Children with Neurofibromatosis Type-1 (NF1) commonly have deficits in executive function. Computerized training programs are increasingly being used in neuropsychiatric disorders, but empirically evaluated interventions are lacking for NF1. This pilot study examined training effects on cognition and resting-state functional connectivity (RSFC) in children with NF1.

Methods: In an open pre-/post-test design, we provided 25 sessions (6-10 weeks) of computerized visuo-spatial working memory training at-home with phone-based coaching assistance (Cogmed®). Sixteen participants (9 males; 11.1 ± 2.3 years) had analyzable pre- and post-test resting-state functional magnetic resonance imaging (fMRI) scans and cognitive task data. Standard data preprocessing and calculation of RSFC indices used the Configurable Pipeline for the Analysis of Connectomes v0.3.3. Two voxel-wise RSFC measures, fractional amplitude of low frequency fluctuations (fALFF) and regional homogeneity (ReHo), were contrasted pre- vs. post-test using paired t-tests.

Results: Both RSFC measures showed statistically significant ($p < 0.05$ corrected) regionally specific differences. Decreased fALFF following treatment was found in a large cluster spanning thalamus, globus pallidus, lingual and parahippocampal gyri, brainstem and cerebellum; a second cluster encompassed precentral cortex, supplementary motor area, extending into middle and superior frontal gyri. Increased ReHo following training was observed in predominantly visual areas (intra- and supra-calcarine cortex, occipital pole and lingual gyrus). Changes in RSFC significantly correlated with facets of behavioral improvement after Cogmed training completion and with performance on tasks tapping executive function and visuo-spatial working memory.

Conclusion: These pilot findings suggest that regionally specific RSFC changes may capture treatment-related improvements in cognitive dysfunction in NF1 and motivate independent controlled replication.

Author List: Yuliya N. Yoncheva, PhD, NYU Langone Medical Center; Kristina K. Hardy, PhD, Children's National Health System; Daniel J. Lurie, University of California, Berkeley; Krishna Somanepalli, MS, University of Southern California; Roger J. Packer, MD, Children's National Health System; Michael P. Milham, MD, PhD, Child Mind Institute; F. Xavier Castellanos, MD, NYU Langone Medical Center; Maria T. Acosta, MD, Children's National Health System

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MEG as Part of Multimodal Investigation of ASD: Towards Biomarkers for Diagnosis, Prognosis, Stratification and Response

Session 6: Monday, June 20, 12:10am – 12:30pm

Timothy Roberts, PhD, *Oberkircher Family Chair in Pediatric Radiology, Children's Hospital of Philadelphia, Professor of Radiology, Perelman School of Medicine, University of Pennsylvania*

There is considerable evidence for ASD-like phenotypes among many children with NF1. Studies suggest up to 25% of children with NF1 might meet ASD diagnostic criteria with a yet higher proportion (~40-60%) scoring in the ASD, or ASD-concern, range on instruments such as the social responsiveness scale (SRS). In particular the ASD phenotype of social communication impairment seems most commonly represented in NF1 populations.

The purpose of this presentation is to discuss strategies to use magnetoencephalography (MEG) in the context of multimodal imaging to establish biomarkers for ASD: diagnostic, prognostic, stratification, response and, indeed, translational. This draws upon the temporal and spectrotemporal capabilities afforded by MEG, and is supported by diffusion-MRI and neurotransmitter MRS, to provide converging evidence and support the biological basis of the proposed biomarkers. In particular focus will be paid to the precise latency of evoked responses in the auditory system as well as stimulus evoked gamma-band oscillatory activity.

It is hoped that the lessons learned in the study of ASD might have both direct and conceptual bearing on the more thorough characterization of the cognitive and behavioral phenotype observed in NF1.

KEYNOTE 3: Defining the Actionable Genome

Monday, June 20, 1:30pm – 2:30pm

David Solit, MD, *Geoffrey Beene Chair in Cancer Research, Director, Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center*

Profound and durable responses are often observed in early stage clinical trials of novel cancer agents in only a small minority of patients. It has long been postulated that these responses have a definable genetic basis but until now it was not feasible to perform a comprehensive genomic analysis of such patients. Rather, at best a few candidate genes were examined. Technical feasibility thus ensured that oncology trials were designed to identify agents that have a statistically significant benefit in a genetically unselected population, a paradigm that has led to the development of many agents that have modest or no benefit in the vast majority of patients. Agents with profound activity in only a small number of patients were on the other hand deemed inactive and abandoned. Our preliminary experience shows that next generation sequencing methodology can now be feasibly applied in the clinical setting to identify previously unrecognized biomarkers of response for agents that show profound responses in only a minority of patients. As an example, we explored the molecular basis of an outlier phenotype, an apparent disease cure of a 51-year old woman with recurrent metastatic small cell carcinoma. The patient was treated on a phase 1 clinical trial that combined topoisomerase I inhibition with an ATP-competitive inhibitor of checkpoint kinase 1 (Chk1). Whole genome sequencing revealed a complex but highly clonal tumor genome with a somatic, missense mutation in the Mre11 complex gene *RAD50*, which functions to initiate double stranded break (DSB) repair by homologous recombination or non-homologous end joining. Through modeling in yeast, we confirmed that this heterozygous *RAD50* mutation, which affects a highly conserved residue and whose wildtype copy was focally deleted, conferred sensitivity to topoisomerase I inhibition. Drug sensitivity was markedly enhanced upon genetic ablation of the DNA damage checkpoint pathway, suggesting a synthetic lethal interaction between checkpoint kinase inhibition and clastogenic chemotherapy. These results and additional examples to be presented demonstrate the feasibility of using whole-genome sequencing in the clinical setting to identify previously occult biomarkers of drug sensitivity that can aid in the identification of patients most likely to respond to targeted anticancer drugs. The use of novel clinical trial designs to confirm genotype-phenotype associations will also be discussed.

SESSION 7A: Beyond the Medical Model: Exploring Psychological, Social and Biological Factors in NF

Chairs: Staci Martin, PhD, *NIH*; Nicole Ullrich, MD, PhD, *Boston Children's Hospital/Harvard University*

Relating the NF Cognitive Phenotype to Environmental and Family Variables

Session 7A: Monday, June 20, 2:35pm – 2:55pm

Jennifer Janusz, PsyD, ABPP-Cn, Colorado Children's Hospital

Much of the NF outcome research has focused on the relationship between disease factors and particular cognitive (e.g., learning, attention) strengths and weaknesses. Minimal attention has focused on other variables that can impact outcome. The research literature in multiple other chronic health conditions has moved to a more complex understanding of outcomes, considering not only biologic disease-related factors but also psychosocial, family, and cultural factors as well. Understanding that outcomes are determined by a complex interplay of disease and non-disease related factors allows for a fuller appreciation of a particular individual's neurobehavioral functioning and also opens up a number of potential options when considering effective clinical management. This talk will focus on factors to consider in this more holistic approach to conceptualizing neurobehavioral outcomes, as well as interventions that have proven effective in other populations that could be readily utilized with individuals and families faced with NF.

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Mind-Body Therapy for Individuals with NF1, NF2 and Schwannomatosis: Class II Evidence for Improvements in Quality of Life

Session 7A: Monday, June 20, 2:55pm – 3:15pm

Ana-Maria Vranceanu, PhD, Harvard University

This talk will focus on the biopsychosocial model of care and its applications for patients with neurofibromatosis. First, I will present a sequential approach to the development of the Relaxation Response Resiliency Program for adults (18 and older) with NF1, NF2 and schwannomatosis (3RP-NF), and discuss the development of a credible attention placebo control, the Health Enhancement Program for patients with NF (HEP-NF). Second, I will present class II evidence on the feasibility, acceptability and efficacy of the 3RP-NF delivered via live video in improving quality of life in adults with NF (N=63). Third, I will discuss adaptations of the 3RP-NF for adolescents (12 to 17) with NF1 and NF2, and present preliminary evidence on the feasibility, acceptability and efficacy of this program delivered via live video in improving quality of life in this population (N=36, ongoing trial). Next, I will discuss adaptation of the 3RP-NF for adults with NF2 who are deaf or have severe hearing loss, and for delivery via live video and Computer Adaptive Real Time Translation (CART), and ongoing efforts of adapting the 3RP-NF for delivery to parents of children with NF. I will end by discussing future plans for dissemination of the 3RP-NF, and advantages of having a psychosocial intervention that teaches the same coping skills for all patients with NF regardless of age or NF type.

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Platform: Parental Coping with Child with NF1

Session 7A: Monday, June 20, 3:15pm – 3:35pm

Taylor Smith, PhD, California Polytechnic State University, San Luis Obispo & Rhode Island Hospital

Background: Very little research has explored the burden and stress experienced by parents of children with NF1 and the mechanisms used to cope with this burden. Therefore the aims of this study were to: 1) characterize strategies utilized by parents to cope with having a child with NF1; 2) measure levels of parenting stress and psychiatric symptomatology among parents of children with NF1; 3) examine differences in coping and stress between parents of children with familial NF1 compared to parents of children with sporadic NF1.

Methods: A total of 172 parents were included in this study. The majority of participants were mothers (94%, $n=161$), married (83%; $n=142$), and had a bachelors/graduate degree (62%; $n=106$). Child mean age was 7.89 years ($SD=4.48$) and 84% of children had spontaneous NF1. Parents completed the WAYS of Coping Questionnaire (WAYS), Parenting Experience of Childhood Illness Scale – Short Form (PECI), Parenting Stress Index –Short Form (PSI), and Brief Symptom Inventory-18 (BSI).

Results: Parents of children with NF1 more frequently used Distancing ($t=3.94$, $df=314$, $p<.01$), Self-Controlling ($t=3.67$, $df=307$, $p<.01$), Seeking Social Support ($t=5.79$, $df=311$, $p<.01$), Escape Avoidance ($t=6.59$, $df=310$, $p<.01$), and Positive Reappraisal ($t=10.03$, $df=317$, $p<.01$) coping strategies compared to the WAYS of Coping normative sample. On average, parental stress (PSI total score mean = 66th percentile) and psychiatric symptoms (BSI General Symptom Index means = 44th-66th percentiles) were in the normal range for parents of children with NF1; however, there is substantial variability in the severity of parental stress and psychiatric symptomatology. Parents of children with spontaneous NF1 had greater long-term uncertainty related to their child's NF1 ($M=2.04$, $SD=.71$) compared to parents of children with familial NF1 ($M=1.73$, $SD=.73$; $t=2.02$, $df=168$, $p<.05$). No other differences in coping, parenting experiences, parental stress, or parental symptoms were observed between parents of a child with spontaneous NF1 and parents of a child with familial NF1. Hierarchical stepwise regression analyses explored the association between child and parenting factors and parenting stress and parental symptomatology. Adjusting for demographic factors, child learning difficulties ($b^*=.22$, $t=2.14$, $p<.05$), parental escape-avoidance coping ($b^*=.39$, $t=3.61$, $p<.01$), and parental emotional resources ($b^*=-.34$, $t=-3.37$, $p<.01$) predict BSI General Symptom Index (GSI). Taken together, the model predicted 46% of variability in parent psychiatric symptoms (results are similar for parenting stress as the outcome).

Conclusion: The way in which parents cope with and experience their child's NF1 is closely related to their mental health risk. Results of the study are limited by a: 1) small number of familial NF1 cases; 2) 50% attrition rate; and 3) cross-sectional design. Accessible interventions aimed at supporting parents to understand/cope with their child NF1 are sorely needed.

Author List: Taylor Smith, PhD, California Polytechnic State University & Rhode Island Hospital, Caroline Stephens, BA, Children's National Health System, Peter Shibuya, CPNP-PC, Children's National Health System; Brian Hoover, MS, Children's National Health System, Madeleine Brown, Children's National Health System, Jessica Kaczorowski, PhD, California Polytechnic State University, Maria T. Acosta, MD, Children's National Health System & George Washington University

Funding: CTF (PEP4NF to Maria T. Acosta, MD) and The Gilbert Family NF Institute (to Maria T. Acosta, MD)

Dedicated Social Media for Adolescents and Parents of Adolescents with Neurofibromatosis Type 1

Session 7A: Monday, June 20, 3:35pm – 3:55pm

Nicole Ullrich, MD, PhD, *Department of Neurology, Boston Children's Hospital, Harvard Medical School*

Objective: To evaluate Children's at Home (C@H), a dedicated social media website created for adolescents with NF1 and parents of adolescents with Neurofibromatosis Type 1 (NF1).

Methods: This interventional study had two cohorts and comprised of 2 phases: (1) Creating Video Intervention/Prevention Assessment (VIA) visual narratives about a) experiences among adolescents with NF1 ages 13-18 and b) parent experiences having an adolescent with NF1; (2) the two cohorts interacted separately on C@H, a secure, medically-moderated social media website. C@H was evaluated qualitatively at 3 time points. Parents also completed the Behavior Assessment System for Children (BASC-II-P) and Impact of Pediatric Illness (IPI) scale.

Methods: This interventional study had two cohorts and comprised of 2 phases: (1) Creating Video Intervention/Prevention Assessment (VIA) visual narratives about a) experiences among adolescents with NF1 ages 13-18 and b) parent experiences having an adolescent with NF1; (2) the two cohorts interacted separately on C@H, a secure, medically-moderated social media website. C@H was evaluated qualitatively at 3 time points. Parents also completed the Behavior Assessment System for Children (BASC-II-P) and Impact of Pediatric Illness (IPI) scale.

Results: Thirteen adolescents and 17 parents participated in the pilot feasibility study and created visual self-narratives for 2-8 months. Many adolescents and parents of adolescents with NF1 reported little experience speaking or interacting with others with NF1. Participants valued the opportunity to speak about the challenges faced with NF1 and their journeys since diagnosis. Adolescents with NF1 were successful in creating visual illness narratives and reported loneliness and desire to connect with others who have NF1. Parents reported connecting with other parents of children with NF1 for the first time, valuing the "real faces" and emotions of shared experiences providing a sense of normalcy. C@H decreased feelings of isolation, provided relief to talk about NF1, new knowledge, a platform to address psychosocial issues never discussed in clinic, and put their lives into perspective.

Conclusions: Many adolescents and parents of adolescents living with NF1 feel isolated in their experience. Innovative applications of social media dedicated to caregivers can provide peer-to-peer support, shared experience, and reliable medical information. Future analyses will also assess the effects of the interactions with others with NF1 through the social media site on quality of life, ratings of disease severity, and social-emotional functioning.

Author List: Nicole Ullrich¹, Christina Akre¹, Julie Polvinen¹, Pamela Wolters², Michael Rich¹. ¹Boston Children's Hospital, Boston, MA; ²National Cancer Institute, Bethesda, MD.

Sources of support: Children's Tumor Foundation/Clinical Research Award (NU); Swiss National Science Foundation (CA).

SESSION 7B: Cellular Pathophysiology in Neurofibromatosis Type 2 and Schwannomatosis

Chairs: Wei Li, PhD, *Penn State Milton S. Hershey Medical Center*; Helen Morrison, PhD, *Leibniz Research Institute for Aging*

Factors secreted by SMARCB1 Mutant Schwann Cells Contribute to Schwannomatosis Pain

Session 7B: Monday, June 20, 2:35pm – 2:55pm

Lawrence Sherman, PhD, *Oregon Health and Science University*

Patients with schwannomatosis often present with intractable pain. This pain can occur in the absence of a detectable mass, and is not always relieved by tumor resection. These clinical findings suggest that the pain afflicting schwannomatosis patients is not strictly linked to tumor growth or mechanical nerve compression by schwannomas. A significant proportion of patients with schwannomatosis have mutations in the *Snf5* gene (also called *INI1*, *BAF47* and *SMARCB1*). We found that inducible conditional disruption of the *Snf5* gene in mouse Schwann cells does not lead to changes in peripheral nerve morphology, Schwann cell proliferation or alterations in cell cycle-related gene expression in peripheral nerves. However, mice with targeted disruption of *Snf5* in Schwann cells demonstrate behavioral phenotypes consistent with chronic pain. We find that dorsal root ganglion (DRG) neurons from mice with Schwann cell-targeted disruption of *Snf5* express elevated levels of the TRPV1, a non-selective cation channel that can be activated by a number of noxious stimuli including capsaicin. We also find that TRPA1, an ion channel that acts as a sensor for environmental irritants, and the calcitonin gene related peptide (CGRP), which has been implicated in pain signaling, are elevated in our *Snf5* mutant mice. Wild type DRG cells grown in *Snf5*-null Schwann cell conditioned media for 1 week demonstrated elevated cobalt uptake, a marker of TRPV1 activity, compared to cells grown with wild type Schwann cell conditioned media. Consistent with these findings, DRG cultures treated with *Snf5*-null Schwann cell conditioned media expressed elevated levels of TRPV1, TRPA1 and CGRP as indicated by immunocytochemistry. Collectively, these data indicate that loss of *Snf5* in Schwann cells leads to the secretion of a factor or factors that induce the expression of pain mediators in sensory neurons, and suggest a mechanism for schwannomatosis pain. We are currently performing proteomic analyses to identify these factors with the aim of identifying therapeutic targets that block pain in schwannomatosis patients.

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Merlin Controls the Proliferation and Repair Capacity of Schwann Cells Following Injury by Regulating Hippo/YAP Activity

Session 7B: Monday, June 20, 2:55pm – 3:15pm

David Parkinson, PhD, Plymouth University, UK

Loss of the Merlin tumour suppressor and activation of the Hippo signalling pathway play major roles in the control of cell proliferation and fate. We have identified completely novel roles for Merlin and the Hippo pathway effector Yes Associated Protein or YAP in events of Schwann cell plasticity and peripheral nerve repair following injury. Loss of Merlin and aberrant signalling through the Hippo pathway drive abnormal Schwann cell behaviour and an ongoing inflammatory state of the nerve following injury. Injury to the peripheral nervous system (PNS) causes the generation of repair-competent Schwann cells, which direct functional repair and we find that loss of Merlin in these cells causes a catastrophic failure of axonal regeneration and remyelination in the PNS. Further analysis shows that it is activation of YAP expression in Merlin null nerves that mediates this effect as loss of YAP restores axonal regrowth, functional repair and resolution of the immune response to injury. This work identifies new mechanisms to understand the regenerative potential of Schwann cells and to understand events in driving the uncontrolled cell proliferation and generation of schwannoma tumours.

Author List: Additional Authors: Thomas Mindos¹, Xin-peng Dun¹, Katherine North¹, Robin D. S. Doddrell¹, Alexander Schulz², Philip Edwards³, James Russell¹, Bethany Gray¹, Sheridan Roberts¹, Aditya Shivane³, Georgina Mortimer¹, Nailing Zhang⁴, Duoia Pan⁴, Helen Morrison²

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Platform: Angiotensin Phosphorylation Regulates Scaffolding Function and Localization of YAP at the Plasma Membrane

Session 7B: Monday, June 20, 3:15pm – 3:35pm

Susana Moleirinho, PhD (Young Investigator Awardee), The Scripps Research Institute

Contact inhibition (CI) of cell proliferation is a critical biological process for the maintenance of normal tissue homeostasis. Increasing evidence has shown that its disruption contributes to tumour development. However, the molecular mechanisms underlying the loss of CI remain poorly understood. Merlin, the NF2-coded protein, localizes at regions of cell-cell contact thus coordinating intercellular contact and subsequent inhibition of growth-promoting signaling. Recent reports identified Angiotensins as critical effectors of Merlin's growthsuppressive function behaving as a positive regulator of Rac1 and Ras/MAPK signaling and the Hippo/YAP signaling, linking it to a number of pathways regulated by Merlin.

Previous findings implicate the Hippo/YAP pathway in regulation of cell CI, proliferation, and tumorigenesis. To establish the function of the Hippo/YAP pathway and determine whether breakdown of the mechanisms known to control CI play a role in NF2, we employed both biochemical and genetic approaches to modulate the expression of wild type and mutated alleles of Angiotensin and effectors of the Hippo/YAP pathway. We found that Merlin, Angiotensin, and YAP form a complex, and Angiotensin is required for this association. Moreover, through diverse immunoprecipitation analyses and subcellular fractionations we found that Angiotensin phosphorylation status is not necessary for the complex formation, but determines the localization of the complex either towards the nucleus or at the plasma membrane. At the plasma membrane, Amot binds Pals1 bringing together the Crb3/Pals1/Patj complex and Merlin/Amot/YAP1 complex. Furthermore, we found that Angiotensin phosphorylation site modulates cell numbers, cell proliferation, and YAP's transcriptional activation. We propose that phosphorylation of Amot is a post translational modification that inhibits YAP's ability to promote tumorigenesis by altering its subcellular localization.

This work is made possible through the Children's Tumor Foundation Young Investigator Award.

Author List: Sany Hoxha, BS, The Scripps Research Institute; Vinay Mandati, PhD, The Scripps Research Institute; Joseph Kissil, PhD., The Scripps Research Institute.

Platform: Global Proteomic Analysis of the Merlin Interactome by Proximity Biotinylation

Session 7B: Monday, June 20, 3:35pm – 3:55pm

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

Neurofibromatosis Type 2 is an inherited neoplastic disease 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 via the proteins with which it interacts. However, there is no consensus as to which protein interactions are necessary for tumor suppression. We used a powerful new technique, proximity biotinylation, to perform a global proteomic analysis of the merlin interactome. We generated fusions between BirA^{R118G} and wild type merlin, a PIP₂ binding mutant, merlin-6N, and two conformational mutants, merlin-FH and merlin-AR. We identified 53 merlin-associated proteins that met our selection criterion, 42 of which were specifically biotinylated by wild type merlin but not the 6N mutant, indicating proteins that require PIP₂ bound merlin to interact. Furthermore, 21 out of the 53 wild type proteins were biotinylated by wild type merlin-BirA^{R118G} but not Merlin-FH-BirA^{R118G}, identifying proteins that either require merlin in the closed conformation or those that interact with the C-terminus. Gene ontology enrichment analysis identified 30 of the 53 wild type biotinylated proteins as components of adherens junctions, tight junctions and/or focal adhesions, 17 actin-binding proteins and 12 members of the Hippo pathway. One potential merlin partner, afadin, is an actin binding protein that is required to form tight junctions and adherens junctions in other cell types. We show that afadin physically interacts with merlin and afadin loss phenocopies the effects of merlin loss on apoptosis, suggesting that afadin is a critical link between cell junction mediated homeostatic signal transduction in Schwann cells. We describe direct interaction assay systems using purified proteins to reveal the structure and function of the merlin complex *in vitro*. This work significantly expands the scope of potential merlin interactions in key subcellular structures and supports an essential role for merlin at Schwann cell junctions. Afadin's role in forming cell junctions, its interactions with multiple signal transduction networks and its physical interaction with merlin, suggests that, together, these molecules are critical for adherens and tight junction mediated tumor suppression in Schwann cells.

Author List: Ami V. Patel, PhD, Cincinnati Children's Hospital; Steven Guard, BS, University of Colorado; Nancy Ratner, PhD, Cincinnati Children's Hospital.

Funded by: Department of Defense, Congressionally Directed Medical Research Programs; Neurofibromatosis Research Program; New Investigator Award NF120118 to Robert Hennigan.

KEYNOTE 4: Using Pluripotent Stem Cells to Understand Human Neural Crest Development and Disease

Monday, June 20, 4:15pm – 5:15pm

Stephen Dalton, PhD, *Center for Molecular Medicine and Department of Biochemistry and Molecular Biology, University of Georgia*

Human pluripotent stem cells (hPSCs) have great potential in regenerative medicine, tissue engineering and disease modeling because of their ability to generate a wide-range of therapeutically-relevant cell types. This presentation will focus on the generation of neural crest stem cells (NCSCs) from hPSCs and how they can be used to understand development of the neural crest lineage and their differentiation towards cells of the peripheral nervous system. Using molecular and cellular approaches we are working towards an understanding of cell division as NCSCs transition towards peripheral neurons and the characterization of gene regulatory networks underpinning these cell fate decisions. These studies should provide foundational information to better understand the molecular and cellular basis of tumors with a neural crest origin.

KEYNOTE 5: New Ways of Targeting RAS

Tuesday, June 21, 8:00am – 9:00am

Frank McCormick, PhD, UCSF Helen Diller Family Comprehensive Cancer Center and the Frederick National Lab for Cancer Research

Activating mutations in K-Ras occur frequently in many human cancers, and are mutually exclusive with other mutations that activate the MAPK pathway, such as EGF-R and other receptor tyrosine kinases upstream, loss of neurofibromin, loss of Spred1 and activation of B-Raf. Neurofibromin and Spred1 form a functional complex that inactivates Ras by converting Ras.GTP to Ras.GDP. Neurofibromin has a GAP domain, and Spred1 is essential for directing neurofibromin to the plasma membrane. Binding of neurofibromin to Spred1 is regulated by phosphorylation, and is constitutively blocked by activated receptor tyrosine kinases (RTKs). This may allow Ras.GTP levels to remain high in cancer cells driven by RTK signaling. We speculate that locking this kinase would activate neurofibromin and so shut down RAS signaling.

Cells transformed by KRAS display stem-like properties This is due to K-Ras's unique ability to bind calmodulin, and to inhibit calmodulin-dependent kinase. Part of the stem-ness program initiated by K-Ras involves secretion of the cytokine LIF, an IL-6 family member with a unique role in maintaining stem-ness. Neutralization of LIF with a monoclonal antibody, or ablation of LIF expression using siRNA or CRISPR, reduces stem-ness and sensitizes established pancreas tumors to gemcitabine. LIF activates a unique set of downstream pathways, distinct from IL-6 and other members of this family of cytokines. This pathway may account for K-Ras's unique ability to promote stem-ness. The existence of a new effector pathway specific for K-Ras therefore offers new opportunities for therapeutic intervention.

The K-Ras protein has been deemed un-druggable, as it does not contain pockets or active sites that can be exploited by traditional medicinal chemistry. However, recent advances in chemical biology have enabled new approaches towards targeting K-Ras, including tethering and fragment-based NMR screening. We have also used a technique referred to as Second Harmonic Generation to screen for compounds that cause allosteric changes in K-Ras. This technique is extremely sensitive to small changes in orientation of a protein relative to a membrane surface, and has enabled us to identify compounds that bind to K-Ras, in a GTP-dependent or GDP-dependent manner, and to promote distinct conformational changes in the protein. We hope that this new method will lead to a new series of compounds that modulate K-Ras function.

K-Ras processing can be prevented by compounds that block the CAAX box cysteine. We have identified compounds that interact covalently with this cysteine and block prenylation by farnesyl transferase or geranylgeranyl transferase. These compounds reduce expression of K-Ras in cells and have specificity for K-Ras relative to H-Ras and N-Ras and represent another approach to targeting K-Ras specifically.

SESSION 8: New Preclinical Models in NF

Chairs: D. Wade Clapp, MD, *Indiana University*; Thomas deRaedt, PhD, *Harvard University/Brigham and Women's Hospital*

The Use of New Mouse Models for Preclinical Development

Session 8: Tuesday, June 21, 10:20am – 10:40am

Thomas deRaedt, PhD, Brigham and Women's Hospital/ Harvard University

Immunotherapy is at the forefront of cancer research. Observed clinical responses, with for example PD1-antibody checkpoint blockade, can be spectacular and durable. Unfortunately however these responses are only observed in a small proportion of the patient population and are often dependent on a favorable immune microenvironment in the tumor. The research community is building on these early successes and many efforts being made to develop combination therapies that improve the response rates of immunotherapy. One approach is the identification of drugs that result in a more favorable immune microenvironment, enhancing the probability of immune checkpoint blockade to work. Unfortunately, when designing classical targeted therapies the cancer community usually solely focuses on the effect a drug has on the malignant cells and rarely considers effects on the microenvironment.

The combination of MEK (PD-0325901) and BRD4 inhibitors (JQ1) potently kills MPNST cells by synergistically inhibiting the RAS transcriptional output. Surprisingly, upon combined inhibition of MEK and BRD4, we observe a rapid (within 3 days) influx of CTLs (CD8 positive T-cells) in the tumor. These CD8 T-cells elicit an anti-tumor response. Our data suggests that for MPNSTs, non-cell autonomous mechanisms contribute to tumor shrinkage induced by MEK and BRD4 inhibition *in vivo*. The elevated number of CD8 positive T-cells present in the tumor dramatically increases the CD8/Treg ratio; a ratio used as a measure for success of immunotherapy in the clinic.

Importantly the majority of the CD8 T-cells present in the MPNSTs express markers of exhaustion (PD1). It was thus not surprising that addition of the anti-PD1 antibody to our PD-0325901/JQ1 therapy significantly enhanced tumor regression in our MPNST model. Markedly, the anti-PD1 antibody as a single agent or in combination with MEK inhibitors has no effect on the tumor response. MPNST cells are exquisitely sensitive to BRD4 and MEK inhibition. Tumor cell intrinsic effects could thus be required to induce the, for immunotherapy, more favorable immune microenvironment. Excitingly however we showed that this is not the case, indicating that BRD4 inhibition could enhance the immune microenvironment in a large number of tumors.

Because of the favorable effects BRD4 inhibition has on the immune-microenvironment we hypothesized that BRD4 inhibition as a mono therapy could prevent formation of MPNSTs by eliciting an immune response against early lesions. Developing a prevention therapy for MPNSTs is important especially for those patients with a high burden of plexiform neurofibromas or NF1 microdeletion patients. Importantly BRD4 inhibition prevents tumor formation in our mouse model of NF1 microdeletion patients (Nf1/P53/Suz12) and in an allograft MPNST model, suggesting that a prevention therapy approach could be a viable option for patients with a high risk for MPNSTs.

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Molecular Mechanisms of Autism Spectrum Disorder Phenotype in NF1

Session 8: Tuesday, June 21, 11:00am – 11:20am

Anantha Shekhar, MD, PhD, Indiana University

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with mutation in one copy of the *NF1* gene (*NF1*^{+/−}) and a substantial number of children with NF1 suffer from learning, attention problems and social and communication deficits similar to autism spectrum disorders (ASD). Thus, mouse models with loss of single *Nf1* allele (*Nf1*^{+/−} mice) that recapitulate human NF1 phenotypes provide a unique opportunity to study the brain circuits and molecular mechanisms underlying the ASD-like symptom phenotypes in a Research Domain Criteria (RDoC) consistent manner. The *NF1* gene encodes neurofibromin, a protein known to function as a GTPase activating protein (GAP) that negatively regulates p21^{ras} (Ras) activity. We have demonstrated that *Nf1*^{+/−} mice exhibit specific deficits in social learning and long-term potentiation in the basolateral amygdala (BLA), a key region for social learning. Either genetic or pharmacological blockade of increased Ras activation in the BLA of *Nf1*^{+/−} mice rescues the social behavioral deficits. *Nf1*^{+/−} mice also exhibit communication difficulties (ultrasonic vocalizations, USVs) but the underlying mechanisms of USV deficits in *Nf1*^{+/−} mice are currently unknown. *Nf1* mutant mice with the 23a alternate splicing in the GAP binding element of neurofibromin (23a^{−/−} mice) exhibit only the communication, but not the social learning deficits. This suggests that disruptions in other than Ras signaling mechanism regulate the communication deficits, providing us with unique model systems to dissociate these two ASD-like symptom networks and study them separately. The presentation will provide approaches to utilizing *Nf1*^{+/−} mice as a tool to understand the different brain networks and molecular pathways involved in two different ASD-associated symptoms of socialization and communication deficits.

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Platform: Modeling the Tumor Microenvironment in MPNSTs: The Impact on Tumor Biology and Chemotherapeutic Response

Session 8: Tuesday, June 21, 11:20am – 11:40am

Rebecca Dodd, PhD, Duke University Medical Center

NF1 haploinsufficiency is a hallmark of Neurofibromatosis Type 1 and is critical for neurofibroma development. The role of the supporting stroma (endothelial cells, immune cells, fibroblasts, etc) in the development of patient neurofibromas is well-established. However, the impact of NF1 haploinsufficiency on MPNST biology is unclear. Sporadic MPNSTs arise in patients with NF1 wild-type stroma (NF1 +/+), while NF1-associated MPNSTs arise in Neurofibromatosis patients with NF1 haploinsufficient stroma (NF1 +/-). Patients with NF1-associated MPNSTs have worse outcome than patients with sporadic MPNSTs, but the cause of this difference is under debate. To define the impact of stromal genetics on tumor biology, we have developed unique mouse models that reflect the genetics of patient-associated MPNSTs.

Using paired littermate mice, we are modeling the genetic status of NF1 in the tumor microenvironment of different MPNST patient populations. We use Adenovirus-Cre injections to generate MPNSTs in NF1 **Flox/Flox**; Ink4a/Arf Flox/Flox and NF1 **Null/Flox**; Ink4a/Arf Flox/Flox paired littermate mice to model tumors from NF1 wild-type and NF1-associated patients, respectively. We have used this system to determine how different genetic contexts in the stroma (NF1 +/+ or NF1 +/-) can influence the biology of genetically identical tumors (NF1 +/-). We have examined multiple tumor phenotypes (growth kinetics, pathology, cell signaling) and stromal features (immune cells, tumor vasculature, and fibroblast function) in MPNST samples from these mice. We have determined that NF1 haploinsufficiency (NF1 +/- stroma) influences tumor onset and the tumor microenvironmental phenotype in these mice.

In parallel, we are examining the contribution of NF1 haploinsufficient stromal cells to therapeutic response. Whether NF1 haploinsufficiency affects the response of MPNSTs to chemotherapy is an important, unanswered clinical question. Currently, we are performing a mouse “co-clinical trial” (based on SARCO06) to compare the efficacy of multi-agent chemotherapy in MPNSTs from paired littermate mice with NF1-haploinsufficient or NF1 wild-type stroma. These experiments will expand our understanding of the tumor microenvironment in Neurofibromatosis, which could have important clinical implications for NF1 patients.

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CONSORTIA UPDATES 2

Chairs: Brigitte Widemann, MD, *NCI*; Brian Weiss, MD, *Cincinnati Children’s Hospital*

The Neurofibromatosis Therapeutic Consortium

Tuesday, June 21, 11:40am – 11:55am

Ophelia Maertens, PhD, Brigham and Women’s Hospital/Harvard University

The Neurofibromatosis Therapeutic Consortium (NFTC) is a multi-center cooperative group that integrates expertise and resources from philanthropic foundations, academia, and industry to promote and accelerate the development of effective new treatments for Neurofibromatosis Type 1 (NF1). Since 2008, the NFTC has utilized genetically accurate mouse models of benign and malignant tumors associated with NF1 as a platform for testing candidate clinical compounds as well as FDA-approved drugs, alone and in combination. To date, the participating labs have conducted 116 individual preclinical trials of 49 agents through collaborations with 20 pharmaceutical companies. Notably, these preclinical studies have directly informed the development of 15 human therapeutic trials. Based on the power of the animal models being utilized and the rate of throughput from this Consortium, the data generated by the NFTC has thus significantly accelerated translation by providing clinical investigators and pharmaceutical companies with a strong rationale for implementing human trials in NF1 patients using the best clinical candidates against the most promising therapeutic targets. By streamlining and expediting the progression of promising therapies to clinical trials, the NFTC is making a significant impact on the clinical care of NF1 patients.

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Specimens and Standards: Banking on Gold for Biomarker Development in Neurofibromatosis

Tuesday, June 21, 12:40pm – 12:55pm

Carolyn Compton, MD, PhD, *Arizona state University, National Biomarker Development Alliance, and Complex Adaptive Systems Institute*

Biomarkers are essential to the central vision of contemporary and future medicine but are sorely lacking for entire classes of diseases, such as neurofibromatosis. Biomarkers support informed treatment decisions, enable earlier diagnosis and drive the development of molecular therapies. Thus, they may represent the greatest potential value in biomedicine today. Despite huge investments in biomarker development and exponential growth in analysis technologies, there remains a staggering asymmetry between the thousands of publications describing candidate biomarkers and the small fraction that progress to clinical implementation. A major contributor to this problem is the huge variation in the quality of patient biospecimens that are the starting materials for molecular analysis in both patient care and translational research, a problem magnified for dispersed patient populations with rare diseases. The quality of biospecimens directly determines the quality and validity of the derived analysis data. However, the method of acquisition as well as the collection, processing, transport and storage variables have the capacity to change both the molecular quality and the molecular composition of specimens. Unless pre-analytical variables are understood, controlled when possible, and recorded to document all critical pre-analytical steps, analysis results may be misleading or frankly incorrect. The analyses may reflect only artifact, not disease biology. As analysis tools become more powerful, patient management becomes more dependent on analysis results that determine therapy, and medical progress dependent on data quality and reproducibility, the impact of pre-analytical variation becomes more profound. For neurofibromatosis, the implementation and enforcement of evidence-based specimen standards for a richly annotated and uniformly consented bank of patient samples will be the essential foundation for biomarker development in this important set of diseases.

Poster Presentation (odd numbers)

SUNDAY, JUNE 19, 2016 (5:20 – 6:50 PM)

LAST	FIRST	POSTER	TITLE
Allaway	Robert J.	1	NF1 Dysregulated Tumor Cells are Susceptible to Perturbation of Proteolytic Mechanisms and Mitochondrial Health
Aschbacher-Smith	Lindsey E.	3	Simultaneous Inhibition of the MAPK and STAT3 Signaling Pathways Demonstrates Efficacy in a Preclinical Mouse Model of Neurofibromatosis Type 1
Bornhorst	Miriam	5	The Role of MEK-inhibitors for the Prevention of Optic Pathway Gliomas in an Nf1-Deficient Mouse Model
Brar	Komalpreet	7	A Comparison of PedsQL scores between Neurofibromatosis-1 and Juvenile Idiopathic Arthritis Patients
Chiara	Federica	9	Mechanotransduction and NF1 Loss: Partner in Neurofibroma Development
Chinthalapudi	Krishna	11	Lipid-binding Mediates Merlin Conformations and Tumor Suppressive Activity
Choi	Kwangmin	13	An Inflammatory Gene Signature Distinguishes Neurofibroma Schwann Cells and Macrophages from their Counterparts in the Normal Peripheral Nervous System
Field	Jeffrey	15	The NF1 and NF2 Pharmacome Project, a Course in High throughput Screening to Identify Targets and Profile the Sensitivity of MPNST Cells to Candidate Drugs
Fuse	Marisa A.	17	Dasatinib, an Approved Src Kinase Inhibitor, Reduces Viability of Human and Mouse Merlin-Null Schwann Cell Lines
Gosline	Sara JC	19	Chemo-Genomic Analysis of Plexiform Neurofibroma Cell Lines
Gosline	Sara JC	21	The Molecular Landscape of Dermal Neurofibromatosis
Guerrant	William	23	Small Molecule Inhibition of the Hippo-YAP Pathway as a Therapeutic Strategy in Neurofibromatosis Type 2
Hanemann	Oliver	25	Cellular Prion Protein (PrP ^C) Promotes Schwannoma Tumour and Genesis
Hsiao	Meng-Chang	27	Alternative Outcomes of Pathogenic Complex Somatic Structural Variations in the Genomes of NF1 and NF2 Patients
Ikuta	Kunihiro	29	Antitumor Effects of 4-Methylumbelliferone, a Hyaluronan Synthesis Inhibitor, on Malignant Peripheral Nerve Sheath Tumor
Jecrois	Emmanuelle	31	The Role of NF1-regulated MAPK/ERK Pathway in Optic Glioma Formation
Keller	Bryant	33	Stromal Targeting in the Schwann Cell Tumor Microenvironment to Enhance Chemotherapy Penetrance
Khanna	Rajesh	35	CRISPR/Cas9-Based Gene Editing of Nf1: A New Rat Model of Neurofibromatosis Type 1 (NF1)?
Ki	Dong Hyuk	37	Drug Discovery for NF1-Associated Malignant Peripheral Nerve Sheath Tumors using the Zebrafish Model
Kraniak	Janice M.	39	Development of 3D Organotypic Models of Human NF1 Plexiform Neurofibromas for Drug Screening
Kuninaka	Shinji	41	Neurofibromatosis Type I Model Medaka Fish
Largaespada	David A.	43	Development and Characterization of a Swine Model of Neurofibromatosis Type I
Li	Kairong	45	Genome Editing of <i>Nf1</i> ^{+/-} iPS Cells Derived from NF1 Patients
Liao	Chung-Ping	47	Contributions of Stem Cell Factor and <i>Nf1</i> Heterozygosity to Neurofibroma Tumor Microenvironment
Look	A. Thomas	49	Molecular Pathogenesis and Drug Synergism in a Zebrafish Model of High Risk Neuroblastoma
Messiaen	Ludwine	51	Changing a Winning Horse: Towards a New Approach for NF1-Diagnostics
Moutal	Aubin	53	Relief of Cephalic Pain by (S)-Lacosamide in an Experimental Model of Headache
Oblinger	Janet	55	ErbB3 and IGF-1R Blockade as a Potential Treatment for Vestibular Schwannomas and Meningiomas
Patel	Ami V.	57	An shRNA Screen Identifies <i>MEIS1</i> as a Driver of Malignant Peripheral Nerve Sheath Tumors
Patil	Dipak N.	59	Understanding the GPCR Driven Interaction of NF1 with G proteins
Rasola	Andrea	61	Neurofibromin Deficiency Induces Oncogenic Metabolic Changes through ERK-Dependent Phosphorylation of the Mitochondrial Chaperone TRAP1
Reilly	Karlyne M.	63	Pathway Analysis of Genes Inhibiting Proliferation of Malignant Peripheral Nerve Sheath Tumor Cells
Reiners, Jr.	John J.	65	MRI Monitoring of Anti-growth Activities of Prenylation Inhibitors on NF1 MPNST Sciatic Nerve Xenografts
Steensma	Matthew	67	Genomic Alterations Drive MET-Dependency in Murine MPNSTs
Tahaei	Seyedmohammad Ebrahim	69	Role of Ectopic EGFR Signaling in NF1 Pseudoarthrosis
Tandon	Preeti	71	Classic Ras Proteins Promote Growth of Neurofibromin Null Schwann Cells
Turner	Ashley N.	73	A Myeloproliferative Nonsense NF1 Mouse Model for Nonsense Suppression Therapy Intervention
Wallace	Peggy	75	Genomic Characterization of Immortalized NF1 Schwann cells
White	Katherine	77	Developing a Novel Porcine Model for Neurofibromatosis Type 1

POSTERS: Basic Science

Poster Presentation (odd numbers)

SUNDAY, JUNE 19, 2016 (5:20 – 6:50 PM)

LAST	FIRST	POSTER	TITLE
Williams	Kyle B.	79	Characterization of NF1 Deficient Immortalized Human Schwann Cell Lines, their Use to Explore Synthetic Lethality and the Synodos for NF1 Drug Discovery Pipeline
Williams	Rory L.	81	<i>In vivo</i> and <i>in vitro</i> Functional Characterization of Mutations Cooperating with NF1 Loss in Schwann Cell Transformation and Tumorigenesis
Wood	Matthew D.	83	Recurrent Sporadic Glioblastoma retains Neurofibromin Protein Expression: An Immunohistochemical Analysis of 20 Cases
Wu	Jianqiang	85	RUNX Function in Neurofibroma Tumorigenesis and Therapy
Xu	Jiajie	87	Yorkie, a Transcriptional Co-Activator that Regulates Growth, also Functions at the Cell Cortex to promote Cytoskeletal Tension
Xu	Lei	89	Blocking HGF/cMET Pathway Enhances Radiation Efficacy in NF2 Schwannoma Model
Yates	Charles	91	AR42 Decreases Growth of Schwannoma in an NF2 mouse model

NF1 Dysregulated Tumor Cells are Susceptible to Perturbation of Proteolytic Mechanisms and Mitochondrial Health

Robert J Allaway, BS, Geisel School of Medicine, Dartmouth College

Neurofibromatosis type 1 (NF) is a disease caused by mutation of neurofibromin 1 (*NF1*), loss of which results in hyperactive Ras signaling and a concomitant increase in cell proliferation and survival. Patients with NF are susceptible to tumors such as malignant peripheral nerve sheath tumors (MPNSTs), and loss of *NF1* is also observed in sporadic cancers such as glioblastoma (GBM) and ovarian cancer. A targeted therapy that inhibits tumor-initiating and -promoting cells would substantially advance our ability to treat tumors that develop as a result of NF1 loss.

To discover molecules that may target tumors with NF1 deficiency, we conducted a synthetic lethal screen using a yeast model of NF1/Ras dysregulation.¹ Wild type and *nf1-delta* yeast were screened against >12,000 compounds; we identified 108 compounds with selectivity for *nf1-delta* yeast. These molecules were subsequently tested in mammalian models of NF1 loss. One lead molecule from this screen (Y100) appears to impact autophagic flux and alter the mitochondria. Overexpression of autophagy or mitochondrial genes in *nf1-delta* cells caused resistance to Y100.

Treatment of NF1 deficient human GBM cells with Y100 led to accumulation of p62 and K63/K48 ubiquitin-linked proteins at concentrations that reduce viability/growth, suggesting that Y100 modulates some form of autophagy. Additionally, Y100 treatment of NF1-deficient cells induced the formation of polarized mitochondrial hotspots, induction of mitochondrial superoxide, and loss of the mitochondrial heat shock chaperone protein Tid1. Finally, the ROS scavengers N-acetyl cysteine and beta-mercaptoethanol abrogated the effect of Y100. Taken together, these data suggest that Y100 targets NF1 deficient cells by modulating proteolytic mechanisms, altering mitochondrial health, and inducing reactive oxygen species.

¹ Wood, M. et al. Discovery of a small molecule targeting IRA2 deletion in budding yeast and neurofibromin loss in malignant peripheral nerve sheath tumor cells. *Mol. Cancer Ther.* 10, 1740–1750 (2011).

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Funding: Children's Tumor Foundation Young Investigator Award 2014-01-12. NINDS R21 NS060940. NINDS R01 NS095411-01A1

Simultaneous Inhibition of the MAPK and STAT3 Signaling Pathways Demonstrates Efficacy in a Preclinical Mouse Model of Neurofibromatosis Type 1

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

Abstract: Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder, which affects approximately 1 in 3,500 individuals. Biallelic loss of the NF1 gene causes the formation of benign peripheral nerve tumors, neurofibromas, which are difficult to treat and associated with morbidity and potential mortality. Using an *Nf1^{flx/flx};DhhCre* mouse model, we show that pharmacological inhibition of the MAPK and STAT3 pathways induces significant reduction of neurofibroma volume. We show that this combination therapy not only inhibits proliferation, but also induces apoptosis within the tumor—a result not yet achieved through previous neurofibroma treatment paradigms.

Author List: Tilat A. Rizvi, PhD, Cincinnati Children's Hospital Medical Center ; Mitch Springer, PhD, Cincinnati Children's Hospital Medical Center ; Jianqiang Wu, MD, Cincinnati Children's Hospital Medical Center ; Eva Dombi, MD, National Cancer Institute; Richard S. Dunn, BS, Cincinnati Children's Hospital Medical Center ; Mi-Ok Kim, PhD, Cincinnati Children's Hospital Medical Center ; Nancy Ratner, PhD Cincinnati Children's Hospital Medical Center

Funding: Children's Tumor Foundation.

The Role of MEK-Inhibitors for the Prevention of Optic Pathway Gliomas in an *Nf1*-deficient Mouse Model

Miriam Bornhorst, MD, *Children's National Health System, Washington, DC*

Background: Neurofibromatosis 1 (NF1) is a genetic syndrome characterized by increased activation of the Ras/ERK pathway. Up to 20% of patients with NF1 are diagnosed with optic pathway gliomas (OPGs) before 7 years of age, suggesting they are formed during development. Previous studies have shown that MEK-inhibitors (MEKi) can be used to prevent structural abnormalities in the developing corpus callosum and cerebellum of *Nf1*-deficient mice (1,2). Thus, we hypothesize that early postnatal treatment with a MEKi that has good blood brain barrier (BBB) penetration can also prevent OPG formation in mice.

Methods: We first tested the biological activity of four different MEKis (PD0325901, GSK1120212, MEK162 (ARRY162), and AZD6244) in the brains of adult and postnatal day 8 (P8) mice. Then, using a previously established MEKi-in-milk preventative protocol, we treated *Nf1*-deficient mice that develop OPGs around P60 with PD0325901 from P0.5-P21. Following preventative treatment, we analyzed the nerves at P21 and P60 to look for short and long term response.

Results: All of the MEKis inhibit pERK in the mouse brain, although PD0325901 has the best potency and very good BBB penetration. Following treatment with PD0325901 from P0.5-P21, *Nf1*-deficient mice had significant improvement in nerve size, glial cell number, and pERK expression at both P21 and P60.

Conclusion: Any of the MEKis tested can be used for treatment/preventative studies in mice, although the potency and BBB penetration of each MEKi is different. Importantly, early postnatal treatment with a MEKi prevents the glial pathology seen in optic nerves both immediately after treatment, and after long term removal of the drug in an *Nf1*-deficient mouse model. This suggests that a similar treatment strategy could potentially delay or prevent OPG formation in patients with NF1, thus improving morbidity and overall outcomes. Additional studies are being done to develop a MEKi treatment plan with the best overall histological and functional response and minimal toxicity.

References: 1. Wang, Y., et al., *Cell*, 2012, 150(4): p. 816-30. PMID: 22901811
2. Kim, E., et al., *Elife*, 2014, PMID: 25535838

Author: Emmanuelle Jecrois, BA, Children's National Health System, Yuan Wang, PhD, Children's National Health System, Daphine Mugayo, BS, Children's National Health System, Yuan Zhu, PhD, Children's National Health System

A Comparison of PedsQL Scores Between Neurofibromatosis-1 and Juvenile Idiopathic Arthritis Patients

Komalpreet Brar, MD, *Cardinal Glennon Children's Hospital, St. Louis University.*

Objective: Quality of life in children and adolescents with neurofibromatosis type 1 (NF1), including related to pain, was compared to children and adolescents with juvenile idiopathic arthritis (JIA), a disorder associated with frequent pain.

Materials and Methods: Data from patients who reported pain in the NF1 study by Chrusciel et al. were compared to JIA data. A literature review of studies on quality of life in JIA was performed, using the publications list provided on the PedsQL (James Varney) website. Criteria for inclusion in the review: articles utilizing the PedsQL Generic core scales version and articles reporting the subdivisions of the PedsQL scale. Scores were statistically compared as a function of NF1 vs. JIA.

Results: No statistically significant differences were found for the PedsQL total scores. PedsQL physical score was lower in JIA, relative to NF1, for the 8-12 year old range ($p=.013$), males ($p=.016$), and in German and Australian samples ($p<.002$ and $.02$, respectively). Females with JIA had significantly ($p=.03$) higher emotional PedsQL scores than females with NF1. JIA patients in Italy ($p=.025$) and the USA ($p=.01$) had significantly higher social PedsQL scores than NF1 patients. JIA patients had higher scores than NF1 patients on the school PedsQL scores in both the German ($p=.038$) and Australian ($p=.008$) samples.

Discussion: The disease process of NF1 has a large physical component of morbidity (café au lait spots and cutaneous neurofibromas), along with pain. Patients with JIA have a physically debilitating disease process during acute flares. This difference may explain lower PedsQL scores in JIA relative to NF1. In NF1, learning disabilities (ADHD and mental retardation) may explain lower PedsQL school and social scores. The results suggest that patients with NF1, with respect to pain, are comparable to patients with a pain-related disorder, JIA, but experience lower quality of life in some domains.

Author: Komalpreet Brar, MD, John T. Chibnall, PhD, Thomas Geller, MD, St. Louis University

Mechanotransduction and NF1 Loss: Partner in Neurofibroma Development

Federica Chiara, PhD, Padova University

A still challenging issue is to understand whether the environmental niche is determinant for NF1^{-/-} Schwann cell progression toward tumorigenesis and thus for neurofibroma onset. Cells haploinsufficient for *Nf1* display hyperactivation of Ras (1), which further increases when LOH of *NF1* occurs (2). The activation of Ras/Raf/ERK signalling in Schwann cells is sufficient to make them more susceptible to proliferative signals provided by a NF1^{+/-} niche [3]. The physiological response to Ras hyperactivation, however, is cell-cycle arrest and/or senescence rather than transformation. Ras-mediated transformation of Schwann cells probably relies on a step-wise process that integrates circuits of amplification signals from the local niche. A major component of the niche is the extracellular matrix (ECM), a complex network of macromolecules whose elasticity (ranging from soft and compliant to stiff and rigid) determines how cells sense and perceive external forces, thus providing a major environmental cue to determine cell behavior (4, 5). To address whether Nf1 loss and ECM elasticity cooperate in the transformation of NF1^{-/-} Schwann cells we have built up a new three-dimensional *in vitro* culture model in order to obtain a suitable experimental platform for the study of the contribution of ECM in cell transformation. In the present report, we provide preliminary findings indicating that both Nf1 loss and ECM stiffness sensitize fibroblasts to the transition toward a myofibroblast phenotype, through activation of both beta-catenin and Snail transcription factors. The process of mechanotransduction involves both the mechanosensor protein kinase focal adhesion kinase (FAK, 6) and the Src kinases/YAP transduction axes, which we found hyper-activated in the absence of Nf1. Importantly, we show that Schwann cells isolated from plexiform neurofibromas grow in 3D only when a mechanical strain is applied on them, strongly suggesting that ECM stiffness is essential for neurofibroma growth. These findings point towards an important role of the fibrotic process not only in sustaining, but also in triggering neurofibroma progression. Hence, preventing fibrosis deposition and ECM stiffness could represent a new strategy to block neurofibroma formation.

References: 1. Cichowski K and Jacks T, Cell; 104:59, 2001; 2. Le LQ and Parada LF, Oncogene 26:4609, 2007; 3. Harrisin MC and Lloyd AC, Cell Cycle; 3:1255, 2004; 4. Aragona M et al, Cell, 2013 Aug 29;154(5):1047-59; 5. Pengfei Lu et al, J Cell Biol-2012-Lu-395-406; 6. Kweh F. et al, Molecular Carcinogenesis 48:1005-1017 (2009)

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Granting: The search has been sustained by Padova University grant (PRAT)

Lipid-Binding Mediates Merlin Conformations and Tumor Suppressive Activity

Krishna Chinthapudi, PhD, Cell Adhesion Laboratory, Department of Cancer Biology, The Scripps Research Institute

The tumor suppressor protein merlin plays an important role in contact inhibition of cell growth. Several mutations of merlin have been identified in Neurofibromatosis type 2 and other cancer patients. Merlin has high sequence similarity to the Ezrin-Radixin-Moesin (ERM) family of proteins. The structural model of ERM protein autoinhibition and cycling between closed/resting and open/active conformational states is often employed to explain merlin function. Despite more than two decades of research, there is no clear mechanism that explained the dynamic merlin states. Here, we show by high resolution crystal structures of merlin in complex with the phosphatidylinositol 4,5-bisphosphate (PIP2) lipid and also in complex with LATS1 FERM Binding Domain (FBD). Merlin bound to lipid shows the opening of an alpha-helix which connects to the merlin tail domain. Importantly, the extended alpha-helix preceding the FERM domain undergoes a 70 degree lever-like swing compared to the unbound structure.

We thus propose that lipid binding induces changes in the FERM domain conformation that extend the central alpha-helix coiled core and separates both the FERM and the tail domain. Our crystal structure of merlin in complex with LATS1 FBD binds to F2 lobe of the merlin FERM domain and is overlapping with the merlin tail domain position indicating that LATS 1/2 only binds to active/open merlin state. Our studies in solution with wild type full-length merlin in the presence of lipid micelles clearly showed the perturbation in the FERM domain where the tail domain binds and also at the extended α -helix region. We observed LATS FBD binding only to the wild type full-length merlin protein which is pre-incubated with lipid micelles. Our findings reveal that merlin undergoes unfurling upon binding to plasma membrane lipids at the cell-cell contacts and thus help in recruiting the interaction partners that are necessary for regulation of Hippo pathway signaling.

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Acknowledgement: Krishna Chinthapudi, Ph.D. is supported by Children's Tumor Foundation's young investigator award.

An Inflammatory Gene Signature Distinguishes Neurofibroma Schwann Cells and Macrophages from their Counterparts in the Normal Peripheral Nervous System

Kwangmin Choi, PhD, *Division of Experimental Hematology and Cancer Biology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital Research Foundation, Cincinnati Children's Hospital*

Neurofibromas are benign nerve tumors driven by Nf1 loss in Schwann cells (SCs), which comprise 20-80% of tumor cells. Neurofibromas also contain macrophages, comprising 20 - 40% of tumor cells. Accurate mouse models provide opportunities to study features of SC-macrophage interaction in early neurofibroma formation. We performed microarray expression analysis on FACS-sorted SCs and macrophages from genetically engineered *Nf1^{fl/fl};DhhCre* mice before and after neurofibroma formation. Prior to tumor formation, neither neurofibroma SCs nor neurofibroma macrophages significantly differ from their wild type counterparts. The expression of inflammatory genes changed significantly after tumor formation, and neurofibroma macrophages were distinct from previously defined macrophage sub-populations. SCs also expressed pro-inflammatory genes. Expression profiles, gene set enrichment, and ligand-receptor interaction analyses revealed potential paracrine and autocrine loops involving cytokines, chemokines, and growth factors. We identified imbalanced type-I/type-II interferon signaling and cytokine expression changes in neurofibromas that were restored by treatment of mice with PEGylated interferon- γ 2b. Our data suggest that SCs and macrophages interact with each other to modulate local chronic inflammation, favoring neurofibroma formation. Identified SC/macrophage pathways provide new avenues to explore for relevance in neurofibroma formation.

This research was supported by a grant from the Neurofibromatosis Therapeutic Acceleration Program (NTAP) to JW, KK, and KC, and a grant from Department of Defense W81XWH-12-1-0133 to NR

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The NF1 and NF2 Pharmacome Project, a Course in High Throughput Screening to Identify Targets and Profile the Sensitivity of MPNST Cells to Candidate Drugs

Jianman Guo, Grace Coggins, Patrick Duggan, Michael Grovola, Jiale Huang, Claire Song, Hong Xie, David Schultz, Simon Berritt and Jeffrey Field, *University of Pennsylvania, Perelman School of Medicine and Department of Chemistry, High Throughput Screening Core, Department of Chemistry, Merck High Throughput Experimentation Laboratory, Department of Systems Pharmacology and Translational Therapeutics*

There are several large-scale efforts to compare the sensitivities of tumor cells to chemotherapeutic drugs and small molecule pathway probes. However, to date, none of these profiling efforts systematically probed Malignant Peripheral Nerve Sheet Tumors (MPNST). Through funding from the Children's Tumor Foundation we initiated a database of drug sensitivities of MPNST as part of a course in High Throughput Screening. We first developed a panel of 130 drugs highly relevant to NF1 and NF2 that included a comprehensive set of MEK, RAF, RAS, FTI, PAK, ERK inhibitors, a representative set of drugs against many other cancer pathways including Wnt, Hedgehog, p53, EGF, HDAC, as well as classical cytotoxic agents such as doxorubicin and taxol. Many of the drugs in our panel are in clinical trials themselves, or closely related to drugs in trials for NF1. The drugs were tested against cells in 384 well plates at eight concentrations ranging from .004 μ M to 10 μ M. Cells were allowed to attach overnight, incubated for 72 hours with drugs and then analyzed for ATP content using ATPlite. To date, we profiled nine MPNST cell lines (ST88-14, ST88-3, 90-8, SNF02.2, STS26, T265, S462TY, SNF96.2, SNF94.3) and one NF2 Schwannoma cell line (HEI193). We also tested several cell lines that were tested in other screens so that we can compare our results to the other databases. The IC₅₀ was calculated as a common measure of how effective each drug is using GraphPad Prism software. NF1 cells were distinguished from NF2 cells and a STS26 cells (a spontaneous MPNST cell line derived from a patient that did not have NF1) by their strong sensitivity to MEK and Bromodomain inhibitors. Some drugs, including cytotoxic agents, Pak inhibitors and HDAC inhibitors, were broadly toxic inhibiting growth regardless of NF1 and NF2 status. None of the drugs in our panel exclusively inhibited HEI193 cells. With this study we initiated a database to archive the drug sensitivities of NF1 and NF2 cells that will be expanded in future versions of our course.

Dasatinib, an Approved Src Kinase Inhibitor, Reduces Viability of Human and Mouse Merlin-Null Schwann Cell Lines

Marisa A. Fuse, MS, *University of Central Florida*

Neurofibromatosis type 2 (NF2) is a tumor disorder characterized by the formation of peripheral nerve schwannomas as a result of a loss of function mutation of the tumor suppressor, merlin. Despite several clinical trials, an effective drug therapy for NF2 has yet to emerge. Previous reports by others, in addition to unpublished antibody array data from our lab, have shown that Src activation is elevated in schwannomas relative to normal human Schwann cells. Thus, we tested several FDA-approved Src inhibitors for their ability to reduce viability and proliferation of merlin-null Schwann cell lines using a multi-parametric assay. One Src inhibitor, Dasatinib effectively reduced proliferation of mouse and human merlin-null Schwann cell lines with IG_{50} of 1 μ M and 60 μ M, respectively. The reduction in cell number resulted from a G1 cell cycle arrest as measured by a decrease in EdU incorporation. Dasatinib reduced phosphorylation of FAK and paxillin, downstream effectors of Src. Current efforts are focused on mapping the signaling cascade whereby changes in Src and FAK activity promote G1 cell cycle arrest. Future studies will evaluate dasatinib efficacy in allograft and xenograft mouse models to provide necessary pre-clinical data to support clinical trials.

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This work was supported by grants to CFV from DOD (Grant Proposal Number NF140044) and the Children's Tumor Foundation (Drug Discovery Award). CTF - Young Investigator Award to M. Fuse, the Industry-Academia collaboration program with Cenix Bioscience and contributions from the CTF compound scouting team.

Chemo-Genomic Analysis of Plexiform Neurofibroma Cell Lines

Sara JC Gosline, PhD, *Sage Bionetworks*

Plexiform neurofibromas (pNFs) that result from NF1 deficiency in the autosomal dominant syndrome neurofibromatosis type I (NF1) can lead to substantial disfigurement, neurologic disability, discomfort and even death (via malignant transformation). There are no approved therapies for pNFs and there is limited understanding of tumorigenesis at a molecular level. Toward this end, we have characterized the genomic and transcriptomic profiles of a panel of human derived pNF cell lines to better understand how they respond to candidate drugs individually and in combination.

We have collected Exome-Seq, RNA-Seq, and DNA copy-number data for a panel of established immortalized pNF cell lines. In addition, we performed a high-throughput chemical screen with a large library of ~1900 approved and experimental compounds (MIPES). Subsets of the drugs analyzed were also measured in combination to identify possible synergy between the compounds.

A preliminary investigation revealed many drugs that were broadly cytotoxic, as well as drugs that were active in an NF1-dependent manner. Of the drugs that exhibit stronger potency in the NF1-/- cells, we identified numerous targets in common such as TUBB, DHFR and PLK1, suggesting that these pathways play a role in the efficacy of these drugs in the pNF cell lines. As we analyze the genomic results we will be able to identify additional molecular characteristics that affect drug sensitivity.

This work represents a collaborative effort between Johns Hopkins University through its Neurofibromatosis Therapeutic Acceleration Program (NTAP), the National Center for Advancing Translational Sciences (NCATS), the Wallace Laboratory of the University of Florida and Sage Bionetworks to assemble a resource for the NF research community. All data will be made available through the Synapse web portal at <http://www.synapse.org/pnfCellCulture>.

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The Molecular Landscape of Dermal Neurofibromatosis

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Neurofibromatosis type I (NF1) is a genetic disorder that disrupts neurological tissue growth and can lead to a diverse set of symptoms including systematic growth of benign tumors, learning disorders and bone deformities. While the disease has been linked to loss of function in the NF1 gene - a known tumor suppressor - there is a high degree of phenotypic diversity in the NF1 patient population. Furthermore, there has been no extensive molecular characterization of dermal NF conducted to date, making it difficult to identify the underlying cause of the disease and treat it effectively. In this work we describe multi-omic profiling of dermal NF to describe the landscape of intra- and inter-patient tumor heterogeneity.

We collected four dermal neurofibromas and peripheral blood from each of 11 NF1 patients. We analyzed each sample using (1) Whole genome sequencing (WGS), (2) SNP Arrays (3) RNA-Sequencing and (4) iTRAQ-labeled mass spectrometry.

Preliminary analysis of the data underscores the complexity of this disease and explains, in part, previous difficulty in identifying effective treatments. For example, copy number analysis using the SNP arrays confirms that the tumors are chromosomally stable across patients. Whole genome sequencing identified common germline variants observed across patients as well as limited somatic mutations across samples. Gene and protein levels suggest more insights into the disease. For example, gene expression analysis reveals driver genes that are enriched in MAPK activation and RAS signaling pathways. The proteomics experiments identify proteins that are enriched in cell adhesion-related processes.

This ongoing analysis is part of a larger effort to make NF1 data available to the public to accelerate research and accelerate the drug discovery pipeline. All data and preliminary results are publicly available at <http://www.synapse.org/dermalNF>.

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Small Molecule Inhibition of the Hippo-YAP Pathway as a Therapeutic Strategy in Neurofibromatosis Type 2

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Cellular mechanisms that control proliferation and cell death are essential for processes that require defined cell numbers, such as developmental control over organ size and repair of injured tissues, and these mechanisms are commonly dysregulated in cancer. A pivotal regulator of anti-proliferative/growth control signaling is Merlin, the product of the *NF2* tumor suppressor gene, originally identified as the gene mutated in Neurofibromatosis type 2 (NF2). NF2 is an inherited disorder with an incidence of approximately 1 in 30,000 births, and characterized mainly by the development of schwannomas of the eighth cranial nerve. Moreover, mutations and loss of heterozygosity of the NF2 locus are associated with multiple nervous system tumors, including schwannomas, meningiomas and ependymomas.

Merlin regulates a wide variety of mitogenic pathways, including Ras, Rac, Src, mTOR, and has recently been linked to the Hippo-YAP pathway. This pathway is comprised of a kinase cascade that leads to phosphorylation of the Yes-associated protein (YAP), sequestering it in the cytoplasm and preventing it from activating the pro-growth/anti-apoptotic TEAD transcription factors in the nucleus. The Hippo-YAP pathway is frequently disrupted in cancer, such that YAP is known to function as an oncogene in multiple tumor types through a variety of genetic and epigenetic mechanisms. Moreover, several tumors have been shown to be YAP-dependent. Thus, the potent antiproliferative phenotype conferred by loss of YAP underscores the promise of inhibiting YAP as an attractive therapeutic strategy in *NF2*-associated cancers.

In order to establish the necessity of YAP in NF2-associated schwannoma, genetic methods were used to silence YAP. Both *in vitro* and *in vivo* studies established the requirement for YAP for the proliferation and survival of *NF2*-null schwannoma cells. Based on these results, we employed a cell-based screen against the Scripps Drug Discovery Library (>640,000 compounds) to identify and evaluate inhibitors of the Hippo-YAP pathway. Details of the screening program along with validation of lead compounds will be presented.

This work is made possible through the Children's Tumor Foundation Young Investigator Award.

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Cellular Prion Protein (PrP^C) Promotes Schwannoma Tumorigenesis

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Loss of tumour suppressor protein Merlin, spontaneously or as part of hereditary condition Neurofibromatosis Type 2 (NF2), causes tumours of the nervous system such as schwannomas, meningiomas and ependymomas. Merlin-deficiency is also seen in other cancers such as proportion of breast cancer, melanoma and glioblastoma. Schwannoma is the most common Merlin-deficient tumour and hallmark of NF2. Current treatment options for NF2-related tumours, surgery or radiosurgery, are invasive and only partially effective therefore new drug therapies are urgently required.

Cellular prion protein (PrP^C), encoded by *PRNP* gene, is involved in tumour development by altering proliferation, adhesion, and survival in some cancers via focal adhesion kinase (FAK) /Src/ NFκB, cyclin D1 and p53 -proteins known to be deregulated in schwannoma and involved in schwannoma development. Our group previously showed a strong elevation of *PRNP* gene activity in schwannoma. We hypothesise that PrP^C may contribute to schwannoma development.

To study the role of PrP^C in Merlin-deficient tumours we have used a well-established in vitro model for schwannoma comprising human primary schwannoma and Schwann cells. We have shown that Merlin-deficiency leads to increased expression of PrP^C protein in schwannoma compared to normal Schwann cells and tissues. PrP^C protein is also released at higher levels from schwannoma cells suggesting it may act in autocrine or/and paracrine manner. PrP^C overexpression leads to strong increase in proliferation, cell-matrix adhesion and survival in schwannoma cells and activation of downstream ERK1/2, PI3K/AKT and FAK signalling pathways.

We conclude that PrP^C overexpression and increased release contributes to schwannoma pathogenesis and could be a potential therapeutic target for these tumours.

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Alternative Outcomes of Pathogenic Complex Somatic Structural Variations in the Genomes of NF1 and NF2 Patients

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Structural variations, including deletions, duplications, mobile-element insertions, translocations and inversions, lead to normal phenotypic variation as well as various genetic disorders such as neurofibromatosis type 1 (NF1) and neurofibromatosis type 2 (NF2). Mosaic *NF1* copy-number variations (CNVs) are not rare and are estimated at ~29% on the basis of a group of 52 sporadic individuals carrying an *NF1* intragenic CNV (Hsiao et al AJHG 2015). Detecting mosaic CNVs has important implications for genetic diagnostics and counseling. Nowadays, multiplex ligation-dependent probe amplification (MLPA) has been widely used to identify CNVs, but MLPA's sensitivity and specificity in mosaic CNV detection is still not well explored. Also, the mutational mechanisms leading to complicated mosaic CNVs remains understudied. Therefore, we present two mosaic deletions identified by MLPA as *NF1* exon 17-21 deletion and *NF2* exon 9-10 deletion. Through genomic-breakpoint-spanning PCR and Sanger sequencing, we further identified the breakpoints at the nucleotide level.

Surprisingly, both *NF1* and *NF2* deletions are each composed of two separate consecutive deletions (exon 17-18 and exon 19-21 in *NF1*; exon 9 and exon 10 in *NF2*), which cannot be recognized by MLPA. Given that these consecutive deletions are either exactly adjacent to each other, or with a 24 bp overlapping fragment, these consecutive deletions most likely originated from a single mutational event. Furthermore, a 2-8 bp microhomology was present in all junction sites. Single nucleotide variation, serial replication stalling and palindromic structures were found close to the breakpoints, strongly supporting a replication-based mechanism leading to these consecutive deletions.

These examples demonstrate that MLPA has limitations to dissect consecutive mosaic CNVs. cDNA-based testing and fragment analysis, complemented with MLPA for CNV detection in *NF1* and *NF2* genetic testing, allowed to separate, visualize and fully characterize these adjacent non-contiguous deletions. Furthermore, we characterized a novel replication-based mechanism leading to complicated consecutive mosaic deletions. This study provides perspective in molecular diagnostics as well as in molecular mechanistics for NF1 and NF2 etiology.

Reference: Decoding *NF1* Intragenic Copy-Number Variations. Hsiao et al AJHG 2015

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Antitumor Effects of 4-Methylumbelliferone, a Hyaluronan Synthesis Inhibitor, on Malignant Peripheral Nerve Sheath Tumor

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Hyaluronan (HA) has been shown to play important roles in the growth, invasion, and metastasis of malignant tumors. Our previous study showing that high HA expression in malignant peripheral nerve sheath tumors (MPNST) is predictive of poor patient prognosis, prompted us to speculate that inhibition of HA synthesis in MPNST might suppress the tumorigenicity. The aim of this study was to investigate the antitumor effects of 4-Methylumbelliferone (MU), an HA synthesis inhibitor, on human MPNST cells and tissues. The effects of MU on cell proliferation, migration, invasion, and apoptotic activity in MPNST cells were analyzed in the presence or absence of MU in an *in vitro* as well as *in vivo* xenograft model using human MPNST cell lines, sNF96.2 and sNF02.2. The effects of MU on HA accumulation and formation of cell-associated matrices were determined with HA binding protein (HABP) staining and particle exclusion assay, respectively. The amount of pericellular and intracellular HA was quantitatively determined with an HA binding assay. Levels of mRNA expression for HA synthases (HASs) in the presence or absence of MU were assessed with quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Inhibitory effects of MU *in vivo* were evaluated with a xenograft model of sNF96.2 cells. MU significantly inhibited cell proliferation, migration, and invasion in both MPNST cell lines. HABP staining, particle exclusion assay, and quantification of HA revealed that MU significantly decreased HA accumulation in the cytoplasm and pericellular matrices in both MPNST cell lines. The expression levels of HAS2 and HAS3 mRNA were downregulated after treatment with MU. MU induced apoptosis of sNF96.2 cells, but not sNF02.2 cells. MU administration significantly inhibited the tumor growth in the mouse xenograft model. To the best of our knowledge, this study demonstrates for the first time the antitumor effects of MU on human MPNST mediated by inhibition of HA synthesis. Our results suggest that MU may be a promising agent with novel antitumor mechanisms for MPNST.

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This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Grant-in-Aid for Young Scientist B)

The Role of NF1-regulated MAPK/ERK Pathway in Optic Glioma Formation

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Low-grade pilocytic astrocytomas are the most common type of glioma found in children. Children with Neurofibromatosis type 1 (NF1) are especially prone to develop optic pathway gliomas (OPGs) – pilocytic astrocytomas that involve the visual pathway. These tumors are normally diagnosed in patients before the age of 7, highlighting a specific window of OPG development during early childhood. Although the majority of these tumors are benign, some may result in partial or total vision loss and cause disruption of the hypothalamic region, which leads to endocrine problems like pituitary gland dysfunction, growth failure and delayed onset of puberty. In addition, there is variability in the disease progression pattern of OPGs in NF1 patients. Thus, there is a critical need to understand the mechanism underlying initiation and progression of NF1-associated optic gliomas and develop targeted therapies to prevent or treat the disease.

Using the genetically engineered mouse model Nf1^{hGFAP}CKO, where the Nf1 gene is inactivated in multipotent radial glial cells that give rise to both neurons and glia, our lab has shown that these Nf1 conditional mutants display brain structural defects and develop OPGs with high incidence. We have found that gliomas in the mutants contain a population of glial cells that abnormally express markers for both astrocyte and oligodendrocyte lineages (Olig2⁺/GFAP⁺) and over activate the mitogen-activated protein kinase (MAPK) pathway (p-Erk⁺). Importantly, these Olig2⁺/GFAP⁺ cells also express markers associated with glial progenitors such as (PDGFR- α , NG2, BLBP). In addition, we have found that the increased number of glial cells observed in OPG was largely established by P21 in Nf1-deficient optic nerves. Lastly, the abnormal triple-positive glial cells (p-Erk⁺/Olig2⁺/GFAP⁺) can be found during early developmental stage in mutant nerves. These results provide insights into critical events during early phases of OPG development and the role of NF1 in developing neural stem and progenitor cells in the optic nerve.

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Funding: NSF GRFP, Gilbert Family Neurofibromatosis Institute

Stromal Targeting in the Schwann Cell Tumor Microenvironment to Enhance Chemotherapy Penetrance

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Neurofibromatosis Type 1 (NF1) is a genetic disease caused by the inheritance of a mutant copy of the Neurofibromin 1 (*NF1*) gene, which encodes a tumor suppressing RAS-GTPase activating protein. Biallelic inactivation (or loss of heterozygosity) leads to dermal or plexiform neurofibroma formation. Subsequent mutations in plexiform neurofibromas give rise to the development of Malignant Peripheral Nerve Sheath Tumors (MPNSTs) in approximately 10% of patients. These MPNSTs, which can also occur sporadically, are the deadliest of the soft tissue sarcomas, showing lethality in 40% of patients after 5 years. Nonspecific and targeted therapies have shown little efficacy in improving prognosis. We hypothesize that this is due to poor drug delivery. Certain tumor types possess increased amounts of hyaluronic acid (HA) in the extra cellular matrix (ECM) which attracts water in to the tumor, raising interstitial fluid pressure. This leads to collapsed vasculature and poor drug penetrance. PEGylated recombinant human hyaluronidase (PEGPH20) has been shown to degrade the accumulated HA in a pancreatic mouse models and human pancreatic adenocarcinoma, which subsequently restore functionality to the vessels and improve drug penetrance. We show that both human and mouse model neurofibromas and high grade PNSTs contain high HA levels and collapsed vasculature. Microarray data from neurofibroma and MPNST Schwann cells also communicate overexpression of Hyaluronan Synthase 2 (HAS2), providing characterization of the increased HA levels. In a PNST mouse model treatment with PEGPH20 not only degrades the surrounding HA but restores vasculature patency and allows for improved penetrance of the small molecule drug doxorubicin. Further experimentation will characterize the ECM response to drug-induced restructuring along with analysis of modulated cytotoxicity/pharmacodynamics of targeted therapies. We believe that degradation of the tumor-associated HA in nerve sheath tumors could will lead to increased drug perfusion and provide a therapeutic benefit.

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Funding: Department of Defense Grant

CRISPR/Cas9-Based Gene Editing of *Nf1*: A New Rat Model of Neurofibromatosis Type 1 (NF1)?

Rajesh Khanna PhD, *University of Arizona*

Neurofibromatosis type 1 (NF1) is a rare autosomal dominant disease, which has been linked with mutations of the *Nf1* gene. The diverse clinical manifestations of NF1 suggest a global impairment of both the central and peripheral nervous system functions. NF1 encodes for neurofibromin, a Ras GTPase-activating protein (RasGAP) that has been implicated in the regulation of long-term potentiation (LTP), Ras/ERK signaling, and learning in mice. Over the last decades, mice with a targeted mutation in the *Nf1* gene, *Nf1*^{-/-} chimeric mice, *Nf1* exon-specific knockout mice, and mice with tissue-specific inactivation of *Nf1* have been generated to model the human NF1. Studies on heterozygous *Nf1* mice have instructive in identifying dysregulation of ion channel activity and function as a cause for the pathophysiology, in particular migraine and headache, in NF1. However, *Nf1*^{+/-} mice reportedly demonstrated no predisposition for either clinical symptom solely due to *Nf1* heterozygosity. In contrast, thermal hyperalgesia and mechanical hypersensitivity, induced by capsaicin, were reported in another study with the same mice. To resolve this apparent discrepancy, we have created a rat model of *Nf1* inactivation with a genome editing approach. Using a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-based genome editing strategy, we edited the C-terminal (after exon 39) of neurofibromin. We selected this region for deletion as the regulatory protein, collapsin response mediator protein 2 (CRMP2), binds here. CRMP2 regulates the activity of N-type voltage-gated calcium channels (CaV2.2), channels key in the control of the nociceptive neurotransmitter calcitonin gene related peptide (CGRP). Consistent with data from *Nf1*^{+/-} mice, transfection of a single guide RNA (sgRNA) for *Nf1*, reduced neurofibromin levels, in line with decreased neurofibromin levels observed in chronic neuropathic pain tissues. Importantly, *Nf1*-sgRNA mediated loss in neurofibromin resulted in a significant increase in CaV2.2 activity as measured by whole cell patch electrophysiology. Finally, we observed a decrease in CaV2.2 with a CRMP2-derived peptide that inhibits the CRMP2/Neurofibromin interaction. Together, our findings support the use of a CRISPR/Cas9-edited *Nf1* rat model for cellular electrophysiological studies to understand NF1 signaling as well as for screening of novel drugs (e.g., (S)-lacosamide – a small molecule that inhibits CRMP2 phosphorylation to reduce Cav2.2 activity, decreases CGRP release, and consequently relieves cephalic pain in an experimental migraine model; see A. Moutal presentation) and biologics for the treatment of the diverse symptoms of NF1.

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Funding: : Neurofibromatosis New Investigator Award NF1000099 from the Department of Defense Congressionally Directed Military Medical Research and Development Program to R.K. A.M. was partially supported by a Young Investigator Award from the Children's Tumor Foundation.

Drug Discovery for NF1-Associated Malignant Peripheral Nerve Sheath Tumors Using the Zebrafish Model

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Children and young adults with type 1 neurofibromatosis (NF1) are at risk to develop plexiform neurofibromas, which can undergo malignant transformation to malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs are among the most frequently occurring sarcomas in children and young adults and are especially problematic in NF1 patients. Currently, complete surgical excision is the only curative therapy for NF1-associated MPNST, but these tumors are often not completely resectable. Therefore, it is very important to identify promising drugs that can be rapidly moved into clinical trials to improve the therapy of patients with MPNSTs.

We are beginning our studies of drugs that are showing promise in Phase I/II trials for human cancer by testing the BET inhibitors, OTX015 and IBET762, because recent papers have described synergy between the BET inhibitor JQ1 and a MEK inhibitor in human MPNST cell lines and mouse models of MPNST. To test the BET inhibitors OTX015 and IBET762 for activity against MPNST tumor cells alone and in combination of the trametinib MEK inhibitor, ~500 Sox10:mCherry expressed MPNST primary tumor cells were transplanted into dechorionized 2 day-post-fertilization (dpf) larval fish using a micro-injector. MPNST-implanted embryos were exposed to individual test compounds added to the fish water at a range of concentrations and to the vehicle control. After 3-days of treatment, quantitative assessment of the remaining mCherry-labeled tumor cell numbers were measured using the ImageJ software for each treated embryo. An advantage of this system is that the MTD is first determined for each drug in zebrafish embryos, so that the activity of each drug is compared at the individual drugs MTD.

In this study, the trametinib MEK inhibitor at 100 nM in the fish water caused marked antitumor effects indicated by reduced numbers of mCherry-labeled *nf1*-deficient MPNST cells compared to the DMSO control. Surprisingly, OTX015 and IBET762 showed even greater activity against *nf1*-deficient MPNST cells than the MEK inhibitor at each of their MTDs. We are now using the in vivo isobologram approach over multiple combined concentrations of each drug to assess the level of synergy between MEK and BET inhibitors in this tumor.

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Development of 3D Organotypic Models of Human NF1 Plexiform Neurofibromas for Drug Screening

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Plexiform neurofibromas (PNs) are present at birth in 25-50% of children with NF1. They are typically highly vascular, involve multiple, large nerve segments and can only be incompletely resected. Our goal is to provide the first in vitro organotypic models of PNs to allow for identification of potential therapeutics. The normal microenvironment of Schwann cells (SCs) is dominated by Type IV collagen (col-IV) and laminin. In vivo growth of PNs results in increased fibroblast infiltration/proliferation and production of Type I collagen (col-I). To define the effects of changes in microenvironment on tumor progression we have used a live-cell proteolysis assay that reports matrix degradation by 3D cultures growing in reconstituted basement membrane (rBM; predominantly col-IV and laminin) with and without admixed col-I (1:1 ratio). Comparison of co-cultures of wild-type or NF1 SCs and NF1 fibroblasts to mono-cultures of the cell types alone demonstrates significantly increased proteolysis in the co-cultures. To better understand the interaction between the SCs and fibroblasts, the two cell types were plated in 3D separately and as a 3D co-culture in a TAME (Tissue Architecture and Microenvironment Engineering) chamber and grown for 30 days. Confocal analysis showed that the SCs proliferated at a higher level in the presence of fibroblasts. These combined results suggest a strong interaction between the fibroblasts and either the wild-type or the NF1 Schwann cell and show an enhanced role in tumor formation for the infiltrating fibroblasts.

We propose that robustly bio-engineered 3D co-culture systems will allow preclinical drug screening that is more representative of likely translational effectiveness than can be achieved through testing in 2D mono-culture. Further, in view of the costs and time required for preclinical screening in animal models of NF1, 3D models represent a reliable and feasible alternative. The effect of several drugs that have exhibited anti-tumor activity in in vitro tumor models were tested for proliferation (luciferase assay) in SCs cultured in 2D and 3D. For example, alvespimycin has significant concentration-dependent cytotoxic activity toward NF1 and wildtype SCs. We have also tested a novel farnesyl transferase inhibitor, IG2 (Borch RF, molecular structure unpublished), with or without lovastatin, and lovastatin alone. Cell proliferation analysis showed a 50% inhibition for the combination drug sample (IG2 plus lovastatin) in 2D, but relative resistance for SCs grown in 3D.

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Funding Source: Neurofibromatosis Therapeutic Acceleration Program

Neurofibromatosis type I model medaka fish

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Fish model of NF1 has an advantage over that of mice regarding its easy husbandry and in vivo high-throughput screening. Thus far, fish model of NF1 has been reported in zebrafish. However, zebrafish has two orthologous genes for *nf1*, namely *nf1a* and *nf1b*. Furthermore, similar to mice, double knockout of *nf1* genes in zebrafish resulted in lethality within 10 dpf. These results imply difficulty for screening of drugs against NF1 using zebrafish because of cumbersome mating to obtain the double mutants and its short living nature.

Contrary to zebrafish, medaka has one *nf1* gene and will be easier to get *nf1* null alleles than zebrafish. We have tried to establish a medaka model of NF1. To this end, we screened a targeting induced local lesion in genomes (TILLING) library of medaka and disrupted medaka *nf1* gene by TALEN (Transcription Activator-Like Effector Nucleases). We obtained a line with a missense mutation (T1157I) in the GAP-related domain of medaka *nf1* and two lines with a nonsense mutation (exon25) before GRD region.

To address whether these lines can be used as a model of NF1, we have tried to show its epistasis with c-kit pathway genes. It is well known that c-kit pathway contributes melanin formation and *nf1* mutation (Nf1 +/-) partially rescued the phenotype of *W⁴¹* (*c-kit*) mutant's white spotty hair coat color. We have identified reduced number of melanophore (melanocyte equivalent) phenotype in medaka mutant was due to mutation in c-kit signaling gene and crossed this mutant with above *nf1* lines. Phenotype of double mutant medaka and relevance of medaka as a model of NF1 will be presented and discussed.

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This research was supported by Keio Gijuku Academic Development Funds.

Development and Characterization of a Swine Model of Neurofibromatosis Type I

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There is a desperate need for new models of NF1 that represent the broad spectrum of disease seen in patients. These may help us to understand the biological and genetic mechanisms underlying disease and disease variability, facilitate early detection, identify biomarkers, uncover novel drug targets, and test new drugs or combination therapies for safety and efficacy prior to human clinical trials. To this end, we have developed a swine model of NF1 by engineering a premature termination codon that mimics an exact human disease allele in the Ossabaw minipig. Fibroblasts isolated from NF1 pigs show hyperactive Ras activity. These animals are enrolled in an ongoing study in which they are phenotypically characterized for the diagnostic criteria of NF1, peripheral nerve hyperplasia, and the development of dermal and plexiform neurofibromas, as well as paralysis, neuropathy, and mobility issues that the enlarged nerves and/or tumors may cause. We are analyzing NF1 swine for other phenotypes typically seen in NF1 patients including skeletal abnormalities, hypertension, epilepsy, optic nerve gliomas, astrocytomas, and the development of Juvenile Myelomonocytic Leukemia. We have observed 100% penetrance of café au lait spots in these NF1 pigs, a phenotype that is seen in patients, but has never been demonstrated in any other animal model. We have also observed tibial dysplasia, excess leptomeningeal fluid reminiscent of hydrocephalus, and abnormalities in the spinal sinus by multiple imaging modalities including X-Ray, magnetic resonance and computed tomography imaging. The FDA has emphasized the need for development and testing of new therapies in large animal disease models, in addition to rodent models, prior to human studies. To this end, we are investigating the potential of this large animal model of NF1 for preclinical drug testing by conducting pharmacokinetic studies in healthy and NF1 swine to see if this animal model displays differential sensitivity to clinically relevant drugs, including targeted therapies such as MEK inhibitors and commonly prescribed drugs for pain, ADHD, and seizures. We hope that this large animal model of NF1 will become a standard in evaluation of new drugs prior to Phase I clinical trials and aid in the discovery of effective treatments and cures for patients with NF1. Additionally, these animals provide an ideal platform upon which to 1) study imaging technology prior to tumor development during growth and through metastasis, 2) understand tumor natural history without intervention, 3) develop minimally invasive surgical techniques and intensity modulated radiation therapy strategies, 4) perform protocol optimization for MRI, CT, PET, 5) improve detection, monitoring and treatment approaches, and 6) support longitudinal blood sampling to detect biomarkers.

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Funding provided by the Children's Tumor Foundation, NF1 Synodos

Genome editing of *Nf1*^{+/-} iPS Cells Derived from NF1 Patients

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Human induced pluripotent stem (iPS) cells reprogrammed from cells of NF1 patients carrying distinct mutations may be helpful in revealing pathogenesis, in modeling the disease *in vitro*, in performing drug screening and in developing “personalized” therapeutics. iPS cell lines obtained from individuals with NF1 are heterozygous for the gene mutation, but it would be helpful to have iPS cells in which both *NF1* alleles are mutated, as is the case in tumor tissue. We are working to introduce a second mutation into the remaining wildtype allele of *Nf1*^{+/-} human iPS cells employing the CRISPR/Cas9 system. We first reprogrammed *Nf1*^{+/-} fibroblasts (harboring a c.204+1G>A splicing mutation) derived from neurofibromas of an NF1 patient into iPS cells by transduction of Cre-excisable constitutive polycistronic lentivirus. These iPS cells share classic features of human embryonic stem cells, such as expression of pluripotency markers Oct-4, Sox-2, SSEA-4 *et al.* and growth as colonies in both feeder-dependent and feeder-free systems. To create *Nf1*^{-/-} iPS cells, we expressed the CRISPR/Cas9 system in these cells. A homology-directed repair (HDR) template was introduced to precisely generate a nonsense mutation c.94_96>TAA. After selection and clonal culture of the transfected single iPS cell, we got 26 colonies. Analysis of these colonies by heteroduplex mobility assay and DNA sequencing identified 22 colonies containing nucleotide insertion or deletion mutations (InDels) in *NF1* gene. The remaining four colonies carry the c.94_96>TAA nonsense mutation. One colony was identified as having incorporated the nonsense mutation into the wildtype allele of NF1 gene. We are currently expanding this novel *Nf1*^{-/-} cell line for detailed biochemical and functional analysis. This study demonstrates that we can perform precise genome editing using the CRISPR/Cas9 technology in *Nf1*^{+/-} iPS cells that carrying unique germ line mutations. This will facilitate the application of iPS cells to better understand NF1 pathogenesis and to search for effective therapeutics.

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Funding Support: UAB Neurofibromatosis Program

Contributions of Stem Cell Factor and *Nf1* Heterozygosity to Neurofibroma Tumor Microenvironment

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder associated with monoallelic germline *NF1* loss. Patients with NF1 are predisposed to develop multiple types of neoplasms and neurofibroma is the most prevalent among them. Neurofibroma is a type of Schwann cell tumor with complex composition of microenvironmental cells developed in the peripheral nerves. One of the diagnostic pathological features for neurofibroma is mast cell infiltration. Distinct from most terminally differentiated blood cells, mast cells express tyrosine kinase receptor c-Kit and can be activated by c-Kit ligand stem cell factor (Scf). Therefore, Scf has been considered as a supportive factor to sustain *NF1*^{+/-} mast cells in neurofibroma microenvironment. In this study, using new genetically engineered *Scf* alleles, we explored the contribution of Scf from neoplastic Schwann cells to neurofibroma development by generating *Nf1/Scf* conditional double knockout mice and performed a side-by-side comparison of neurofibroma progression between mice with germline *Nf1*^{+/-} and *NF1*^{+/-} status. We also generated *Nf1/Scf* double knockout skin-derived precursor cells (as the cell of origin for cutaneous neurofibroma) to examine their tumorigenicity in allotransplanted sciatic nerves. We found that Scf status in Schwann cells minimally affects neurofibroma progression, suggesting other sources of Scf in the microenvironment are crucial for tumor development. In addition, the disease progression in germline *NF1*^{+/-} mice is significantly faster than germline *Nf1*^{+/-} mice, showing the important role of germline *Nf1* heterozygosity. Our research addresses the contributions of tumor-derived Scf and germline *Nf1* heterozygosity to neurofibroma development and provides insights for the design of future neurofibroma therapies in targeting its tumor microenvironment.

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Funding: Children's Tumor Foundation Young Investigator Award

Molecular Pathogenesis and Drug Synergism in a Zebrafish Model of High Risk Neuroblastoma

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We have developed a transgenic zebrafish model of childhood neuroblastoma that overexpresses *MYCN* in the peripheral sympathetic nervous system and harbors loss-of-function mutations of the *nf1* tumor suppressor. In this model, loss of *nf1* leads to aberrant activation of RAS-MAPK signaling, promoting both increased tumor cell survival and rapid tumor cell proliferation. These neuroblastomas are very aggressive in that almost all of the fish develop neuroblastoma by 3 weeks of age. Three-week old juvenile fish are very small, making it feasible to test the effectiveness of many drugs and drug combinations in vivo for activity against the primary tumors. We demonstrate these advantages of the model by showing marked synergistic anti-tumor effects of a MEK inhibitor (trametinib) and a retinoid (isotretinoin) *in vivo* at several different dosage combinations by in vivo isobologram analysis. Thus, inhibition of RAS-MAPK signaling can significantly improve the treatment of this very aggressive form of neuroblastoma when it is combined with the inhibition of other key pathways. Because of the very high penetrance and rapid onset of neuroblastoma in our *nf1*-deficient, *MYCN*-transgenic zebrafish model, it is one of the only model systems in which extensive analysis of the synergistic activity of two or more drugs can be evaluated in primary tumors in vivo. This capability is especially valuable given that mutations causing RAS-MAPK pathway hyperactivation, including acquired mutational loss of the NF1 tumor suppressor, have been shown to arise frequently at the time of relapse of childhood neuroblastomas, indicating the need to eliminate these mutated tumor cells as a component of the primary treatment.

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Funding: Young Investigator Award from Children's Tumor Foundation and Young Investigator Award from Alex's Lemonade Stand Foundation; by the Department of Defense (W81XWH-12-1-0125).

Changing a Winning Horse: Towards a New Approach for NF1-Diagnostics

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We developed a customized next generation sequencing (NGS) panel for 16 rasopathy genes: *NF1*, *SPRED1*, *PTPN11*, *BRAF*, *CBL*, *HRAS*, *KRAS*, *NRAS*, *MAP2K1*, *MAP2K2*, *RAF1*, *RIT1*, *RASA2*, *SHOC2*, *SOS1*, *SOS2*, using Agilent Haloplex and Illumina sequencing chemistry. The *NF1* component comprises all regions we encountered through comprehensive RNA-based *NF1* analysis of >15,000 individuals, including >8,100 unrelated *NF1*-positive carrying 1 out of >3,100 different unique *NF1* mutations. Included in the NGS assay are >65 different deep intronic splice locations, accounting for 2.6% of all mutations found. The average coverage of the panel is 1,800x with <0.01% of the *NF1* regions covered at <100x but still >50x, allowing the detection of very low level mosaicism.

After validation of the bio-informatics pipeline using positive controls, including substitutions, insertions/deletions/duplications up to 64bp and one-to-multiple exon deletions/duplications, we performed *in duplo* double-blind analysis of 118 samples consecutively received for clinical testing due to a suspected diagnosis of NF1 or Legius syndrome: 45 samples for *NF1*-only (with 24/45 *NF1*-mutation-positive), 73 samples for *NF1*+*SPRED1* analysis (with 40/73 *NF1*- and 1/73 *SPRED1*-positive). We compared results of comprehensive state-of-the-art RNA-based Sanger approach with NGS deep coverage approach. *NF1*/*SPRED1* results included 2 deep intronic mutations (2/64, 3.1%). Results between both assays were fully concordant except for one mosaic patient fulfilling NIH criteria with only 4% mutant allele in blood detected only by the NGS approach. Mosaicism in blood was found in 2/29 sporadic cases. A deep intronic NF1 mutation was identified in 2/64 (3.1%) positives by both approaches. Through further analysis of all genes on the NGS panel a differential diagnosis could be made in a sporadic 40-yo man with >6 CALMs, bilateral axillary freckling, 2-6 cutaneous neurofibromas (no histology review) and bilateral spinal tumors: a recurrent known pathogenic *PTPN11* mutation p.Arg498Trp was found, in line with our previous report. Furthermore, 6 unrelated individuals carried a variant of suspected pathogenicity (based on absence in control databases, in silico predictions, single report as pathogenic in literature) in the following genes: *KRAS*, *CBL*, *BRAF*, *MAP2K1*, *SOS1*, *SOS2*. These variants require further studies in relevant relatives to assist with classification.

In conclusion: the customized NGS rasopathy approach identified all mutations found by comprehensive RNA-based testing, but *additionally* identified a low level mosaic NF1, and allowed to make one *definite* as well as 6 possible alternate diagnoses.

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Relief of Cephalic Pain by (S)-Lacosamide in an Experimental Model of Headache

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Chronic headache and migraine are major healthcare and societal issues. Migraine has a higher incidence in Neurofibromatosis type 1 (NF1) patients. Current treatments have limited efficacy. Targeting calcitonin gene related peptide (CGRP), a key integrator of nociceptive signal transmission, whose signaling is increased in NF1, has commanded wide attention. While targeting CGRP itself or its receptor appears promising, the toxicity of these agents remains unknown and early clinical data reported only marginal improvement, compared to placebo, for migraine relief. Capitalizing on the cardinal role CGRP plays in migraine headache, here, we introduce the axonal growth/specification collapsin response mediator protein 2 (CRMP2) as a novel “druggable” target for curbing CGRP release. We previously demonstrated that CRMP2 phosphorylation by Cyclin dependent kinase 5 (Cdk5) regulates N-type voltage gated Ca^{2+} channel (CaV2.2) activity and Ca^{2+} -dependent CGRP release in sensory neurons. Of relevance to this study, we observed increased CRMP2 phosphorylation and CaV2.2 activity in the Nf1^{+/-} mouse model, supporting the hypothesis that dysregulation of the CRMP2/CaV2.2/CGRP signaling axis may account for migraine pathophysiology in NF1 patients. CRMP2 was co-expressed with CaV2.2 and CGRP in trigeminal ganglia (TG) sensory neurons, suggesting a novel axis coordinating CGRP release in the trigeminal system. The small molecule (S)-Lacosamide ((S)-LCM), an inactive analog of the clinically-approved anti-epileptic drug (R)-Lacosamide (Vimpat®), inhibited CRMP2 phosphorylation by Cdk5 in TG slices and decreased depolarization-evoked Ca^{2+} influx in TG cells in culture. (S)-LCM significantly blocked capsaicin-evoked CGRP release from dural nerve terminals in an *ex vivo* cranial cup preparation. Finally, cutaneous allodynia (CA) of the periorbital region and the hindpaw, induced by activation of dural nociceptors with a cocktail of inflammatory mediators, was inhibited by oral administration of (S)-LCM. Our results identify CRMP2 as a novel upstream modulator of CGRP release and propose (S)-LCM as a new small

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Funding: Neurofibromatosis New Investigator Award NF1000099 from the Department of Defense Congressionally Directed Military Medical Research and Development Program to R.K. E.T. was supported by a T35 HL07479-31A1 training grant from the NIH/NHLBI to Marlys H. Witte (Department of Surgery, University of Arizona). A.M. was partially supported by a Young Investigator Award from the Children’s Tumor Foundation.

ErbB3 and IGF-1R Blockade as a Potential Treatment for Vestibular Schwannomas and Meningiomas

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Vestibular schwannomas (VS) and meningiomas are intracranial tumors that are frequently caused by inactivation of the *NF2*/merlin tumor suppressor gene. These neoplasms incur significant patient morbidities, such as deafness, vertigo, facial paralysis, hydrocephalus, cranial nerve palsy, seizures, and brainstem compression. Currently, treatments for these tumors include surgical excision or radiation; however, an FDA-approved targeted therapy is not available. One of merlin’s functions is to suppress aberrant signaling from receptor tyrosine kinases (RTKs) on the cell surface. Indeed, VS and meningiomas often exhibit abnormal activation of RTKs, including members of the epidermal growth factor receptor (EGFR) family and insulin-like growth factor 1 receptor (IGF-1R). However, the EGFR inhibitors erlotinib and lapatinib exhibit only minimal efficacy in VS or meningioma patients, suggesting that additional RTKs provide survival signals for these tumors. We show that treatment of NF2 patient schwannoma and Ben-Men-1 benign meningioma cells with MM-121, an antibody that targets the EGFR member ErbB3, abrogates ligand-induced receptor activation and AKT phosphorylation. Similarly, treatment with MM-141, a bispecific antibody which blocks ErbB3 and IGF-1R signaling, also reduces activation of these receptors and downstream AKT. Importantly, prolonged treatment with MM-121 or MM-141 strongly suppresses ligand-mediated cell proliferation of NF2 schwannoma cells by 65% and 81%, respectively. We also found that Ben-Men-1 cells possess autocrine ErbB3 activation, which drives robust AKT signaling. Addition of MM-121 or MM-141 to Ben-Men-1 cells reduces ligand-induced cell growth and S-phase entry. These promising results warrant further *in vivo* evaluation of MM-121 and MM-141. Our study implicates ErbB3 and IGF-1R as important in VS and meningioma cell growth and indicates that blockade of these RTKs should be considered in the treatment of these tumors.

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Funding: Galloway Family Fund, Advocure NF2, CTF, Department of Defense

An shRNA Screen Identifies *MEIS1* as a Driver of Malignant Peripheral Nerve Sheath Tumors

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Malignant peripheral nerve sheath tumors (MPNST) are rare soft tissue sarcomas that are a major source of mortality in NF1 patients. To identify MPNST driver genes, we performed a lentiviral short hairpin (sh) RNA screen, targeting 130 genes up-regulated in neurofibroma and MPNST versus normal human nerve Schwann cells. *NF1* mutant cells show activation of RAS/MAPK signaling, so a counter-screen in RAS mutant carcinoma cells was performed to exclude common RAS-pathway driven genes. We identified *MEIS1* as one of 8 possible novel sarcoma oncogenes using this method. *MEIS1* was frequently amplified or hypomethylated in human MPNSTs, correlating with elevated *MEIS1* gene and protein expression. Confirming the validity of this approach to identify driver genes, in cultured MPNST cells *MEIS1* expression rescued the shRNA phenotype, and in a genetically engineered mouse model of MPNST *MEIS1* expression in developing nerve glial cells was required for MPNST growth. Mechanistically, *MEIS1* enables cell cycle progression in MPNST cells. *MEIS1* drives MPNST cell growth via the transcription factor ID1, thereby suppressing expression of the cell cycle inhibitor p27^{Kip} and maintaining cell survival. We conclude that inhibitors targeting cell cycle checkpoints and/or upregulating p27^{Kip} may have therapeutic value for MPNST patients, and perhaps for other tumor types in which *MEIS1* is believed to act as an oncogene.

Supported by NIH R01NS28840 to N.R. A.V.P. was the recipient of Postdoctoral Fellowship DAMD W81XWH1110144 and a Pelotonia Postdoctoral Award.

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Understanding the GPCR Driven Interaction of NF1 with G Proteins

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Neurofibromatosis type-1 (NF1) is an inherited autosomal dominant disorder caused by loss-of function mutations in the NF1 gene. The NF1 gene encodes neurofibromin protein which contains several domains, including a cysteine-rich domain (CSR), a Ras-Gap domain (GRD), a Sec14-like (SEC14) and a tightly interacting pleckstrin homology (PH)-like domain. NF1-GRD domain that serves as a negative regulator of Ras by virtue of its GAP (GTPase Activating Protein) domain is thought to be responsible for most of the biological activities of NF1 protein. Our study is focused on understanding the role of NF1 protein in the nervous system and mechanisms behind prominent neuropsychiatric symptoms in NF1 patients.

A wealth of available information show that cAMP and MAPK signaling systems are essential for learning, memory and motor coordination and are also implicated in chronic pain. Both cAMP and MAPK pathways are commonly activated by the G-protein-coupled receptors (GPCRs). However, the mechanisms by which GPCRs transduce these signals and their relevance to neuropsychiatric disease are still unknown. We found that activation of GPCR can dynamically regulate association of G protein beta gamma subunits with NF1. We found that G beta gamma directly binds to NF1 Sec14/PH module using bioluminescence resonance energy transfer (BRET) assay, co-immunoprecipitation experiments in co-transfected HEK293T cells and GST-pull-down assay of purified recombinant G beta gamma with GST-tagged recombinant Sec14/PH protein. Using Ras GTP hydrolysis assay, we observed that the binding of G beta gamma to NF1 inhibits its ability to inactivate Ras.

Based on this newly discovered phenomenon and the previous wealth of documentation on GPCRs, we hypothesize that the residues of GRD domain (which is located before the SEC14/PH domain) interacting with Ras are masked by G beta gamma due to conformational changes upon G beta gamma -Sec14/PH interaction. Interestingly, many pathogenic mutations in NF1 patients with neurofibromatosis have been mapped to Sec14/PH domain. In this direction, to evaluate the role of residues involved in the interaction and basis of inhibition of GAP activity, the structure of the NF1-G beta gamma complex and the physiological consequences of this interaction on MAPK pathways are currently under investigation.

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Funding acknowledgement: Dipak N. Patil is a recipient of a Children's Tumor Foundation Young Investigator Award (Grant ID: 2015-01-009). This research is supported by a Department of Defense grant (W81XWH-14-1-0074) to K.A.M.

Neurofibromin Deficiency Induces Oncogenic Metabolic Changes through ERK-Dependent Phosphorylation of the Mitochondrial Chaperone TRAP1

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The Ras/ERK transduction axis dysregulated in Neurofibromatosis type 1 (NF1) is emerging as an important player in metabolic rewiring. Even if this adaptive process is crucial for the progression of many cancer types, nothing is known about metabolic changes occurring in NF1-related tumors.

We report that enhanced and pro-tumorigenic activation of Ras/ERK signaling in neurofibromin-deficient cells increases glycolysis and concomitantly down-regulates mitochondrial oxidative phosphorylation by inhibiting respiratory complex II (succinate dehydrogenase, SDH). We had previously proven in other tumor models that SDH inhibition by the mitochondrial chaperone TRAP1 leads to accumulation of the oncometabolite succinate and to the ensuing stabilization of the transcription factor HIF1alpha, thereby establishing a pseudohypoxic state required for tumor growth. Here we demonstrate that in neurofibromin-deficient cells a fraction of active ERK locates into the mitochondrial matrix and phosphorylates TRAP1, and that this regulatory axis is required for TRAP1-dependent inhibition of SDH. TRAP1 silencing abrogates the neoplastic growth of cells lacking neurofibromin both *in vitro* and following their xenograft in nude mice.

Our study indicates that aberrant activation of Ras/ERK signaling in neurofibromin-deficient cells involves a mitochondrial branch of this transduction pathway, which activates TRAP1 and leads to inhibition of mitochondrial respiration. In turn this metabolic rewiring could be mandatory for the growth of NF1-related tumors and could constitute a potential target in the development of novel strategies for their treatment.

Granting: AIRC (Italian Association for Cancer Research) IG 15863 to AR

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Pathway Analysis of Genes Inhibiting Proliferation of Malignant Peripheral Nerve Sheath Tumor Cells

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Despite over a decade of clinical trials with targeted therapies for malignant peripheral nerve sheath tumors (MPNST), there remains no effective treatment for these tumors. MPNSTs occur in Neurofibromatosis type 1 patients with a lifetime risk of up to 15.8% [1]. Candidate targeted therapies are being tested in preclinical models of MPNST [2-5], but there are currently no published studies on siRNA screens to identify candidate targets to inhibit MPNST growth. We have conducted an siRNA screen of the druggable genome in human MPNST cell lines. We present here enrichment analysis of hits from the first and second passes of the screen, as well as the top hits from the 3rd pass of the screen. We also compare our results to other datasets from MPNST cells. These data highlight the pathways necessary for active proliferation of MPNST cells and suggest new targets for intervention.

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Funding: This work was supported by the Intramural Research Program of the National Cancer Institute, NIH, and by funds from the Children's Tumor Research Foundation to Cenix BioScience.

MRI Monitoring of Anti-growth Activities of Prenylation Inhibitors on NF1 MPNST Sciatic Nerve Xenografts

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Neurofibromatosis type 1 (NF1) is a disorder in which Ras is constitutively activated due to loss of the Ras-GTPase-activating activity of the protein neurofibromin. Ras must be prenylated (i.e., farnesylated or geranylgeranylated) to traffic and function properly. Previous studies have shown that the toxicity of prodrug farnesyl monophosphate farnesyl transferase inhibitors (FTIs) to human NF1 malignant peripheral nerve sheath tumor (MPNST) cells is potentiated by cotreatment with lovastatin. Unfortunately, the poor aqueous solubility of such drugs limits their in vivo usefulness. To address this problem we synthesized pro-drug FTI PAMAM G4 dendrimers that compete with farnesyl pyrophosphate for farnesyltransferase, and assessed their effects on human NF1 MPNST S462TY cells. A prodrug 3-*tert*-butylfarnesyl monophosphate FTI-dendrimer (hereafter called IG 2) exhibited improved aqueous solubility and no effect as a single agent on colony formation or prenylation up to 7 micromolar. Cotreatment of cultures with non-cytotoxic concentrations of IG 2 and lovastatin (0.1 to 0.5 micromolar) resulted in synergistic suppression of colony formation and protein prenylation. Injection of 40,000 S462TY cells into the thigh sciatic nerve resulted in successful tumor xenografts in 25 of 25 SCID mice. Tumor xenografts expanded along the nerve in both directions from the point of injection (as monitored by T2 MRI analyses), and in several cases tracked along the tibial and common peroneal nerve branches following bifurcation. Twice-daily i.p. injections of 15 mg/kg lovastatin, or i.v. injections of 10 micromole/kg IG 2, relative to saline-injected mice, did not suppress the growth of established S462TY sciatic nerve xenografts. However, combinational treatment with lovastatin and IG 2 significantly suppressed xenograft growth in 6 out of 6 mice (regression or complete cessation of growth in 3 mice). Tumor xenografts reinitiated growth upon cessation of combinational treatment. These studies indicate that prodrug farnesyl monophosphate FTIs can be rendered water-soluble by conjugation to PAMAM G4 dendrimers, and exhibit potent in vitro and in vivo anti-growth activity towards a NF1 MPNST cell line when combined with a statin.

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Funding acknowledgement: Supported in part by a gift from Jennifer and Daniel Gilbert, and pilot funding from the Molecular Therapeutics Program, Karmanos Cancer Center

Genomic Alterations Drive MET-Dependency in Murine MPNSTs

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Previous studies have identified overexpression or amplification of the *MET* protooncogene and its ligand, *Hepatocyte Growth Factor/Scatter Factor (HGF)*, in a substantial proportion of NF1-related MPNSTs. Previously, we presented a novel mouse model of spontaneous tumorigenesis resulting from conditional enhanced *MET* expression in combination with *NF1* loss-of-heterozygosity in myelinating cells (*NF1^{fl/KO};R26^{stopMET};Plp1-cre/ERT^{tg/+}*). The observed phenotype was enriched in MPNSTs (mean tumor-free survival=420 days versus litter mate controls>600 days); sporadic plexiform neurofibromas were also observed. Derived MPNST tumorgrafts demonstrated *MET* duplications and amplifications (FISH analysis), whereas MPNSTs derived from *NF1^{+/-}* or *NF1^{+/-};p53^{+/-}* background mice demonstrated chromosomal losses and gains, but no specific *MET* or *HGF* alterations. We observed a dose-dependent response to the single agent *MET* inhibitor, capmatinib (Novartis), in MPNST allografts derived from *NF1^{fl/KO};R26^{stopMET};Plp1-cre/ERT^{tg/+}* founders, whereas MPNSTs derived from *NF1^{+/-}* or *NF1^{+/-};p53^{+/-}* background mice did not respond to *MET* inhibitor. Doxorubicin chemoresistance was noted in each model. Capmatinib suppressed both *MET* (pMET) and *RAS*/*MAPK* (pERK) activation at 48 hours in the *MET*-activated MPNST allografts, whereas control tumors exhibited a low level of basal *MET* activation and unaltered *RAS*/*MAPK* activation in the presence of *MET* inhibitor. These results confirm that genomic *MET* alterations are capable of activating *MET* signaling in MPNSTs, and create a *MET*-dependent state that can be successfully targeted.

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Role of Ectopic EGFR Signaling in NF1 Pseudoarthrosis

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Forty percent of patients with Neurofibromatosis type 1 (NF1) present with skeletal manifestations, ranging from low BMD to scoliosis and tibial pseudoarthrosis (TPA). Pseudoarthrosis or bone non-union after fracture has unknown etiology and can result in amputation of affected limbs, most often the tibia. Data from human biopsies and mouse models of this condition have shown that adherent stromal cells from the affected site are unable to differentiate towards the osteoblast lineage. RNA-Seq data have shown that EGFR and its ligand epiregulin are ectopically expressed in human undifferentiated stromal cells collected from the NF1 TPA site compared to cells from normal bone, an observation we confirmed in *Nf1*^{-/-} bone marrow stromal cells (BMSCs) extracted from a mouse model of NF1 TPA. Excessive EGFR signaling is known to inhibit the differentiation of osteoprogenitors and bone healing. In order to determine if increased and ectopic EGFR signaling observed in *Nf1*-deficient BMSCs has any functional relevance to the impaired differentiation of these cells, *Nf1*^{+/-} and *Nf1*^{-/-} BMSCs were treated with various EGFR inhibitors and their differentiation potential was measured *in vitro*. We found that EGFR inhibition in *Nf1*^{-/-} BMSCs was unable to rescue their osteogenic differentiation defect, despite the use of several EGFR inhibitors and dose. It had, however, a stimulatory effect on the differentiation of WT BMSCs, demonstrating potency but also suggesting that EGFR blockade might improve bone healing in NF1 TPA, as increased epiregulin might impair the differentiation of *Nf1*^{+/-} BMSCs adjacent to NF1-deficient BMSCs in a paracrine manner. We conclude that the increase in epiregulin and EGFR expression associated with *NF1* loss-of-function mutations in BMSCs does not contribute to their osteogenic differentiation phenotype, but may negatively impact the differentiation of adjacent osteoprogenitors in the NF1 TPA site, which remains to be addressed *in vivo*.

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Funding sources: Young Investigator Award: 2015-01-015

Classic Ras Proteins Promote Growth of Neurofibromin Null Schwann Cells

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Mutations in the NF1 gene cause Neurofibromatosis type 1 (NF1), a disease characterized by the formation of benign and malignant tumors of the peripheral nervous system. Neurofibromin, the protein encoded by the NF1 gene, functions as a tumor suppressor, largely by inhibiting multiple small G-proteins from the classic Ras (H-Ras, N-Ras and K-Ras) and R-Ras (R-Ras, TC21 and M-Ras) subfamilies. While Ras activation has been implicated in the pathogenesis of NF1 associated tumors, the relative contributions of specific classic Ras family proteins to tumorigenesis remain poorly understood. To better understand the role of Ras family proteins in NF1, we first investigated if NF1 GAP-related domain (NF1-GRD), responsible for stimulating Ras-GTP hydrolysis and inactivating Ras proteins, is essential for neurofibroma development. Mice carrying a missense mutation in the active site of NF1-GRD develop neurofibromas and have a greatly reduced lifespan as compared to *Nf1*^{fl/fl}*DhhCre* mice, confirming that Ras GAP function of NF1 is critical in neurofibroma genesis. However, genetic ablation of H-Ras did not prevent neurofibromas from developing in *Nf1*^{fl/fl}*DhhCre* mice, suggesting compensation by other Ras family proteins. Individual knockdown of H, N or K Ras proteins in NF1^{-/-} Schwann cells did not inhibit MAPK pathway activation and survival. However, dominant negative (DN) H-Ras, a pan inhibitor of Classic Ras family prevented Erk activation and growth of NF1^{-/-} Schwann cells. These results suggest that multiple Ras proteins are involved in NF1 pathogenesis and therapies based on targeting specific Ras proteins may prove ineffective in NF1 patients.

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NIH F32 NS083249-02

A Myeloproliferative Nonsense NF1 Mouse Model for Nonsense Suppression Therapy Intervention

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Neurofibromatosis type 1 (NF1) is a genetic disorder with patients developing a wide variety of cancers, including juvenile myelomonocytic leukemia (JMML). NF1 is caused by mutations in the NF1 gene with nearly 20% nonsense alleles. We created a novel NF1 mouse line carrying a recurrent nonsense mutation found in NF1 patients at exon 18 (c. 2041 C>T; p.Arg681Ter, *NF1*^{Arg681*}) for intervention studies testing nonsense suppression therapy (NST). To establish the JMML model, the *NF1*^{Arg681*} allele is combined with a conditional knockout allele (*NF1*^{Flox}, *Nf1* gene with lox P sites flanking exon 4) and a Csf1r-iCre knock-in to induce recombination system in early myeloid precursors. The Csf1r-iCre line was constructed by targeted knock-in into exon 22 of the Csf1r gene. Tail biopsies were collected and DNA was extracted for genotyping by PCR. To date, we have bred Csf1r-iCre mice to obtain *Csf1r*^{iCre}; *NF1*^{Flox/Flox} and *Csf1r*^{iCre}; *NF1*^{Arg681*/Flox} animals that are currently being phenotyped. We are focusing our efforts on using this model to elicit onset of myeloproliferative disease in a timely and reproducible manner. Therapeutic drugs have been developed that can suppress nonsense mutations and partially restore protein activity, and are being used clinically. Using mice harboring a nonsense mutation along with a single floxed Nf1 allele (*Csf1r*^{iCre}; *NF1*^{Arg681*/Flox}) will provide an in vivo JMML NF1 model for assessment of NST to restore significant *Nf1* activity.

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Funding Support: UAB Neurofibromatosis Program

Genomic Characterization of Immortalized NF1 Schwann cells

Peggy Wallace, PhD, University of Florida

In neurofibromatosis 1 (NF1), an autosomal dominant condition, tumors are considered to result from a “two-hit” phenomena, in which a somatic cell suffers an inactivating *NF1* gene mutation. Such cells thus have two mutated alleles and can't produce normal levels or versions of neurofibromin. Primary Schwann cell cultures from individuals with NF1 (plexiform neurofibromas and non-tumor nerve (heterozygous)) as well as wild-type Schwann cell primary cultures, were immortalized with viral delivery of two transgenes: human *TERT* (telomerase reverse transcriptase) and murine *Cdk4*. We describe the resulting set of stable immortalized cell lines in comparison with the primary cultures. Akin to the heterogeneity found in cell biology properties of the primary cultures, the phenotypes of the immortalized lines had a range of phenotypes (e.g. proliferation rate, contact inhibition), but many were similar to the primary cultures. In addition, genetic characterization was performed on a subset of immortalized cells and primary cultures. Cell authentication work showed that all cells derived from the same patient had identical profiles for all 17 STR loci, plus gender determining loci. The STR profiles were also the same for primary and immortalized cells, and no cells showed contamination or misidentification with lines held in established repositories. Similarity between immortalized and parent cells were also demonstrated with SNP array studies. With regards to the latter, cell lines generally appeared diploid, although cytogenetics suggested some had clones closer to tetraploid, beyond detection by SNP array. LRR, BAF, and CNV analyses from chromosome 17 aneuploidy plots showed distinct deletion in 17q11.2 in both primary and immortalized pNF05.5 (population and set of 6 mixed clones), and pNF95.11b C, thus confirming their *NF1* somatic LOH status. In contrast, ipNF95.6 (and parent tumor cells) did not show deletion LOH patterns, which was consistent with the somatic *NF1* nonsense mutation detected in our lab. Also, the IGV plots of chromosome 17 confirmed the ploidy plots and show that most segmented values of copy number data (generated from SNP arrays) were matched between parental and immortalized cells. Exome sequencing showed that there were no consistent mutations at other genes beside *NF1* in either primary or corresponding immortal cell lines. Transcriptome (RNAseq) data are also being generated, and these data will be also presented to further illuminate the relationship between primary and immortalized cells.

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Supported by: Neurofibromatosis Therapeutic Acceleration Program (NTAP), U.S. Department of Defense Neurofibromatosis Research Program, Children's Tumor Foundation.

Developing a Novel Porcine Model for Neurofibromatosis Type 1

Katherine White, BS, Sanford Research

Neurofibromatosis type 1 (NF1), a rare autosomal dominant RASopathy caused by mutations in the *NF1* gene, affects approximately 1 in 3000 individuals worldwide. Clinically, NF1 manifests with a well-described phenotype with variable expressivity. Hallmark features of the condition include café au lait spots, neurofibromas, Lisch nodules and skinfold freckling. Additional health concerns include learning disabilities, short stature, plexiform neurofibromas, optic gliomas, skeletal abnormalities, seizures, headaches, neuropathic pain, hypertension or vasculopathy. Although much has been learned about the function of neurofibromin, the protein product of *NF1*, attempts to model neurofibromatosis type 1 in animals have failed to fully recapitulate the disease. A variety of genetically engineered mice have been generated with alterations in the *NF1* gene, however these models are limited to just a few of the disease-associated phenotypes seen in humans, such as abnormal growth phenomena and cognitive deficits. Other key aspects of the human disease, including formation of café au lait spots, enhanced pain perception, migraines and the spectrum of benign and malignant tumors, have not been effectively modeled in *NF1* mutant mice. Based on our past successes in accurately phenotyping other human diseases in genetically modified pigs, our team recently created a porcine model of NF1. This initial study provides details on the development and characterization of NF1 related phenotypes in a novel porcine model of NF1, including the presence of café au lait spots and abnormalities in RAS activity. As efforts accelerate in the NF1 research community towards development of novel therapies and technologies for early detection of tumor growth, this innovative animal model could provide a much sought after system for testing novel therapies. Funding Source: Children's Tumor Foundation and Sanford Research.

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Characterization of NF1 Deficient Immortalized Human Schwann Cell Lines, Their Use to Explore Synthetic Lethality and the Synodos for NF1 Drug Discovery Pipeline

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

Among other symptoms, Neurofibromatosis Type 1 (NF1) predisposes individuals to formation of benign tumors (neurofibromas), which can cause significant pain and mobility problems. Some of these tumors (10%) progress further to malignant peripheral nerve sheath tumors (MPNSTs) and are a leading cause of death among NF1 patients. The majority of NF1 MPNSTs arise in preexisting plexiform neurofibromas. Treatment options for the benign tumors and MPNSTs are extremely limited, mostly relying on surgical resection and broad-spectrum chemotherapy. There are however recent promising results with drug therapies targeting signaling pathways implicated in disease development. Finding new molecular targets for therapeutics effective against both benign tumors and MPNSTs is critical for improved patient outcomes and quality of life.

As part of the Synodos for NF1 initiative, we have developed a drug discovery pipeline to identify targeted therapeutics for treatment NF1-related neoplasia, including MPNSTs. To identify useful therapeutics, we have created and characterized isogenic pairs of NF1 proficient and deficient immortalized human Schwann cells. The *NF1* deficient cells exhibit increased oncogenic phenotypes, including increased anchorage independent growth under low serum conditions, higher basal levels of Ras-GTP, and a tendency to form tumors in athymic nude mice.

Following our characterization of these cell lines, we are now using them for a number of synthetic lethal screens, including: 1. A large-scale screen (~11,000 compounds from focused libraries) for drugs that selectively kill/inhibit the NF1 deficient cells. 2. Synthetic lethal genetic screens using genome-wide RNAi and CRISPR/Cas9 approaches to knockdown/out expression of additional genes.

Promising hits from the therapeutic molecule screening, showing selectivity toward *NF1* deficient cells, will be further validated for efficacy in xenograft and genetically engineered mouse models of these tumors. Our collaborators at Recombinetics have developed a porcine model for NF1, which clearly has numerous exciting uses for the NF1-related disease modeling. For this focus of the Synodos for NF1 project, we will use this more physiologically relevant large animal model to study the pharmacokinetics and pharmacodynamics of our most promising hits identified in the screening projects.

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Funding Support: Children's Tumor Foundation, NF1 Synodos, K.B.W. is supported by Children's Cancer Research Fund Emerging Scientist Award and 5T32CA009138-04

***In vivo* and *in vitro* Functional Characterization of Mutations Cooperating with NF1 Loss in Schwann Cell Transformation and Tumorigenesis**

Rory L. Williams, BS, *Masonic Cancer Center, University of Minnesota*

A *Sleeping Beauty* forward genetic screen in mice identified many potential drivers of MPNSTs, including previously implicated genes, as well as novel drivers (Rahrmann et al., *Nature Genetics*, 2013). Additionally, this screen identified numerous genes recurrently co-mutated with *Nf1*, indicating their probable importance in the development or progression of NF1 associated Schwann cell tumors. For one such gene, *Creb binding protein (Crebbp)*, we developed an *in vivo* mouse model utilizing the *Dhh-Cre* transgene to biallelically knockout floxed alleles of *Nf1* and *Crebbp* in the Schwann cell lineage. Concurrent loss of *Crebbp* and *Nf1* caused a reduction in life span, increased peripheral nerve hyperplasia, and the development of more, larger, and higher-grade Schwann cell tumors than when either gene was lost alone. Though validating the utility of the SB screen for identifying cooperating mutations, generating a GEMM for each potential cooperating mutation is costly in time and resources, and does not ensure that mutations will cooperate in the same manner in human tumors. In order to more rapidly characterize this and other mutations that cooperate with *NF1* loss, we utilized a normal human Schwann cell line immortalized with hTERT and activated CDK4, and CRISPR/Cas9 to functionally knockout one or both copies of *NF1*. Phenotypic characterization of multiple independently derived *NF1*^{-/-} and *NF1*^{+/-} clones in comparison to isogenic *NF1* WT clones revealed a dose dependent increase in anchorage independent growth under low serum conditions, and the ability to form tumors in immunodeficient mice for *NF1*^{-/-} but not *NF1*^{+/-} clones. These cell lines provide a novel and useful platform for characterizing potential driver mutations in NF1 associated peripheral nerve sheath tumors. Utilizing CRISPR/Cas9 mediated gene knockout and co-transposition with a drug resistance *piggybac* transposon to enrich for modified cells (Moriarty et al., PLoS ONE, 2014), anchorage independent growth and other assays can then be conducted on pools of transfected cells of various *NF1* status to characterize cooperation. Surprisingly, using this method for *CREBBP* demonstrated decreased anchorage independent growth regardless of *NF1* status. The reasons for this deviation from *in vivo* data are currently being investigated. Additionally, *PTEN*, *TOP2B*, *SRGAP2*, *TAOK1*, *DYRK1A*, *PP6R3*, *CCNY*, and *PICALM* were all identified as potential cooperating mutations through the SB screen, and these and other genes recently implicated in NF1 associated MPNSTs (i.e., *SUZ12*, *PTCH1*) are currently being characterized using this strategy.

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Recurrent sporadic glioblastoma retains neurofibromin protein expression: An immunohistochemical analysis of 20 cases

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Background: Alterations in the *NF1* tumor suppressor gene occur in approximately 20% of cases of sporadic primary glioblastoma (GBM), with changes including point mutations, small insertions/deletions, and heterozygous gene deletion. *NF1* alterations may be characteristic of a subset of GBM with mesenchymal or stem-like properties. Upon recurrence, GBM can shift toward a mesenchymal gene expression pattern and tumor phenotype. New *NF1* alterations have been reported in recurrent GBM, and in recurrent lower grade gliomas following temozolomide treatment. These findings raise the possibility that new *NF1* alterations in recurrent GBM could promote a mesenchymal transition in recurrent tumors by disrupting neurofibromin protein expression. We therefore questioned whether neurofibromin protein is present in recurrent GBM.

Methods: Tissue microarrays with paired initial and recurrent GBM samples from 20 patients were immunostained using an antibody against neurofibromin protein (clone NFC). Neurofibromin was scored as present or absent, based on the intensity of staining in tumor cells.

Results: Fifteen cases had evaluable immunostained tissue from both initial and recurrent tumors. This included two tumors that were positive for IDH1^{R132H} mutant protein. Thirteen of the initial tumors (87%) were positive for neurofibromin and two tumors (13%) were negative. All of the tumors had the same neurofibromin immunostaining pattern on recurrence. Both neurofibromin deficient tumors showed spindle or sarcomatous morphology in the recurrent specimens. Three cases had evaluable tissue only from the recurrent samples; two were positive for neurofibromin and one was negative. Two tumors had evaluable tissue only from the initial samples, and both were positive. All samples from IDH1^{R132H} mutant cases were positive for neurofibromin.

Conclusions: In this cohort of 20 specimens, neurofibromin protein was detected by immunohistochemistry in the majority of initial tumors, and protein expression was retained at tumor recurrence in all of the evaluable paired cases. This result suggests that complete loss of neurofibromin is an uncommon event in recurrent GBM; however, potentially significant heterozygous mutations would not necessarily be detected by immunohistochemical staining.

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Funding Sources: The Children's Tumor Foundation and the University of California San Francisco Department of Pathology.

RUNX Function in Neurofibroma Tumorigenesis and Therapy

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Neurofibromatosis type 1 (NF1) patients are predisposed to neurofibroma formation but the driver(s) that contribute to neurofibroma formation are not fully understood. The Runt-related transcription factor (Runx) family of genes (Runx1, 2, & 3) have shown paradoxical effects in cancers. They can function either as tumor-suppressors or oncogenes according to context. Targeted genetic deletion of Runx1 in Schwann cells (SCs) and Schwann cell precursors (SCPs) transiently delayed neurofibroma formation, suggesting Runx1 plays a role in neurofibroma formation. However, Runx1 deletion was accompanied by compensatory increased Runx3 expression based on qRT-PCR and immunohistochemistry. Dual deletion of Runx1 and Runx 3 in SCs and SCPs significantly delayed neurofibroma formation. To explore potential Runx1 down-stream targets, we performed microarray on shRUNX1 or control vector infected human MPNST cells. By comparing genes down-regulated in shRUNX1 with genes expressing in EGFR⁺/P75⁺ tumor initiating cells, we identified the transcription factor 4 (TCF4) as a potential RUNX target. Western blots showed Tcf4 expression decreased in *Runx1^{fl/fl};Nf1^{fl/fl};DhhCre* mouse neurofibromas compared to *Nf1^{fl/fl};DhhCre* mouse neurofibromas. Overexpression of Tcf4 partially increased *Runx1^{fl/fl};Nf1^{fl/fl};DhhCre* sphere numbers compared to control. Pharmacological inhibition of Runx/core binding factor beta (Cbf-beta) function with a Runx/Cbf beta interaction inhibitor, Ro5-3335, inhibited mouse neurofibroma cell proliferation and induced cell apoptosis *in vivo*, implicating a novel signaling pathway involving the oncogenes Runx1 and Runx3 in neurofibroma initiation and/or maintenance. Targeting the transcription factors Runx/Cbf-beta interaction might provide a novel therapy for neurofibroma patients.

Supported by a Cincinnati Children's Hospital Trustee grant to JW.

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Yorkie, a Transcriptional Co-Activator that Regulates Growth, also Functions at the Cell Cortex to Promote Cytoskeletal Tension

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NF2 gene product Merlin regulates the activity of the Hippo signaling pathway, a conserved signaling pathway involved in controlling organ size and tissue homeostasis. Yorkie (Yki) is well known as a transcriptional co-activator that functions downstream of the Hippo pathway to positively regulate transcription of genes that promote tissue growth. Recent studies have shown that increased cytoskeletal tension activates both Yki and YAP (a mammalian orthologue of *Drosophila yki*), resulting in increased nuclear localization and tissue growth. To better understand the effects of tension, as well as upstream pathway activity, on Yki function in living tissues, we generated a tagged *yki* transgene that is expressed at endogenous levels. Using this Yki reporter, we find that tension, generated either mechanically or genetically, results in increased nuclear Yki accumulation in the wing epithelium. Unexpectedly, we also find that tension induces Yki to accumulate in the cell cortex at the apical junctional region (AJR) in live cells. To ask if Yki might have a previously unrecognized, non-transcriptional function at the cell cortex, we added a myristoylation signal to the Yki N-terminus to tether Yki to the membrane. Remarkably, when expressed transgenically in the wing myristoylated Yki promotes cytoskeletal tension and folding in the epithelium by activating the *Drosophila* myosin regulatory light chain Spaghetti squash (Sqh). In addition, we found that activating Yki by genetically inactivating the Hippo pathway also causes Yki to accumulate at the AJR and increases Sqh activity. Conversely, depletion of *yki* using RNAi resulted in reduced Sqh activity. Based on these results, we suggest that active Yki functions in a feed forward 'amplifier' loop that promotes cytoskeletal tension, and thereby greater Yki activity, in response to tension such as that generated in the peripheral regions during imaginal disc growth. We are testing this hypothesis by generating *yki* alleles that lack the ability to activate Sqh, and are also dissecting the molecular mechanism underlying this novel function of Yki.

This research is funded by a CTF Young Investigator Award to Jiajie Xu and a National Institutes of Health grant (NS034783) to Richard Fehon.

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Blocking HGF/cMET Pathway Enhances Radiation Efficacy in NF2 Schwannoma Model

Lei Xu, MD, PhD, *Department of Radiation Oncology, Massachusetts General Hospital, Harvard Medical School*

Over the past few decades, ionizing radiation has become a standard treatment for vestibular schwannoma (VS). However, it is associated with a high risk of toxic effect of debilitating hearing loss. More importantly, the progressive lesions after radiation are resistant to further radiation therapy (RT). Therefore, novel adjunct therapy that can enhance radiosensitivity is urgently needed. c-MET, the hepatocyte growth factor (HGF) receptor, has been shown to be activated by RT in various tumors and contributes to radiation resistance. Previous study showed that HGF is a mitogen for Schwann cells, making the HGF/c-MET pathway a valid target in VS. However, whether HGF/c-MET plays a role in VS tumor progression after radiation is not known. Here, we showed that in NF2 patient VS samples, HGF/c-MET expression levels correlate with tumor size. We established radiation recurrent schwannoma mouse models that mimic human disease by progressing after RT. In this model, we found that RT induces the expression of HGF and activates c-MET signaling; indicating that c-MET is a potential escape mechanism contributing to tumor relapse after RT. Blocking cMET signaling with crizotinib, that inhibits cMET tyrosine tyrosine kinase, enhanced RT efficacy. Our results provide compelling rationale for testing combined therapy in NF2 VS.

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AR42 Decreases Growth of Schwannoma in an NF2 Mouse Model

Charles Yates, Waylan Bessler, Li Jiang, and D. Wade Clapp, *Indiana University School of Medicine*

Objective/Hypothesis: A genetically-modified mouse model of NF2 may be an *in vivo* system for testing therapeutics that may have an effect on NF2-related vestibular schwannoma (VS). The mouse model is generated through excision of the *NF2* gene driven by Cre expression under control of a tissue-restricted 3.9-kb Periostin (*postn*) promoter. 100% of the *postn-Cre; NF2^{flx/flx}* mice develop schwannomas in the dorsal root ganglia (DRG) that are histologically similar to human schwannomas. AR42 is a novel histone deacetylase inhibitor that has previously been shown to suppress schwannoma growth in xenograft and allograft models. The objective of the current study is to validate the *postn-Cre; NF2^{flx/flx}* conditionally *NF2*-deficient mouse model as a model for experimental therapeutics for NF2-related VS.

Study Design: *In vivo* mouse study.

Methods: AR42 was dosed by oral gavage at a dose of 25 mg/kg/day. Pharmacodynamics and pharmacokinetics were confirmed in mice prior to the study. Mice were treated for a 3-month period with AR42 and compared to a control group that was gavage fed vehicle. Auditory brainstem response (ABR) testing was performed throughout the study period at monthly intervals. At the end of the study period, mice were examined via necropsy section and DRG were assessed for tumor size and compared.

Results: The tumor size, as determined by the volume of DRG schwannoma, was reduced by AR42 compared to controls. Treated mice tolerated medication at this dose and gained weight appropriately. The behavior of the mice was similar to the control. ABR testing showed a statistically-significant trend toward less decline in hearing for the treated mice when compared to controls.

Conclusions: AR42 suppresses growth of DRG schwannoma in a novel genetically-engineered mouse model of NF2. This is further data that supports the use of AR42 in human trial. The *postn-Cre; NF2^{flx/flx}* mouse model is a model for further assessment of compounds in an *in vivo* model that may hopefully translate testing of compounds to future human trials.

Poster Presentation (even numbers)

MONDAY, JUNE 20, 2016 (5:15 – 6:45 PM)

LAST	FIRST	POSTER	TITLE
Ayter	Sükriye	2	The Effect of Parental Age in Turkish NF 1 Patients
Babovic-Vuksanovic	Dusica	4	Unilateral Vestibular Schwannoma in a Patient with PIK3CA-Related Segmental Overgrowth: Co-occurrence of Mosaicism for Two Rare Disorders
Bellus	Gary A.	6	Plexiform Neurofibroma in a 13 Year Old Boy with Hemimegalencephaly Due to a Somatic <i>PIK3CA</i> p.H1047R Mutation
Bergner	Amanda L.	8	Evaluating Patient-Reported Speech, Spatial and Qualities of Hearing Scale Responses to Bevacizumab among Individuals with Neurofibromatosis 2 and Hearing Loss
Bradford	Diana	10	A Phase II Trial of the Mitogen Activated Protein Kinase Kinase (MEK1/2) Inhibitor Selumetinib (AZD6244; ARRY-142886) in Patients with NF1-mutated Gastrointestinal Stromal Tumors (GIST)
Campen	Cynthia J.	12	Decreased White Matter Integrity of the Corpus Callosum in Children with NF1 Compared to Age-Matched Controls
Cannon	Ashley	14	Longitudinal Natural History Study of Dermal Neurofibromas
Cox	Stephany M.	16	Neurocognitive Abilities, Social Functioning, and Facial Expression Recognition in Children with NF1
Dai	Annie	18	Not Quite There: Access to Specialty Care for Neurofibromatosis and Schwannomatosis Patients
de Blank	Peter	20	Comparison of Automated and Hand-Drawn Tractography of the Optic Radiations in Children with NF1 - Associated Optic Pathway Gliomas
de Souza	Juliana Ferreira	22	Identification of Three Novel Pathogenic Mutations In NF1 Gene
de Souza	Juliana Ferreira	24	Gene Deletion is Associated With Second Toe Signal Phenotype in Neurofibromatosis Type 1
de Souza	Juliana Ferreira	26	Retinal Optical Coherence Tomography Contributes To The Diagnosis Of Neurofibromatosis Type 2
de Souza	Juliana Ferreira	28	18F-Fdg Pet/Ct In Plexiformes Neurofibromas In Nf1
Engelson	Celia	30	Addition of a Genetic Counselor to the Multidisciplinary Team at a Large Neurofibromatosis Center in New York City: A Retrospective Analysis
Gross	Andrea	32	Association of Clinical Morbidities and Plexiform Neurofibroma Volume Changes in Neurofibromatosis Type 1
Gruber	Lucinda M.	34	Case Detection Testing for Pheochromocytoma and Paraganglioma in Patients with Neurofibromatosis Type 1
Hu	Xiaoje	36	Evaluation of Orbital Asymmetry in 58 Unilateral Peri-Orbital NF patients
Hu	Xiaoje	38	Initial Exploration on Temporal Branch of Facial Nerve Function Preservation in PNF Resection
Jonas	Rachel	40	Neural Maturation Trajectories Underlying Risky Decision Making in Youth with Neurofibromatosis type 1
Kallionpää	Roope A.	42	Alterations of the <i>NF1</i> gene in Breast Cancers of the General Population
Lee	Beom Hee	44	Identification of NF1 Mutations by Long-PCR Method of Genomic DNA
Leppävirta	Jussi	46	The Pregnancies and Fertility in Neurofibromatosis 1
Merker	Vanessa	48	Using Health Services Research to Identify Barriers to Early Diagnosis of Schwannomatosis
Mitchell	Carole Wind	50	Summary of 2 Year Experience Conducting Monthly a Multidisciplinary NF Patient Management Conference at an Academic Medical Center
Moodley	Mani	52	Neurofibromatosis Type 2 in Children: A Single Center Experience
Murray	Jeffrey C.	54	Pediatric Intracranial Glioblastoma Multiforme in Association with Neurofibromatosis Type 1
Nishida	Yoshihiro	56	Roles of FDG-PET in the evaluation of deep-seated peripheral nerve sheath tumor
Nutakki	Kavitha	58	Development of the Pediatric Quality of Life Inventory™ Neurofibromatosis Type 1 Module for Children, Adolescents and Young Adults: Qualitative Methods
Payne	Jonathan M.	60	Face perception in children with neurofibromatosis type 1: emotion recognition and scan paths
Prada	Carlos E.	62	Magnetic Resonance Imaging Screening for Optic Pathway Gliomas in Neurofibromatosis type 1
Rezende	Nilton Alves de	64	Evaluation Of Insulin Resistance And Adipocytokines In Neurofibromatosis Type 1
Rezende	Nilton Alves de	66	Amusia Is A Common Feature In Neurofibromatosis Type 1
Rezende	Nilton Alves de	68	Increased Telomere Length In Neurofibromatosis Type 1
Rezende	Nilton Alves de	70	Auditory Training: A New Approach To Learning Disabilities In Neurofibromatosis Type 1 Patients
Riccardi	Vincent M.	72	NF1 Breast Cancer: The Mitochondrial Connection
Riccardi	Vincent M.	74	Purinosomes And Nf1 Pathogenesis
Sellmer	Laura	76	Non-optic Gliomas in Adults and Children with Neurofibromatosis 1
Sellmer	Laura	78	Prevalence and Natural History of Optic Pathway Gliomas in Neurofibromatosis 1
Shea	Stephanie	80	Advanced Practice Provider Use in a Neurofibromatosis Clinic: Development of a Neurology Neurofibromatosis Type I Clinic to Compliment a Multidisciplinary Program at a Larger Tertiary Care Center

POSTERS: Clinical Science

Poster Presentation (even numbers)

MONDAY, JUNE 20, 2016 (5:15 – 6:45 PM)

LAST	FIRST	POSTER	TITLE
Sheridan	Monica	82	Preliminary evaluation of pain in schwannomatosis patients
Shibuya	Peter	84	Outpatient Treatment with Ultra Low Dose Ketamine Infusion for Neuropathic pain in patients with Neurofibromatosis
Shofty	Ben	86	Isolated Gliomas of the Optic Nerve in NF1 Children; A Multi-Center Historical Cohort Study
Talaei-Khoei	Mojtaba	88	Health Literacy in Adults with Neurofibromatosis
Talaei-Khoei	Mojtaba	90	Patient Reported Outcomes (PROs) in Adults with Neurofibromatosis
Talaei-Khoei	Mojtaba	92	Predictors of Patient Satisfaction in Adults with Neurofibromatosis
Walsh	Karin S.	94	The Impact of Ras/MAPK Signaling Pathway-Targeted Therapies on Neurocognitive Functioning in NF1: Preliminary Results of Feasibility, Validity, and Monitoring of Possible Neurotoxicity using a Novel Clinical Trials Approach
Wolters	Pam	96	Development of Patient-reported Outcomes (PROs) to Assess Pain in Individuals with Neurofibromatosis (NF1) and Plexiform Neurofibromas (PNs) for Clinical Trial Endpoints: Preliminary Data from Qualitative Research

The Effect of Parental Age in Turkish NF 1 Patients

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

Neurofibromatosis type 1 (NF1) is the most common neurogenetic disorder, affecting in 3,000 - 3,500 individuals worldwide. Clinic presentations of NF1 are highly variable. Typical manifestations are cafe-au lait spots, freckling, peripheral nerve sheath tumors (benign: Neurofibromas; malignant: Neurofibrosarcomas) and other malignancies (intracranial astrocytomas, gastrointestinal stromal tumors, pheochromocytomas, and juvenile monocytic leukemia. NF1 is caused by mutations of the NF1 gene and 50% of patients represent sporadic NF1 occurs in the absence of a family history of the disease and usually results from a new mutation in the germ cell of one of the parents. Advanced paternal age may increase the risk for new germinal NF1 mutations, although some dominant conditions including neurofibromatosis show a lesser association with paternal age but there are conflicting results on this subject in the literature. Therefore we investigated paternal and maternal age in 241 NF1 patients (121 sporadic and 120 familial) who were seen in Hacettepe hospital which is a reference center for genetic diseases in Turkey. For statistical analysis "SPSS (Statistical Package for Social Sciences) - Windows 20" program was used. Spearman's and Chi – square test were used for statistical analysis. In this study we evaluate the paternal and maternal age at birth in sporadic and familial cases. The mean NF1 paternal and maternal age was almost the same for both sporadic and familial cases as seen in Table.

	Paternal age (Mean)	Maternal Age (Mean)
Sporadic cases (n=121)	31,82 ± 7,19 (20-62)	27,04 ± 6,35 (16-45)
Familial Cases (n=120)	31,58 ± 6,18 (19-52)	27,19 ± 5,34 (17-40)

We also compared the effect of parental age on the appearance and the coexistence of different number of the NF1 symptoms. There were no significant statistical differences between them. However, a slightly negative correlation has been observed between the paternal age and the coexistence of NF1 symptoms in familial cases ($p < 0.05$). As a result we did not find a strong evidence for parental age effect on the clinical severity of the NF1 patients.

This study was supported by Hacettepe University Scientific Research and Development Office (Project Numbers: H.U.BAB. 010 T02 102).

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Unilateral Vestibular Schwannoma in a Patient with PIK3CA-Related Segmental Overgrowth: Co-occurrence of Mosaicism for Two Rare Disorders

Dusica Babovic-Vuksanovic, MD, Mayo Clinic

A 28-year-old female with a history of PIK3CA-related segmental overgrowth presented to Mayo Clinic with headaches and was found to have 5.4 cm left-sided vestibular schwannoma, as well as three small (<2 cm) meningiomas. At birth, she was noted to be large (10 pounds, 1 ounce) and had macrocephaly, mottling of her skin, and by 6-months of age she was noted to have generalized body asymmetry, with her right side being larger than her left. Her hemihypertrophy persisted and required multiple surgeries. She has no significant family history. She underwent surgical resection of the presumed unilateral vestibular schwannoma, which was confirmed by pathology.

According to the current NIH consensus diagnostic criteria for neurofibromatosis 2 (NF2), the presence of a unilateral vestibular schwannoma along with three meningiomas is sufficient for a clinical diagnosis of NF2. While prior genetic analysis of DNA extracted from blood revealed the presence of a mosaic heterozygous c.2740G>A (p.G914R) *PIK3CA* mutation, confirming the diagnosis of PIK3CA-related overgrowth, no mutations in *NF2* were detected on analysis of blood lymphocytes. Although vestibular schwannoma has not previously been reported in PIK3CA-related segmental overgrowth, meningiomas have been associated, raising the question of whether this patient's vestibular schwannoma and meningiomas represent coincidental NF2 or additional manifestations of her overgrowth syndrome. Therefore, we tested the vestibular schwannoma for *NF2* mutations.

We identified a heterozygous *NF2* mutation c.784C>T (p.R262X) and loss of 22q, including *NF2*, *SMARCB1*, and *LZTR1* genes, confirming *NF2* involvement in the development of the acoustic schwannoma. These results suggest that the patient has two different mosaic mutations, *PIK3CA* and *NF2*. We also confirmed presence of *PIK3CA* (c.2740G>A) mutation in the vestibular schwannoma tissue, indicating that the same cell likely received two different mutation hits. Confirmation of the clinical diagnosis of NF2 in this patient has implications for her health monitoring and highlights the possibility of co-occurrence of mosaicism for multiple rare disorders in a single patient.

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Plexiform Neurofibroma in a 13 Year Old Boy with Hemimegalencephaly due to a Somatic *PIKC3A* p.H1047R Mutation

Gary A Bellus, MD, PhD, Irellyn Shepard

We report the case of a 13 year old boy with severe developmental delays who presented shortly after birth with nystagmus and seizures. A brain MRI revealed right sided hemimegalencephaly and ophthalmology evaluation was notable for chorioretinal lacunae and RPE lesions with indistinct borders in his right eye. A high resolution karyotype analysis was performed as was normal. At approximately age 3 years, he was noted to have facial asymmetry with the left cheek being larger than the right. He underwent a MRI study that was negative for hemangioma and revealed mild atrophy of the left optic nerve and globe compared to the right. At age 12 years he was noted to have a protruding nasal mass involving the left septum and presented to the otolaryngology service. MRI revealed inflammatory changes surrounding two left maxillary molars suspicious for an inflammatory process and slightly asymmetrically enlarged fatty tissues in the left cheek. Shave biopsy of the nasal mass was performed and histology was consistent with a plexiform neurofibroma. He was referred to our neurocutaneous clinic and physical examination did not reveal any systemic signs of neurofibromatosis, type 1. Biopsy material from the plexiform tumor was sent for gene sequencing of *AKT3*, *HRAS*, *KRAS*, *NF1* and *PIKC3A*. No pathogenic mutations were detected in any of the genes except for a *PIKC3A* p.H1047R mutation which is one of the most common hotspot mutations reported in the *PIKC3A*-related overgrowth spectrum (PROS). To our knowledge, this is the first report of a plexiform neurofibroma occurring in a patient with PROS.

Evaluating Patient-Reported Speech, Spatial and Qualities of Hearing Scale Responses to Bevacizumab among Individuals with Neurofibromatosis 2 and Hearing Loss

Amanda L. Bergner, MS CGC, Johns Hopkins University

Previous studies aimed at halting or improving neurofibromatosis 2-related hearing loss (NF2-HL) have primarily utilized tumor volume and word recognition scores (WRS) to measure response. We used the Speech, Spatial and Qualities of Hearing scale (SSQ), a comprehensively validated questionnaire for hearing that has not yet been applied to NF2, to assess the patient-reported effect of bevacizumab on hearing disability. We then compared this data to WRS outcomes.

Fourteen subjects with NF2-HL received bevacizumab (7.5mg/kg) every 3 weeks for up to 48 weeks and were monitored after treatment for 6 months to evaluate response durability. Subjects completed the SSQ at baseline, 24 weeks, 48 weeks, and 6 months after the final dose (72 weeks). WRS were measured at baseline and then every 12 weeks on drug, and for 6 months post-drug. Subjects were labeled as WRS responders if they experienced an improvement in WRS at any point during the study above the 95% critical difference compared with baseline.

The overall SSQ completion rate was 91%. Total and domain-specific raw scores were calculated and converted to a percentage score for each subject at each time point. Average total SSQ scores improved from 44.1% at baseline (SD = 18.8) to 48.8% at 24 weeks (SD = 11.7), and 55.3% at 48 weeks (SD = 17.0) with decline to 47.3% by week 72 (SD = 16.2). 50% of subjects (n=7) had a WRS response at some point in their evaluation. Although WRS responders demonstrated higher total SSQ scores at every time point than non-responders, the same longitudinal pattern of improvement and decline was seen among both the responders and non-responders. The majority of the improvement was explained by the Speech domain of the SSQ, which measures the ability to listen to speech in noisy and quiet situations.

NF2-HL can be multifactorial and the mechanism of improvement in hearing with bevacizumab is not fully delineated. Inclusion of the SSQ measure in the analysis of response to bevacizumab further contextualizes the perceived benefit by patients and provides some insight into the aspect of the hearing experience affected by bevacizumab. Interestingly, there is a pattern of improvement even in patients who did not meet criteria for WRS response. This may suggest that there is subjective improvement in hearing, primarily in the ability to listen to speech. Overall, the SSQ appears to be sensitive to change in people with NF2-HL and may be a useful tool to measure the impact of therapies targeting hearing disability in this population.

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A Phase II Trial of the Mitogen Activated Protein Kinase Kinase (MEK1/2) inhibitor Selumetinib (AZD6244; ARRY-142886) in Patients with NF1-mutated Gastrointestinal Stromal Tumors (GIST)

Diana Bradford, MD, *Pediatric Oncology Branch, National Cancer Institute/Children's National Medical Center*

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasm of the gastrointestinal tract. While the tyrosine kinase inhibitors imatinib and sunitinib prolong survival in many patients, they are not effective in patients with "wild type" (wt) GISTs, which lack KIT/PDGFR mutations. Wt GIST comprise 85% of pediatric and 15% of adult GISTs, and include GIST associated with Neurofibromatosis type 1 (NF1) and NF1-mutated GIST in patients without a clinical diagnosis of NF1. NF1-related tumors result from upregulation of the Ras pathway due to NF1 mutations. Selumetinib is an oral MEK1/2 inhibitor, which may mediate anti-tumor effects in NF1 GIST by inhibition of Ras signaling. Our phase I study of selumetinib in children with NF1 related plexiform neurofibromas (PN) has demonstrated a 70% partial response rate. NF1-mutated GIST has no established medical therapy; given the response to selumetinib in other NF1 tumors, there is a strong rationale for its evaluation in NF1 GIST.

Objectives: We will determine the 1) response rate (imaging response RECIST v1.1) of selumetinib in children and adults with NF1-mutated GISTs, 2) the toxicities and progression-free survival, 3) the utility of FDG-PET/CT as a biomarker of early response, 4) response rate determined by the Choi criteria in comparison to RECIST, 5) analyze the molecular pathways altered in NF1-mutated GIST tumor samples, 6) attempt to establish cell lines from patient tumor samples, and 7) evaluate quality of life (QoL) to understand the clinical benefit of selumetinib. Patients will undergo whole body MRI, counting of dermal neurofibromas and NF1 genotyping.

Methods: This is a single site phase II study of selumetinib in children and adults with NF1-mutated GIST. Patients must have a clinical diagnosis of NF1 or a germline or tumor NF1 mutation, and demonstrate measurable disease (RECIST) which has progressed within 12 months. Selumetinib will be given continuously in 28-day cycles at 75 mg BID (≥ 18 years old) or 25mg/m²/dose (< 18 years old). Imaging evaluations and QoL evaluations will occur after every 3 cycles; there is an optional FDG-PET on day 11. With a Simon's two-stage design, if $\geq 1/9$ of the initial cohort demonstrate response, the study will proceed to enroll the full cohort of 24 patients.

Results: The Letter of Intent for this study has been approved by the NCI Cancer Therapy Evaluation Program and the protocol is in development.

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Decreased White Matter Integrity of the Corpus Callosum in Children with NF1 Compared to Age-Matched Controls

Cynthia J. Campen, MD, *Department of Neurology, Lucile Packard Children's Hospital, Stanford University, Palo Alto, CA*

Background: Children with NF1 have learning challenges including decreased processing speed and working memory. These neurocognitive functions have been localized, in part to the corpus callosum (CC). In addition, adults with NF1 have differences in CC white matter (WM) tract diffusor tensor imaging (DTI) compared to controls, with fractional anisotropy (FA) showing decreased organization of the white matter in adults with NF1.

Methods: FA was measured in 24 children (17 months–18 years) with NF1 and 26 age-matched controls on 3T MR imaging. Measurements were obtained at the genu, splenium, and body of the CC using region of interest (ROI) method. Data were acquired using a 25-direction DTI at 3T MRI. Two-tailed student t-test was used with p values < 0.05 deemed significant. Analysis was performed on the group as a whole and breaking the group into three age groups: 17 months–5 years; 6–10 years; 12–18 years.

Results: A significant difference in FA of the CC between patients with NF1 (mean = 0.643, SD = 0.059) and controls (mean = 0.686; SD = 0.127) was identified ($p = 0.0002$). This difference was largely driven by the youngest ages with a mean FA in NF1 = 0.620; SD = 0.115 while that for the controls was 0.658; SD = 0.132 ($p = 0.009$). Children aged 6–10 years also had a significantly different FA; NF1 mean was 0.631 (SD = 0.134), while control mean was 0.678 (SD = 0.131; $p = 0.037$). The oldest ages showed no significant difference in mean FA values ($p = 0.193$).

Conclusions: Young children with NF1 have significantly lower FA values in the CC compared to age-matched controls indicating either decreased myelination or less organization of myelinated fibers. This difference could be implicated in the learning issues most commonly seen in NF1. The difference is more apparent at younger than older ages, a recapitulation of the natural history of optic pathway gliomas and unidentified bright objects in NF1, which stop growing and disappear, respectively with age in NF1. The difference in WM organization may be due to alterations in oligodendroglial lineage precursor dynamics, which have implications in cognition and tumor formation. Future investigation of DTI in other WM tracts and in neoplastic processes of children with NF1 is warranted.

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Longitudinal Natural History Study of Dermal Neurofibromas

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Dermal neurofibromas (DN) manifest in >99% of adults with neurofibromatosis type 1 (NF1) and are responsible for major negative effects on quality of life in individuals with NF1. Previous reports have correlated increased burden of dermal neurofibromas with age and pregnancy, but longitudinal data are not available to establish a quantitative natural history of these tumors. We have previously developed an approach to quantify the number and size of DNs using photographs, calipers, and paper frames, which provides reliable outcome measures. For the present study, we have used this approach to monitor tumor number and size in a cohort of 22 patients with NF1 followed over an eight-year period. A minimum of 6 and maximum of 18 tumors were monitored for each patient in three different body sites. Preliminary data from the first two years of the study showed significant ($p = 0.0185$) tumor growth, although the trend of increased tumor volume was slight. The final 8-year study visit measurements are ongoing and are expected to be available by the time of the conference. This will be the first reported longitudinal natural history study of DNs. Furthermore, these results will provide insight into DN development and may be useful to researchers considering clinical trials targeting DNs.

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This study was supported by internal funds from the University of Alabama at Birmingham.

Neurocognitive Abilities, Social Functioning, and Facial Expression Recognition in Children with NF1

Stephany M. Cox, PhD, *Children's National Medical Center*

Impairments in cognitive and social functioning among children with neurofibromatosis type 1 (NF1) have been well documented. Further, structural and functional brain abnormalities have also been identified as a feature of NF1 and have been shown to contribute to cognitive impairments. However, little is known about the relationship between neurocognitive impairments and social deficits for this population. Aspects of facial expression recognition have been associated with various cognitive and social impairments in children with autism spectrum and attentional disorders, and there is some evidence indicating that facial recognition skills may be impaired in individuals with NF1. The aim of this investigation was to examine the relationship between neurocognitive ability, social functioning, and multiple aspects of facial expression recognition in children with NF1. Thirty-one children with NF1, ages 8 to 15 ($M = 11.54$, $SD = 2.58$, 51.6% male), were administered a traditional cognitive measure (WISC-IV), a computerized battery measuring neurocognitive processes (e.g., attention, working memory, processing speed; CogState), and a social emotional cognition task (SECT). The SECT uses an odd-man out paradigm to measure social emotional cognition, focusing on the perception of three areas of facial expression recognition: eye direction, eye emotion, and face emotion. Parent questionnaires regarding social, emotional, and executive functioning were also administered (CBCL, BRIEF). Overall cognitive ability was significantly correlated with all three tasks of the SECT: eye direction ($r = .523$, $p < .05$), eye emotion ($r = .487$, $p < .05$), and face emotion ($r = .598$, $p < .05$). The Working Memory Index of the WISC-IV was also moderately correlated to performance of all three SECT tasks ($.384 < r < .484$). Speed and accuracy on a visual-spatial learning task was moderately correlated to participants' ability to discriminate eye direction ($.393 < r < .561$). Of interest, performance on facial expression recognition tasks also significantly correlated with parent report of social problems and engagement in extracurricular activities ($.403 < r < .502$, $p < .05$). Additional analyses showed differences in aspects of cognitive and social skills as a function of whether or not participants showed impaired performance on the SECT. Children with NF1 exhibit impairments in cognitive abilities, social functioning, and facial expression recognition. This study builds upon the current literature by highlighting the relationship between these deficits, demonstrating that overall cognitive ability and working memory is associated with social functioning as measured by performance on facial expression recognition tasks and parent-report.

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Funding Source: The Jennifer and Daniel Gilbert NF1 Research Institute

Not Quite There: Access to Specialty Care for Neurofibromatosis and Schwannomatosis Patients

Annie Dai, *Massachusetts General Hospital/Harvard University*

Methods: We retrospectively reviewed reports submitted annually by NFCN clinics from 2008 to 2015. We classified clinics as having a specialty in NF1, NF2, and/or SchW if at least 12 patients were seen with that condition per year (an average of 1 patient per month). We classified clinics as having a pediatric focus if they were part of a Children's Hospital or $\geq 85\%$ of patients seen were ≤ 18 years of age. We also reviewed data from patients in the CTF-sponsored NF Patient Registry, including patients' diagnosis, age, state of residence and place of NF care. For regional analyses, the United States was divided into eight regions, as defined by the U.S. Bureau of Economic Analysis.

Results: The NFCN grew from 33 to 50 clinics between 2008 and 2015, and annual patient volume rose from 6,817 to 10,245 patients per year. The average annual number of patients seen in NFCN clinics from 2013 to 2015 included 8987 (92%) NF1 patients, 601 (6%) NF2 patients, and 149 (2%) SchW patients. 74% of patients were age ≤ 18 and 26% were age > 18 . Of the 50 clinics, 49 (98%) had a NF1 specialty, 10 (20%) had a NF2 specialty, and 4 (8%) had a SchW specialty. 30 (60%) of clinics had a pediatric focus. The Mideast had the highest concentration of clinics per capita (3.3 million people per clinic) and the Far West region had the lowest (14.2 million people per clinic). As of 10/14/2015, 4476 living U.S. patients with a known diagnosis were enrolled in the NF Registry. 285/3848 (7%) of NF1 patients, 94/496 (19%) of NF2 patients, and 53/82 (65%) of SchW patients did not live in a region with an NFCN clinic seeing both their disease and age group (i.e. for adult patients, a non-pediatric focused clinic had to be present). Of the 2270 registry patients who entered information about which clinic they attended, only 999 (44%) received care in an NFCN clinic

Conclusions: Since 2008, the number of NFCN clinics and the volume of NF patients seen in these clinics have increased, but there are still many NF patients who are not seen in NFCN clinics and/or travel a significant distance for their care. Potential measures to improve access to specialty NF care include establishing new clinic sites in the Western region; recruiting/partnering with additional clinicians specialized in NF2/SchW; and increasing capacity of adult care.

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VM is supported by a Children's Tumor Foundation Young Investigator Award.

Comparison of Automated and Hand-Drawn Tractography of the Optic Radiations in Children with NF1-Associated Optic Pathway Gliomas

Peter M.K. de Blank, MD, MSCE, Rainbow Babies & Children's Hospital

Introduction: Fractional anisotropy (FA) of the optic radiations has been associated with visual acuity in children with NF1-associated optic pathway gliomas (OPGs).[1] Previous tractography methods have relied on hand-drawn regions of interest, which limits consistency between sites and studies. An automated process that quickly and reliably identifies white matter tracts would improve consistency in and access to DTI tractography biomarkers. We created an automated method to identify white matter tracts in the optic radiations and compared this method to tractography using hand-drawn regions of interest as previously reported.[1]

Methods: In 50 children with NF1-OPG (26/50 with abnormal visual acuity), two methods for OR tractography were assessed (Figure). In the hand-drawn method, a section of the optic pathway was isolated between regions of interest posterior to Meyer's loop and anterior to tract branching near the calcarine cortex. Automated tractography of the complete optic radiation was performed using probabilistic streamline fiber tracking between the lateral geniculate nucleus of the thalamus and the occipital cortex. The association of FA and visual acuity deficit was evaluated for both methods on univariate and multivariate analysis adjusting for age, tumor location, and DTI parameters.

Results: Hand-drawn tractography methods required 20-25 min/scan; automated methods were performed with <1min/scan of operator time. FA of the optic radiations was significantly different between the two methods. On univariate analysis, FA was associated with visual acuity loss in both methods. On multivariable analysis, FA from both methods demonstrated an association with visual acuity. The model using automated tractography had a better coefficient of determination (R^2) (Table).

Conclusion: Automated tractography of the optic radiations offers a fast, reliable and consistent method of tract identification that is not reliant on operator time and expertise. Unlike the prior hand-drawn method, automated tractography measures the complete tracts from thalamus to cortex and may better conform to anatomic expectations of the radiations. This method of tract identification may be useful as DTI is developed as a potential biomarker for visual acuity.

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Acknowledgement: Neurofibromatosis Therapeutic Acceleration Program. 1. de Blank et al. Neuro-oncology, 2013.

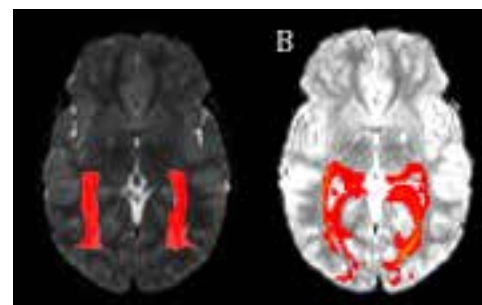


Figure: Optic radiation tractography using hand-drawn regions of interest (A) and automated tractography (B).

	Association with Visual Acuity		
	Unadjusted OR	Adjusted OR	R ²
FA Hand-drawn	-5.59 [-9.40 to -1.77]	-6.08 [-10.32 to -1.84]	0.32
FA Automated	-6.36 [-10.85 to -1.87]	-7.90 [-12.64 to -3.17]	0.36

Identification of Three Novel Pathogenic Mutations In NF1 Gene

Juliana Ferreira de Souza, MD, Neurofibromatosis Reference Center, Federal University of Minas Gerais (UFMG), Brazil

Neurofibromatosis type 1 (NF1) is a genetic autosomal dominant disease characterized by café au lait spots, Lisch nodules, neurofibromas, osseous dysplasia and optical gliomas. NF1 is the most common genetic disease caused by a single gene [1]. The gene NF1 is located at chromosome 17 (17q11.2) near the centromere, whose product is a protein (Neurofibromin - 2,485 amino acids) predominantly expressed in neurons, Schwann cells, oligodendrocytes, and leukocytes. The Neurofibromin is a tumor suppressor protein, that functioning as negative regulator of cellular RAS-MAPK signaling pathway. Any mutation that disrupts the function of protein could lead to uncontrolled cell growth and potentially tumorigenesis. More than 90% of these mutations are small deletions and insertions, splicing mutations, and nonsense or missense mutations. A minority of patients (4-5%) has exonic or whole-gene deletions/duplications. The NF1 gene has the highest rate of mutation in a human gene and the causes remain unclear. In this work, we described three novel mutations in the NF1 gene, related to typical NF1 clinical features. The mutations were identified by Next-generation sequencing complete of NF1 gene of individuals previously diagnosed for NF1 and result negative for exonic or whole-gene deletions/duplications test. The clinical diagnosis of NF1 was based on the clinical diagnostic criteria outlined in the National Institutes of Health (NIH) consensus development conference in 1988. The products of PCR were loaded on Ion 316 chip and sequenced with an Ion Personal Genome Machine (PGM) System (Thermo Fisher). Entire NF1 coding exons and their intron boundaries (25 bp) were coverage (99.44%). Screening for NF1 deletions was performed using the SALSA P081/082 (MRC-Holland) NF1 MLPA assay following instruction's manufacturer. All the three mutations (g.248760_248763del, g.250559dup and g.69115delG) resulted in a frame shift predicted to generate a premature stop codon at amino acid positions 2268, 2305 and 102, respectively. All of them were classified as pathogenic following the Standards and Guidelines for interpretation of sequence variants by the American College of Medical Genetics and Genomics (ACMG – 2015). The knowledge of new pathogenic mutations is helpful to best clinical decisions for NF1 treatment and diagnosis.

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Funding Support: FAPEMIG, CAPES, CNPQ

Gene Deletion is Associated With Second Toe Signal Phenotype In Neurofibromatosis Type 1

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The early diagnosis of Neurofibromatosis types 1 (NF1), based on consensus criteria, is useful for the management of clinical aspects and genetic counseling. Additional specific congenital lesions might assist in the early diagnosis of NF1. Our group previously reported, through questionnaire (12%) and photographic register (5.8%), the prevalence of a not yet described NF1 phenotype component: bilateral superposition of the second toe over the first and the third toes, which we referred to as the “Second Toe Signal” (STS) (Figure 1). Regarding the fact that the most severe NF1 phenotypes are associated with microdeletions (the former whole-gene deletions), we assessed the association between STS and microdeletions. Multiplex Ligation-dependent Probe Amplification (MLPA) was performed for 21 NF1 patients presenting at least three NIH diagnostic criteria. The kits used were P081 e P082 – version C1. The samples’ results were analyzed by Coffalyser v.140721.1958 software. Statistical analysis was performed with the open source calculator OpenEpi (version 3, www.openepi.com), using the Fisher exact test. Results: We found three microdeletions, including the regions of the flanking probes of the NF1 gene.

All three microdeletion subjects have STS, one patient has STS but not microdeletion and the 17 others have neither STS nor microdeletion ($P=0.006$). These three microdeletions patients with STS present the generally accepted microdeletions clinical phenotype. As we increase our sample size, we wish to suggest that STS, particularly regarding its presence at birth, is a useful clinical sign of NF1 microdeletion.

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Funding Support: UAB Neurofibromatosis Program

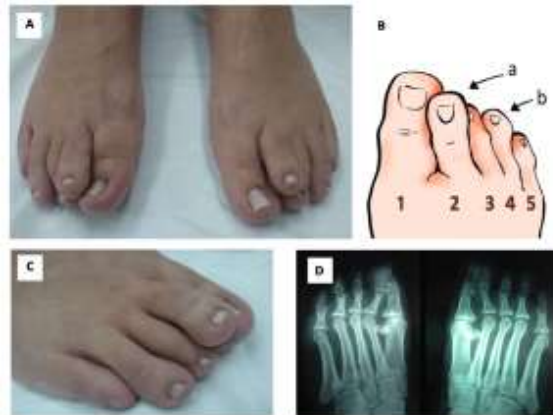


Figure 1 – Pictures (A, C) of one NF1 volunteer (three NIH criteria at least) with bilateral superposition of the up of second toe (diagram B, a) and enlargement of the forth (diagram B, b) and feet XR (D). The patient presents the NF1 gene microdeletion phenotype and gene deletion at MLPA.

Retinal Optical Coherence Tomography Contributes To The Diagnosis Of Neurofibromatosis Type 2

Juliana Ferreira de Souza, MD, Neurofibromatosis Reference Center, Federal University of Minas Gerais (UFMG), Brazil

Background: Neurofibromatosis type 2 (NF2) is an autosomal-dominant disease, characterized by bilateral vestibular schwannomas, multiple central nervous system (CNS) tumors, skin tumors and juvenile cataract. A well-defined spectrum of ocular features has been specifically associated with NF2. The present study evaluated retinal abnormalities using spectral domain optical coherence tomography (SD-OCT) in a case-series of NF2 patients.

Methods: Nine NF2 patients from the neurofibromatosis outpatient reference center of Federal University of Minas Gerais, Brazil, underwent a detailed ophthalmic and medical history and a comprehensive ophthalmic evaluation, including SD-OCT to detect retinal lesions. From nine NF2 patients evaluated, five had early onset (< 20 years) of NF2 and four patients had late onset (>20 years) of symptoms.

Results: SD-OCT scans revealed retinal abnormalities in all patients with early onset (EOS) and in two patients with late onset (LOS). In the EOS group SD-OCT scans revealed flame-shaped epiretinal membranes (ERM) with peculiar characteristics in four eyes of three patients. Two patients had fine undulations of the inner retinal surface with a subtle ERM. Retinal hamartomas were presented in four eyes of three patients with EOS; in two eyes they were subclinical, detected only by SD-OCT scans. In two patients with LOS and one patient with EOS, SD-OCT scans revealed retinal tufts of nerve fiber layer.

Conclusion: SD-OCT revealed retina and vitreous alterations in most of patients with NF2 and is a valuable exam for their evaluation. It was well tolerated for all patients with NF2, including children and/or patients with disabling conditions. ERM in NF2 have unique features that distinguished it from idiopathic ERM, or membranes associated with other diseases. We suggest that flame-shaped ERM seems to be specific of NF2 and that ERM could be included in the NF2 diagnostic criteria.

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18F-Fdg Pet/Ct In Plexiformes Neurofibromas In Nf1

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Malignant tumors of peripheral nerve sheath (TMNP) are a leading cause of death among patients with neurofibromatosis type 1. The lesions were classified according to increasing metabolism, metabolism aspect, the presence of hypermetabolism associated with increased radiation density and the presence of low uptake suggestive of central necrosis. The values of SUVmax, SUVav, and the average value of the Hounsfield scale close to the maximum point in the lesion SUV and the largest diameter of each lesion were discriminated. In this dissertation forty two individuals with NF1 were studied with 18F-FDG PET / CT. Radiological and functional parameters were examined for 55 lesions (18 diffuse neurofibromas and 37 nodular neurofibromas). A highly significant correlation was found between the values of SUVmax ($p < 0.001$), SUVav ($p < 0.001$), the relationship between the SUVmax value of the lesion and SUVav the hepatic parenchyma ($p < 0.001$), the mean value of Hounsfield unit area close to SUVmax ($p < 0.011$) for the presence of malignant transformation ($p < 0.001$). It was also observed a significant correlation between the type of neurofibroma ($p < 0.028$), the presence of other lesion in a different segment ($p < 0.038$), the increase of metabolism ($p < 0.001$), the appearance of intratumoral metabolism ($p < 0.001$), the presence of hyper metabolism associated intralesional radiological hyperdensity ($p < 0.002$) and presence of low uptake suggestive of central necrosis ($p < 0.001$). The aim of this study was to evaluate the potential of qualitative and quantitative predictors of 18F-FDG PET / CT images that can contribute to more accurate diagnosis of patients with neurofibromatosis type 1 (NF1) on suspicion of malignant transformation. The studies of 18F-FDG PET / CT represent a breakthrough in detecting TMNP due to its high sensitivity. Conclusion: The present study showed that addition of radiological and functional predictors provided some increase in specificity of the method. Its use has the potential to reduce the false positive case number and unnecessary surgical procedures.

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Addition of a Genetic Counselor to the Multidisciplinary Team at a Large Neurofibromatosis Center in New York City: A Retrospective Analysis

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Genetic counselors are highly trained professionals with expertise in supporting individuals and families with genetic disorders. Genetic testing can be an important adjunct to clinical care for these individuals, and genetic counselors can serve as experts in the provision of this testing. While some clinics in the United States that care for patients and families with neurofibromatosis (NF) and schwannomatosis (SWN) offer genetic services as part of the multidisciplinary approach to care, many do not have dedicated genetics services available as part of their team.

Using a retrospective, qualitative approach, we analyzed the impact that the addition of a genetic counselor has made on care provided at a busy urban clinic specializing in NF and SWN. We qualitatively analyzed the impact of this team member on the provision of genetic testing services within our clinic, specifically identifying the types of patients for whom genetic testing was offered and performed.

Multiple barriers to providing genetic counseling and testing within the NF Center, including: time constraints of existing providers, lack of a staff member dedicated to overseeing all aspects of the genetic testing process, insurance regulations regarding genetic testing; and the need for follow up counseling after test results were received. Therefore, a minority of patient for whom genetic testing was appropriate could receive these services within the NF Center. Prior to the addition of a genetic counselor to the team, most patients and their families for whom genetic testing was appropriate were referred to an outside Genetics clinic within the medical center genetic counseling and genetic testing at an appointment separate from their NF clinic appointment, resulting in long wait times for these services and inconsistent follow-through by patients.

The addition of a genetic counselor to the NF Center has resulted in increased counseling encounters for our NF patients and availability of genetic testing and related services for all appropriate patients within our Center, thereby reducing wait times and improving patient follow-through. Our analysis highlights the importance of the inclusion of an individual with specialized training in genetic counseling in the comprehensive approach to care of patients and their families at a specialty NF clinic.

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Association of Clinical Morbidities and Plexiform Neurofibroma Volume Changes in Neurofibromatosis Type 1

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Background: Neurofibromatosis Type 1 (NF1) is an autosomal dominant disorder characterized by mutation in *NF1*. Approximately 25-50% of patients with NF1 will develop plexiform neurofibromas (PN), which can be associated with significant morbidities. Ongoing clinical trials are evaluating the effect of targeted therapies on PN volume. However, no previous study has assessed for an association between an increase in PN volume and development of morbidities.

Objective: To utilize data from the NIH NF1 Natural History study to assess the hypothesis that increasing PN volume in NF1 is associated with increased morbidity.

Method: Retrospective chart review of patients enrolled on the NF1 Natural History study with ≥ 10 years of historical data available. Patient morbidities were assessed at two time-points: time of baseline PN MRI with volumetric analysis and time of MRI with maximum PN volume. Morbidities evaluated included the presence of pain, motor dysfunction, airway compromise, bowel or bladder dysfunction, vision loss and PN related surgery.

Results: Twenty-four patients with 35 distinct PN were included, with median PN volume 163 mL at baseline (range 2.3 - 4895 mL) and 469 mL at maximum assessment (range 10.2 - 7210 mL). At baseline, 15 of 35 PN had at least one associated morbidity. There was an increase in the number of PN requiring scheduled pain medications when comparing baseline (2/35) to maximum volumes (11/35). Based on their locations, 24 of the 35 PN had the potential for motor related morbidity. Of these, the PN that had motor impairment at baseline (8/24) or maximum volumes (14/24), had larger volumes compared to those that did not have motor morbidity (514 mL larger at baseline (95% CI 27, 1092), 719 mL larger at maximum (95% CI 31, 3271)).

Conclusion: Many patients with NF1 had significant PN related morbidities even at baseline assessment, which reinforces the need for long-term evaluation to see changes in PN associated morbidities. There was a trend towards increased need for pain medications with increased PN volume, as well as an increase in motor morbidity associated with larger PN volumes. Future analyses of larger patient populations are ongoing and may help elucidate these associations.

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Case Detection Testing for Pheochromocytoma and Paraganglioma in Patients with Neurofibromatosis Type 1

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Context: Individuals with neurofibromatosis type 1 (NF1) are at an increased risk of developing a pheochromocytoma or paraganglioma (PHEO/PGL). However the best case detection strategy is unknown.

Methods: This is a retrospective cohort study of 41 patients with NF1 and PHEO/PGL who were identified from 1959 to 2015 using the Mayo Clinic PHEO/PGL and NF1 databases: 3289 and 1415 patients, respectively.

Results: The prevalence of PHEO/PGL in patients with NF1 was 6.6%. The 41 patients included 23 men (56%) and 18 women. The median age at diagnosis was 41.0 years (range 14-67). The median tumor size was 3.4 cm (range 0.8-9.5). Bilateral PHEO was identified in 17% (n=7) of patients, all women. Metastatic or recurrent disease occurred in 7.3% (n=3). In the last 25 years, PHEO/PGL was diagnosed after incidental finding on computed imaging in 31% of patients (n=11). Only three patients (7.3%) had PHEO/PGL discovered because of biochemical case detection testing.

Conclusions: We recommend patients with NF1 have biochemical case detection testing for PHEO/PGL every 3 years starting at age 10 to 14 years. Biochemical case detection testing should also be done prior to elective surgical procedures and prior to conception.

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Evaluation of Orbital Asymmetry in 58 Unilateral Peri-Orbital NF Patients

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Background: Severe peri-orbital NF is characterized by orbital morphological alterations which may result in orbital deformation or even eye-ball dislocation. Purpose: The objective of this study was to evaluate the orbital asymmetry in unilateral peri-orbital NF patients.

Methods: This was a retrospective study by review of medical records and computerized tomography examination of 58 peri-orbital NF patients between 2011 and 2016, who were divided into 2 groups: group of peri-orbital NF with SGWD (sphenoid great wing defect) and group without SGWD. The orbit width, height, and diagonals were measured with MIMICS software. Statistical analyses were conducted using Student t test.

Results: Patients' age ranges from 6 to 49 years, in an average of 20 years' old. The average orbital width of affected and unaffected sides of orbital were 37.67 ± 4.01 mm and 35.75 ± 3.09 mm in peri-orbital NF group with SGWD, respectively, and 36.04 ± 2.38 mm and 35.37 ± 2.60 mm in group without SGWD. The average orbital height of affected and unaffected sides of orbital were 44.54 ± 4.64 mm and 38.24 ± 2.47 mm in SGWD group, respectively, and 38.41 ± 2.70 mm and 35.79 ± 2.07 mm in group without SGWD. The average orbital diagonals of affected and unaffected sides of orbital were 49.40 ± 5.21 mm and 41.07 ± 2.72 mm in SGWD group, respectively, and 43.34 ± 4.69 mm and 40.6 ± 3.38 mm in group without SGWD. Significant differences were observed in the orbital width ($p=0.016$), height ($p<0.01$) and diagonals ($p<0.01$) between the affected and unaffected sides of peri-orbital NF groups with SGWD. Significant differences were observed in orbital height ($p<0.01$) and diagonals ($p<0.01$) between the affected and unaffected sides of peri-orbital NF groups without SGWD. The average variance of orbital width, height and diagonals between affected and unaffected sides of orbital were $8.52 \pm 5.90\%$, $15.35 \pm 8.27\%$ and $17.97 \pm 8.81\%$ in SGWD group, respectively, and $6.00 \pm 4.55\%$, $7.07 \pm 5.37\%$, $7.96 \pm 6.93\%$ in group without SGWD. Significant differences were observed in variance of orbital height ($p<0.01$) and diagonals ($p<0.01$) between groups with and without SGWD.

Conclusions: Whether or not with SGWD, the orbital Bone dysplasia accompany with peri-orbital NF in our patients. The peri-orbital osteal malformation will be more obvious when SGWD.

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Initial Exploration on Temporal Branch of Facial Nerve Function Preservation in PNF Resection

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Background: Large temporal plexiform neurofibroma (PNF) is an irritating problem that causes facial disfigurement. Surgical resection of PNF is the only effective way to remove the tumor as well as to improve the patient's facial appearance. However temporal branch of the facial nerve (TBFN) in the tumor is prone to be destroyed during PNF removal. Thus, TBFN palsy is the inevitable complication after surgery and might induce other malformation & dysfunction. Therefore, the aim of this study is to reconstruct a nearly normal face contour while preserving the facial nerve function.

Purpose: Selective PNF removal technique was designed to protect TBFN during PNF lesions resection in our cases.

Methods: From May 2011 to June 2015 we had 10 patients that suffered from PNF in the temporal region with facial disfigurement and underwent selective PNF removal to correct the facial disfigurement while preserving TBFN as well.

Results: All patients obtained the improvement of facial appearance after surgery. The temporal PNF was removed and the TBFN function successfully maintained. PNF recurrence has not been relapsed during 6 to 49 months' follow-up.

Conclusions: In our initial exploration, TBFN function maintenance and facial appearance improvement can be achieved simultaneously by using PNF selective removal surgery technique.

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Funding supported by 1: Wang Kuan Cheng Medicine Reward Funding; 2: Grant for national clinic key disciplines and specialties for plastic and reconstructive surgery department.

Neural Maturation Trajectories Underlying Risky Decision Making in Youth with Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) is a neurogenetic disorder caused by a mutation in the neurofibromin gene, and is one of the most common single gene disorders affecting cognitive function. About one-third of children with NF1 meet diagnostic criteria for ADHD and the cognitive phenotype is characterized by impairment in prefrontally-mediated functions, which encompass attention, working memory, and inhibitory control. Additionally, individuals with NF1 are thought to have irregularities in dopaminergic metabolism, particularly in the striatum. Due to this neurobehavioral and physiological profile, we hypothesized that the NF1 population would show abnormal behavior on a task of risk-taking shown to be reliant on the orbitofrontal cortex and striatum. We anticipated that neural activity during risky decision-making would be irregular in the NF1 group. Healthy individuals show an adolescent-specific peak in the striatum during risky decision-making, and we predicted that individuals with NF1 would not show this typical trajectory.

Methods: Youth with NF1 (N=28, mean age=11.96 +/-2.69 years) and typically developing (TD) controls (N=22, mean age=12.55 +/- 3.45 years) were administered a developmentally sensitive gambling task (Van Leijenhorst et al., 2008), in which they chose between low-risk gambles with a high probability of obtaining a small reward and high-risk gambles with a low probability of obtaining a large reward. Primary behavioral analyses included assessing how risk-taking changes as potential reward increases. We used functional MRI (fMRI) to investigate neural activity associated with risky decision making, as well as age-associated changes in these behavioral and neural processes

Results: Youth with NF1 made fewer risky decisions, particularly when the potential reward was high. Neuroimaging analyses revealed a significant age by group interaction during risky decision-making in the medial prefrontal cortex (mPFC); in TD controls increased age was associated with diminished mPFC activity, whereas the opposite relationship was found in the NF1 population.

Conclusions: Individuals with NF1 have high rates of attention-related problems which may be related to underlying abnormalities in dopaminergic metabolism. Youth with NF1 show risk-averse behavior, particularly during high-reward conditions. Neuroimaging findings suggest that developmental trajectories of prefrontal neural activity during risky decision-making may be disrupted in youth with NF1.

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Funding: DOD W81XWH-12-1-0081

Alterations of the *NF1* Gene in Breast Cancers of the General Population

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Background: Germline mutations of the *NF1* gene cause neurofibromatosis type 1 (NF1) that is notorious for the associated high cancer risk. Recent sequencing studies have also identified somatic alterations of *NF1* in a variety of tumors from patients without the NF1 syndrome, such as pulmonary, mammary, and ovarian cancers, the glioblastoma and melanoma (<http://www.cbioportal.org/>). The aim of this study is to examine the role of *NF1* alterations in breast cancer.

Methods: Data generated by the TCGA Research Network (<http://cancergenome.nih.gov/>) was analyzed to examine the role of *NF1* alterations in breast invasive carcinoma. The database contains tumor sequencing and copy number variation data together with clinical characteristics of the cancers and patients. Receptor status and patient survival were compared between samples with normal *NF1* and those that harbored mutation or some level of deletion in *NF1*. Chi-squared test and Cox proportional hazards model were used for statistical analysis. Expression of drug metabolizing or transporting proteins was analyzed by fitting a linear regression model between expression levels and *NF1* copy number.

Results: Mutations or deletions of the *NF1* gene were observed in 33% of the TCGA breast cancers. The presence of *NF1* mutations or deletions seemed to be associated with poor 5-year survival, although the difference was not statistically significant: 86.6% (95% CI 81.7% to 91.7%) with normal *NF1* vs. 77.1% (95% CI 70.5% to 84.3%) with *NF1* mutation or deletion ($P=.281$). Cancers with *NF1* mutation or deletion were significantly more often estrogen and progesterone receptor negative and *HER2*-amplified than those with *NF1* intact ($P<.001$ for all). The expression of 10 transporter proteins and 7 drug metabolizing enzymes was significantly associated with *NF1* copy number.

Discussion: The results show that *NF1* alterations in breast cancer are associated with unfavorable prognostic factors. Similar characteristics have been observed in breast cancers of NF1 patients (Uusitalo et al., unpublished), which suggests that these effects are specific to the *NF1* gene. Changes in the expression of drug metabolizing and transporting proteins may contribute to the treatment outcome of cancers with *NF1* alterations.

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Identification of NF1 Mutations by Long-PCR Method of Genomic DNA

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The importance of genetic diagnosis is increasing in terms of genetic counseling and adequate management of patients with Neurofibromatosis I (NF1, OMIM 162200). Currently multi-step mutation detection protocol is recommended for the genetic diagnosis of NF1; both genomic DNA and cDNA analyses with duplication/deletion analyses. With this protocol, more than 95% of the patients can be diagnosed genetically. However, the RNA analysis is a labor intensive and sensitive study. On the other hand, genomic DNA sequence analysis alone can detect *NF1* mutations in only ~ 60% of the patients. A reason of this low detection rate is that the *NF1* gene is a large sized gene that consists of 58 exons. Moreover, it contains the homologous domains in exon 9-31, cystein-serine rich domain (CSD), GTPase related domain (GRD), and adenylated kinase pseudogene (AK3), that are dispersed in multiple chromosomal loci. Thus, routine medical genomic DNA sequencing can yield the wrong results derived from the homologous sequences located outside *NF1*. In our current study, 5 long-PCR products (6-13 kbs) were amplified using NF-1 specific primers to avoid the amplification of these homologous domains. The nested PCR was done in each of the 5 long PCR products. With this method, 49 patients with NF1 were tested and 42 patients (86%) were genetically diagnosed; 5 missense, 10 nonsense, 21 frameshift and 6 splicing NF1 mutation were identified and 21 mutations (51%) were novel mutations. Multiplex ligation-dependent probe amplification (MLPA) analysis identified whole genomic deletion of NF1 in two additional patients (4%). Overall, 90% of the patients were genetically diagnosed by long-PCR and MLPA methods. The results of our current study indicate that high mutation detection rate can be achieved using long-PCR of genomic DNA.

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The Pregnancies and Fertility in Neurofibromatosis 1

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Purpose: The purpose of this retrospective total population study was to form an extensive view of the fertility, abortions, pregnancies and deliveries of the patients with neurofibromatosis type 1 (NF1).

Methods and patients: The cohort of 1,417 patients with NF1 was acquired by searching NF1 related hospital admissions and confirming the diagnoses reviewing the medical records. Ten control persons per patient with NF1 were collected by Population Register Centre of Finland. Study persons were linked to data from Medical Birth Register with personal identity code. Standardized fertility ratio (SFR), abortion rate, duration of the pregnancy and delivery-related variables were analyzed.

Results: The SFR of patients with NF1 was reduced to 0.87 (CI 95%: 0.766-0.983, $p=0.027$). The mean duration of the pregnancy was 39.17 weeks among mothers with NF1 while being 39.82 weeks in the control group. The difference was highly significant ($p < 0.001$). The mean length of the pregnancy of healthy mother with a child having NF1 was 0.45 weeks shorter than in the matched control group leading to the significant difference between these groups ($p < 0.001$). Cesarean deliveries and hospitalization for hypertension during pregnancy were significantly more common among mothers with NF1.

Conclusions: NF1 of the mother or the fetus is associated with the decreased duration of the pregnancy and increased pregnancy complications. This is the first study describing the effect of the NF1 of the fetus on the pregnancy. Considering the increased risk of the pregnancy complications among NF1 related pregnancies, careful evaluation is needed when assessing these pregnancies.

This study was funded by grants from Finnish Society of Dermatology, Turku University Hospital and University of Turku Doctoral Programme of Clinical Investigation.

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Using Health Services Research to Identify Barriers to Early Diagnosis of Schwannomatosis

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Background: Delay in diagnosis is a critical issue for schwannomatosis (SWN) patients since lengthy delays can lead to psychological distress and sub-optimal care management. We sought to investigate provider and system level factors contributing to delays in SWN diagnosis in order to identify target areas for improvement.

Methods: We conducted a retrospective chart review of patients with definite SWN (as defined by Plotkin et al., 2013) seen in neurofibromatosis (NF) specialty clinics at two U.S. academic medical centers. Narrative summaries detailing key processes and events in each patient's diagnostic journey were generated by the first author based on all available information in the electronic medical record. We applied qualitative thematic analysis to these summaries to understand clinicians' diagnostic decision-making and identify recurrent barriers to timely SWN diagnosis.

Results: Medical records of 27 patients were analyzed. The median age at first symptom was 32 years (range, 11 to 63 years), with a median of 7 years between first symptom and diagnosis (range, <1 year to 30 years.) Qualitative analysis revealed 4 major patterns related to diagnostic delay: lack of awareness of SWN, dismissal of pathologically benign tumors, difficulty interpreting nonspecific symptoms, and impaired information flow between medical institutions. Multiple clinicians did not appear to know the distinguishing features between NF1, NF2, and SWN, leading to a misdiagnosis of NF1/NF2 in 5/27 cases. Pathologists sometimes had difficulty interpreting schwannoma samples, and in two cases, misdiagnosed a schwannoma as a malignancy. Multiple surgeons did not refer patients for further evaluation after schwannoma resection based on benign histology, even in patients with multiple prior resections. Impaired information flow between institutions led to clinicians not knowing that a diagnostic test had already been performed, or not having the results available at the time of their encounter with a patient. This led to duplicative testing (most often MRIs) and unnecessary work-ups for alternate conditions that had already been ruled out.

Conclusions: Diagnostic delay is a significant problem in SWN. However, there are potentially remediable provider and system level barriers to early diagnosis. Targeted education of medical professionals and improved sharing of medical records may reduce diagnostic delay in the future.

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This work was supported by a Children's Tumor Foundation Young Investigator Award to VM.

Summary of 2 Year Experience Conducting Monthly a Multidisciplinary NF Patient Management Conference at an Academic Medical Center

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Background: A diverse group of surgical and medical specialists as well as fellows, residents and nurses attend a monthly NF patient management conference. We have established multiple goals for a monthly multidisciplinary NF patient management conference including: 1. To review the complex medical history, MRI's and pathology of patients with NF1, NF2 or Schwannomatosis who have progressive symptoms usually related to a tumor. 2. To propose additional diagnostic and surgical procedures or enrollment on a clinical trial. We have attempted to objectively evaluate the success and limitations of this forum after a 4 year period by circulating a survey to the participants.

Observations: The annual accrual of new patients to our NF Center in 2015 was 190 which is reflective of the recent addition of the patient's formerly seen at Weil Cornell. We have presented an average of 12 patients per month and, 144 patients per year with management or diagnostic concerns. The average attendance at this conference is 14 specialists and the distribution of diagnoses was: NF1 40%; NF2 59% and Schwannomatosis 1%. Complex surgical procedures were planned for 16 patients, 8 patients were treated medically with chemotherapy and 18 patients were enrolled on clinical trials. The result of our survey was this was a valued conference which met the educational needs of the participants and improved patient outcomes.

Conclusions: 1. Our multi-disciplinary NF team has become increasingly reliant on this forum to optimize treatment planning and we believe that patient care has improved. 2. Clinical trials awareness and enrollment has increased significantly during this period due to enrollment on institutional (22) and NF Clinical Trials Consortium (6) protocols. 3. Residents and attending value this educational exchange and patients are reassured by shared decision making.

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Neurofibromatosis Type 2 in Children: A Single Center Experience

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Objective: The aim of this study was to describe the earliest clinical presentation, clinical course and complications of NF2 in children.

Methods: A retrospective chart review (1975-2016) of all children and adolescents between ages 0-21 years of age seen at the Cleveland Clinic with a diagnosis of NF 2.

Results: 30 children who fulfilling the Manchester criteria for NF 2 were identified, 11 males and 19 females. All were Caucasian; mean age of symptoms 8.3 years. 33 % patients had a family history of NF 2

Presentation at onset: The most common presentation of NF-2 were: ocular complaints 11 patients. Skin tumors 5 patients, motor weakness 4 patients, hearing loss 4 patients and 1 patient with café au lait macules. Other symptoms at presentation included headaches, pain and paresthesias.

At diagnosis: Males were diagnosed earlier than females (10.5 yrs. vs. 14.5 yrs.) with hearing loss present in 15 (50%) of patients. Imaging revealed bilateral vestibular schwannomas in 16 patients (53%) and unilateral schwannomas in 7 patients (23.3%).

Clinical Course: The mean duration of follow up 9.6 years .Severe hearing loss developed in 23 (77%) patients. Bilateral vestibular schwannomas were present in 28(93%) patients and brain and spinal cord tumors in 24(80%). 25 patients (83%) developed skin lesions. CAL macules and schwannomas were the commonest skin lesions (CAL) macules in 10 (33%) and peripheral schwannomas in 15 (50%) patients. Motor weakness 15 (50%) of patients. CNS tumors developed in 24 patients (80%).

Conclusion: NF 2 is more frequent in adults and its presentation and natural history differs in children. Early recognition and appropriate counselling with follow up leads to a better final outcome. In the absence of a family history of NF2, a high index of suspicion is required for early diagnosis.

Pediatric Intracranial Glioblastoma Multiforme in Association with Neurofibromatosis Type 1

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Introduction: Neurofibromatosis type 1 (NF1) predisposes to the development of central nervous system (CNS) neoplasms, primarily low-grade benign gliomas (most commonly optic pathway pilocytic astrocytomas). There is an acknowledged risk of developing high-grade glial CNS tumors as well, though these are rarely reported, particularly in young children.

Report: A 7-year-old boy with known NF1 (no OPG or other CNS lesions) presented with several weeks of progressive headaches, nausea and emesis. Brain imaging revealed a large paraventricular solid and cystic mass with rim enhancement and diffusion restriction of the solid tumoral components. A near-total gross resection of the tumor was achieved without complication. Neuropathology revealed a highly cellular tumor with multiple mitotic figures, focal necrosis, INI-1 nuclear retention, GFAP and Ki-67 expression and a suggestion of vascular endothelial proliferation, all diagnostic of a W.H.O. grade IV astrocytoma, glioblastoma multiforme (GBM). Adjuvant therapy included fractionated photon radiation and oral valproate with parenteral bevacizumab. He continues adjuvant chemotherapy and maintains stable MRI scans without evidence of tumor progression.

Review/Discussion: While benign CNS gliomas are relatively common in children with NF1, malignant CNS neoplasms are infrequent. There is a paucity of reports (< 10-15 cases) of pediatric NF1/GBM in the medical literature¹. Reported cases have demonstrated an increased survival in such children, suggesting that the molecular biology of GBM in NF1 may be different. It is uncertain whether different treatment approaches should be applied to the child with NF1/GBM, in comparison to non-NF1/GBM. Our case is illustrative of the risk of malignant CNS tumor development in patients with NF1, even the young child, and adds to the few cases that have been reported in the literature. We suggest ongoing reporting of this association in order to broaden our understanding of NF1 and malignant neoplasia.

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Reference: 1. Huttner AJ, Kieran MW, Yao X, et al. *Pediatr Blood Cancer* 2010;54:890-896.

Roles of FDG-PET in the Evaluation of Deep-Seated Peripheral Nerve Sheath Tumor

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Background: The most crucial event for patients with neurofibromatosis type 1 (NF1) patients is development of malignant peripheral nerve sheath tumors (MPNST), which most affect patients' prognosis. We have established in-hospital NF1 care network in our institution partly in order to detect and treat MPNST early during clinical course. In this study, we evaluated the significance of FDG-PET prospectively conducted to NF1 patients who harbored deep-seated tumors.

Patients and methods: Since 2008, a new patient, who referred to our hospital with main complaint of deep-seated tumors, has been prospectively subjected to FDG-PET examination to evaluate SUVmax of the tumor and existence of other deep-seated tumors. Based on the value of SUVmax and patients' complaint, we actively biopsy the deep-seated tumors and confirm the histological diagnosis of the tumor. We examined the significance of SUVmax in the differentiation of malignant tumors from benign ones, and roles of FDG-PET for NF1 patients.

Results: Among 93 NF1 patients with medical record, 50 patients were referred since 2008. Thirty-six of fifty patients were referred with chief complaint of deep-seated tumors. All 36 patients with deep-seated tumors received FDG-PET examination. There were 13 cases with MPNST and 23 with neurofibroma (NF). There were 77 evaluable lesions with FDG-PET (MPNST; 14, NF; 54, other; 9). The mean value of SUVmax were 6.8 in MPNST and 4.03 in NF, which is significantly different ($P=0.002$). If cutoff value set as 5.5, sensitivity was 75%, specificity was 81%, positive predictive value was 53%, negative predictive value was 94%. Intriguingly, there were 5 thyroid-related diseases, and 2 gastrointestinal tumors (GIST) detected by FDG-PET.

考察および結語: FDG-PET is useful for the differentiation of MPNST from NF. However, SUVmax value of FDG-PET is not sufficient for differentiation. As shown in some treatment guidelines of NF1, patients' symptoms should be taken into account to decide the necessity of biopsy, in addition to the results of FDG-PET. In patients with NF1, other neoplasms than neurogenic tumors occur relatively frequently including GIST and breast cancer. Physician should be aware of development of various kinds of neoplasms in NF1 patients in clinical course.

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Development of the Pediatric Quality of Life Inventory™ Neurofibromatosis Type 1 Module for Children, Adolescents and Young Adults: Qualitative Methods

Kavitha Nutakki MBBS, MPH, *Indiana University School of Medicine*

Background: Health-related quality of life (HRQOL) is arguably one of the most important measures in evaluating effectiveness of clinical treatments. At present, there is no disease-specific outcome measure to assess the HRQOL of children, adolescents and young adults with Neurofibromatosis Type 1 (NF1). Hence, the objective of this qualitative study was to develop the items and support the content validity for the Pediatric Quality of Life Inventory™ (PedsQL™) NF1 Module for children, adolescents and young adults.

Methods: The iterative process included multiphase qualitative methods. Based on existing literature and the expertise of the research team and expert clinical consultants, semi-structured interview guides were developed for the four patient age groups (5-7, 8-12, 13-17 and 18-25) and parents. A total of 39 children ages 5-17 with NF1 and their parents and 12 young adults ages 18-25 with NF1 participated in the semi-structured interviews, cognitive interviews and pilot testing.

Results: Fifteen domains were identified from the narrative data obtained from the patient and parent interviews and from expert opinions. The domains include skin, pain, pain impact, pain management, cognitive functioning, speech, fine motor, balance, vision, perceived physical appearance, communication, worry, treatment, medicines and gastrointestinal symptoms. The PedsQL™ NF1 Module contains 115 items in the 15 domains (except for young child report ages 5-7y which has 112 items).

Conclusions: Qualitative methods support the content validity for the PedsQL™ NF1 Module for children, adolescents and young adults. The PedsQL™ NF1 Module is now undergoing national multisite field testing as the next iterative phase.

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Face Perception in Children with Neurofibromatosis Type 1: Emotion Recognition and Scan Paths

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Neurofibromatosis type 1 (NF1) is a neurodevelopmental disorder associated with elevated risk of specific cognitive impairments and a high prevalence of psychological comorbidities. Social dysfunction is also common with impaired social skills, high rates of peer rejection, increased social isolation and an elevated autistic trait burden reported. While specific facial emotion recognition deficits have also been described, we know little about visual scanning of faces and face perception abilities in NF1 and how these might relate to emotion recognition abilities in this population.

We investigated facial emotion recognition, face scan paths and face perception in 29 children with NF1 compared to 29 chronological age-matched typically developing controls. Relationships between facial emotion recognition, face scan paths, face perception and social competence in children with NF1 were examined.

Children with NF1 displayed significantly poorer recognition of fearful expressions compared to controls, as well as a non-significant trend towards poorer recognition of anger. While there was no significant difference between groups in time spent viewing individual core facial features (eyes, nose, mouth and non-feature regions), children with NF1 spent significantly less time than controls viewing the face as a whole. Children with NF1 also displayed significantly poorer face perception abilities than typically developing controls. Facial emotion recognition deficits were not associated with aberrant face scan paths, social competence problems or face perception abilities in the NF1 group, although a non-significant trend was observed whereby better recognition of anger was associated with increased face perception accuracy.

These results suggest that impairments in the perception, identification and interpretation of information from faces are important aspects of the social-cognitive phenotype of NF1. Our findings highlight a need to incorporate screening for face perception and emotion recognition problems into the general clinical management of individuals with NF1.

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Magnetic Resonance Imaging Screening for Optic Pathway Gliomas in Neurofibromatosis type 1

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Optic pathway gliomas (OPGs) in neurofibromatosis type 1 (NF1) frequently remain indolent, but can lead to vision loss and significant morbidity in a subset of children with NF1. The objective of this study was to evaluate the outcomes of magnetic resonance imaging (MRI) screening for this complication. A retrospective analysis of clinical and imaging data from 826 children with NF1 seen at Cincinnati Children's Hospital over a 20 year period was performed.

Results: Of 826 children screened with MRI of brain and orbits, 149 individuals (18%) were identified with OPGs, at a median age at detection of 3 years. 955 surveillance MRIs were performed in the subset of 149 patients at established intervals to monitor tumor growth. OPGs were more frequent in females than males and in Caucasians compared to African Americans. Fifteen percent of patients (22/149) with OPGs had radiological and/or clinical progression requiring therapy. Patients with chiasmatic (15/42) and postchiasmatic (4/11) tumors were much more likely to require therapy compared to patients with prechiasmatic OPGs (3/96) ($p < 0.0001$). Patients with visual signs at time of diagnosis were more likely to experience visual decline despite therapy (10/12) when compared to patients with OPGs treated based on radiological progression (2/10) ($p < 0.012$). No patients who had negative MRI screening performed at or before age 15 months later developed symptomatic OPGs.

Conclusions: This data confirms that chiasmatic and postchiasmatic OPGs have the highest risk for progression and vision loss. Early identification of OPGs by screening MRI prior to visual abnormalities may lead to better visual outcomes. Based on this finding, the utility of baseline MRI screening for young children with NF1 should be further evaluated.

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Evaluation of Insulin Resistance and Adipocytokines In Neurofibromatosis Type 1

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Introduction: Evidences indicates a lower incidence of diabetes mellitus type 2 (DM2) and lower fasting blood glucose levels in individuals with neurofibromatosis type 1 (NF1). Insulin resistance (IR) is a predictor of DM2. The Homeostasis Model Assessment Insulin Resistance (HOMA-IR) is one of the methods used to assess IR and studies indicates that decreased levels of adiponectin and increased leptin, resistin and visfatin are associated with IR and DM2.

Aim: To evaluate the IR and adipocytokines in individuals with NF1 and compare with people unaffected by the disease.

Methods: Individuals with NF1 were matched by sex, age and body mass index (BMI) to controls (1:1). We evaluated fasting blood glucose, glucose two hours post-dextrose, glycated hemoglobin, fasting insulin, adiponectin, leptin, resistin and visfatin. IR was assessed by HOMA-IR.

Results: Forty patients with NF1 and 40 controls were enrolled in the study, 70% female, mean age 40.65 ± 11.80 years in NF1 group. The median BMI was 24.17 kg/m^2 (21.59 - 26.22) in NF1 group. No differences were observed in age and BMI between the groups ($p > 0.05$). The median of HOMA IR was 1.01 (0.69 - 1.64) in NF1 group and 1.32 (0.94 - 2.27) in control group, $p = 0.147$. The median of fasting plasma glucose in NF1 group was 83.50 mg/dL (78.00 - 90.00) and control group 86.00 mg/dL (83.00 - 94.00), $p = 0.008$. The median of adiponectin in NF1 group was 23.70 ng/mL (17.00 - 39.35) and control group 14.98 ng/mL (6.19 - 20.88), $p < 0.001$. The median of leptin in NF1 group was 5.71 ng/mL (3.05 - 11.65) and control group, 11.92 ng/mL (6.79 - 20.68), $p = 0.042$ and the median of visfatin in NF1 group was 118.20 ng/mL (105.77- 124.75) and control group, 138.22 ng/mL (124.50-147.65), $p < 0.001$. No significant differences were observed in resistin, post dextrose glucose, glycated hemoglobin and fasting insulin levels.

Conclusion: Individuals with NF1 have IR similar to controls. However, they have lower levels of leptin and visfatin and higher levels of adiponectin, suggesting that the lower occurrence of DM2 and lower levels of fasting blood glucose may be associated with changes in the adipocytokines in NF1.

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Amusia Is A Common Feature In Neurofibromatosis Type 1

Nilton Alves de Rezende, MD, *Neurofibromatosis Reference Center, Federal University of Minas Gerais (UFMG), Brazil.*

Background: Common cognitive impairments described in neurofibromatosis type 1 patients (NF1) are speech, language, motor and learning deficits, but we have observed some NF1 people with musical deficits. Congenital amusia has been hypothesized as a disconnection syndrome resulted of white matter neural dysfunction. Our previous studies showed auditory central processing disorder in most of affected NF1 individuals, but we did not know if it could be related to some degree of amusia in neurofibromatosis type 1.

Aim: To investigate the occurrence and levels of amusia in NF1 patients.

Methods: 15 NF1 volunteers (presenting at least three NIH diagnostic criteria for NF1) and 14 healthy controls matched by sex and age were evaluated using Montreal Battery Evaluation of Amusia (MBEA). The record of potential long latency Mismatch Negativity (MMN) was done to assess the electrophysiological behavior of auditory cortex of subjects, who also answered a questionnaire of their musical history.

Results: Assuming as a diagnostic criteria for amusia the total score of MBEA lower than two standard deviations of the control group average, we have found a Strong relationship between NF1 and amusia ($P = 0.001$).

Discussion: These results indicates amusia is common in NF1 individuals. Considering that most of them present auditory processing disorderis, we could suspect it would be the main cause for NF1 reduced musical perception.

Conclusion: Amusia is a common disorder in neurofibromatosis type 1 affected peoples.

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Increased Telomere Length In Neurofibromatosis Type 1

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Background: – Telomeres are tandem repeats of DNA located at the end of eukaryotic chromosomes that play an essential role in maintaining chromosomal integrity. Several studies have highlighted the role of telomere length (TL) as a biomarker in several diseases, including cancer, but there is little information regarding telomere biology on neurofibromatosis type 1 (NF1) subjects.

Aim: To investigate whether NF1 is associated with telomere shortening.

Methods: 24 NF1 subjects and 24 healthy controls were matched by age (range 19-45 years, mean 29.75 ± 6.94 years) and sex (n=15 females; n=9 males). Patients were classified in three subgroups: asymptomatic plexiform neurofibroma (PNF) (n = 6), symptomatic PNF (n = 8) and malignant peripheral nerve sheath tumors (MPNST) (n = 6). Measurement of relative TL was determined using a quantitative real-time polymerase chain reaction (RT-PCR). Statistical analyses were performed using R (version 3.2.2). Mann–Whitney U test was used for TL comparison to investigate significant difference between cases and controls. Kruskal-Wallis test was performed to compare TL among NF1 subgroups.

Results: NF1 patients showed larger TL when compared to control subjects (NF1: 625.3 ± 169.57 ; Control: 438.3 ± 133.67 ; $p = 0.0003$). No difference was observed across NF1 subgroups (Figure 1B).

Discussion: Since NF1 is a syndrome associated to tumor predisposition, it was reasonable to hypothesized larger telomeres in NF1 patients, based on previous described data on cancer. Nevertheless, possible explanations for the present results would be higher telomerase activity (enzyme responsible for telomere replication). Further studies are needed to elucidate this original result.

Conclusion: NF1 subjects presented larger TL compared to control subjects, but TL could not be used as a biomarker for prediction of malignancy process.

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Auditory Training: A New Approach To Learning Disabilities In Neurofibromatosis Type 1 Patients

Nilton Alves de Rezende, MD, *Neurofibromatosis Reference Center, Federal University of Minas Gerais (UFMG), Brazil.*

Introduction: Individuals with neurofibromatosis type 1 (NF1) often show auditory processing deficits related to their overarching language impairment (Batista et al, 2014). Auditory training (AT) acoustically controlled may potentially alleviate these deficits through training-induced improvements in auditory processing (AP) (Weihing et al. 2015).

Objectives: Verify the efficacy of AT in NF1 patients with auditory processing disorder (APD) and verify the maintenance of auditory skills trained one year after the end of the intervention in this population.

Methods: To assess the efficacy of AT on auditory function in individual with NF1, auditory behavioral tests performance (speech in noise - SN, staggered spondaic word - SSW, duration patterns - DP and gaps in noise - GIN) were collected from 2 groups (G1: individuals with NF1 and G2: individuals without NF1). These groups completed 8-week AT program and were reevaluated after 12-weeks from the initial assessment. The G1 was also reevaluated after one-year that finish the AT to verify the maintenance of auditory training. This group was called G3. Statistical analysis was performed using the Mann-Whitney test to compare inter-group differences before and after training and the Wilcoxon test to verify intra-group differences. Statistical significance was set at a p value ≤ 0.05 .

Results: The groups G1, G2 and G3 were composed of 22, 11 and 13 individuals, respectively. All participants presented normal auditory peripheral hearing, but altered central AP. The comparison between the evaluations made before and after the AT pointed to a statistically significant result for SN right ear (RE) ($p = 0.000$), SN left ear (LE) ($p = 0.004$), SSW RE ($p = 0.001$), SSW LE ($p = 0.000$), GIN RE ($p = 0.027$) and GIN LE ($p = 0.038$), suggesting improvement in auditory closure, figure-ground and temporal resolution abilities, respectively. No improvements were observed in PD humming ($p = 0.073$) and PD labeling ($p = 0.572$). Comparisons between G1 and G2 indicate that the groups were similar before the intervention and remained similar after AT, except for the DP humming test ($p = 0.013$). It was observed in G3 that trained auditory skills were maintained one year after the end of the AT.

Conclusion: These results provide an indication of the efficacy of training in auditory closure, figure-ground and auditory resolution abilities, and that these benefits keep one year after the end of the AT.

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NF1 Breast Cancer: The Mitochondrial Connection

Vincent M. Riccardi, MD, MBA, *The Neurofibromatosis Institute, La Crescenta, CA*

Finnish nation-wide registry data unequivocally establish that breast cancer is a significant risk for NF1 persons, especially for young women.¹ The standardized incidence ratio for breast cancer in NF1 women <40 years was 11.1 ($p < .001$). Not only was the risk high for relatively young women, their associated breast cancer standardized mortality ratio was excessive at 5.2 ($p < .001$). In addition, mitochondrial derangement in NF1 is already well-known² and there is recent evidence for mitochondrial dysfunction in the origin and progression of breast cancer in the general population.³ Moreover, circulating peripheral blood cell mitochondrial biomarkers employed in the general population appear to be predictive both of an individual's breast cancer risk and of more aggressive progression of an established breast cancer.^{4, 5}

These facts sum to suggest three considerations for both further NF1 scientific investigations and immediate NF1 clinical imperatives. 1) Women with a proclivity for mitochondrial dysfunction are inter alia at increased risk for breast cancer. 2) Women with NF1 are at increased risk for (especially aggressive) breast cancer respecting the known NF1 mitochondrial dysfunction. 3) Peripheral blood cell mitochondrial biomarker screening should be standard of care for NF1 women.

In this presentation, I shall elaborate on the details of the relevant published data in terms of a) the increased NF1 breast cancer risks, b) their potential application to other female-gender-biased elements of NF1⁶⁻⁸ and c) consider their broader application to other aspects of the NF1 syndrome. For example, monitoring peripheral blood cell mitochondrial markers in NF1 may not be restricted to concerns about breast cancer, particularly respecting the already-established importance of mitochondria to skeletal muscle function, S100 protein dynamics, mast cell activities and the oxidative phosphorylation generation of ATP apropos of the latter's central role in the Ras-GTP overdrive that is the biochemical hallmark of NF1.⁹

Author List: ¹Uusitalo E, Leppavirta J, Koffert A et al. *J Invest Dermatol* 2015;135:904-906. ²Tong JJ, Schrinier SE, McCleary D, Day BJ, Wallace DC. *Nat Genet* 2007;39:476-485. ³Feeley KP, Bray AW, Westbrook DG et al. *Cancer Res* 2015;75:4429-4436. ⁴Shen J, Wan J, Song R, Zhao H. *Carcinogenesis* 2015;36:1307-1313. ⁵Lemrroux A, Brook MN, Fletcher O et al. *Cancer Res* 2015;75:2844-2850. ⁶Diggs-Andrews KA, Brown JA, Gianino SM, Rubin JB, Wozniak DF, Gutmann DH. *Ann Neurol* 2014;75:309-316. ⁷Koelsch BU, Fischer C, Neibecker M et al. *Int J Cancer* 2006;118:108-114. ⁸Prada CE, Hufnagel RB, Hummel TR et al. *J Pediatr* 2015;167:851-856. ⁹Jiang XP, Elliott RL, Head JF. *Breast Cancer Res Treat* 2015;153:519-529.

Purinosomes And Nf1 Pathogenesis

Vincent M. Riccardi, MD, MBA, *The Neurofibromatosis Institute, La Crescenta, CA*

I have previously proposed that *NF1* is a disorder of purine nucleotide imbalance and that one of its key biochemical aspects involves relative ATP depletion. I have suggested that NF1 is a Purine Nucleotide Balance (PNTB) disorder and the *NF1* gene is a PNTB gene. NF1 determines how the purine nucleotide, GTP, controls the activities of three G-proteins collectively known as Ras (H-Ras, K-Ras, N-Ras). Deficiency of neurofibromin directly and immediately accounts for an excess of GTP-bound Ras that drives multiple overactive phosphorylation cascades. In turn, there is excessive consumption of the purine nucleotide, ATP, donor of the phosphate in the phosphorylation events. Thus, Ras-GTP overdrive adversely determines the availability and disordered utilization of two biologically critical purine nucleotides, GTP and ATP: by definition, a PNTB disorder.

In addition to overdrive, there is a presumed consequent deficit in the timely availability of ATP for its critical function in fueling myriad physiological phenomena, including almost all CNS and PNS functions. Since the brain uses 20% or more of a human's ATP production, I presume an ongoing deficit in ATP production is a significant physiological depletion. One must consider potential cerebral-neuromuscular compromise and one or more metabolomic adjustment, such as documented absence of high fasting blood glucose levels.¹ Respecting my previous scientific focus on PNTB in the Lesch-Nyhan syndrome, I suggest that *the wildtype NF1 interactome involves genetic networks that optimize organismal and cellular purine nucleotide production and availability.*

Although the purine salvage pathway may warrant some consideration, the primary metabolomic adjustments in NF1 likely involves de novo purine synthesis pathways. Two recent articles describing the involvement of *mTORC1* in purine *de novo* synthesis in circumstances of purine depletion are consistent with a role for the purinosome in NF1 pathophysiology.^{2,3} The relevance of purinosomes to the initiation and progression of cancer, and the ancillary involvement of tubulin, heat shock proteins 70 and 90 and the MAPK cascades, adds cogency to this approach. The complex assemblage of the purinosome is known to develop and persist in response to purine deficits. The primary consequences of the activated system are increased amounts of AMP, GMP and their precursor, IMP. NF1 mass lesions, including gliomas, neurofibromas, neurofibrosarcomas, etc., as well as non-tumor defects, require consideration of purinosome involvement in their development and progression

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Non-Optic Gliomas in Adults and Children with Neurofibromatosis 1

Laura Sellmer, BSc, *University of British Columbia*

Background: Gliomas are one of the most common tumours in patients with NF1 and may involve either the optic nerves or other parts of the CNS. The frequency and natural history of non-optic gliomas in children and especially in adults are incompletely understood.

Methods: 1727 head MRI scans were obtained from 573 unselected individuals with NF1 at the NF outpatient department of the University Hospital Hamburg-Eppendorf between 2003 and 2014. We analyzed the clinical features of non-optic gliomas and focused on the differences between affected adults and children.

Results: The number of scans per patient ranged from 1 to 11; patients were followed for a median of 4.1 years. We identified 24 patients (4.2%) with non-optic gliomas, a prevalence about twice that reported in most previous studies. Only 6 of the 24 glioma patients were symptomatic. Median age of these patients at first scan was 22.4 years, much higher than previously reported in the literature. Tumours were located in the brainstem (33.3%), cerebellum (29.2%), corpus callosum (12.5%), temporal lobe (12.5%), frontal lobe (8.3%) and internal capsule (4.2%). Histology was available on nine tumours and showed seven pilocytic astrocytomas, one dysembryoplastic neuroepithelial tumour and one ganglioglioma. Three individuals developed a tumour after having at least one tumour-free scan of the affected region, and all three of these patients had pre-existing optic gliomas. Four cases showed increase in tumour volume over 20% during follow-up, and three individuals had more than one non-optic glioma.

Conclusion: The number of scans per patient ranged from 1 to 11; patients were followed for a median of 4.1 years. We identified 24 patients (4.2%) with non-optic gliomas, a prevalence about twice that reported in most previous studies. Only 6 of the 24 glioma patients were symptomatic. Median age of these patients at first scan was 22.4 years, much higher than previously reported in the literature. Tumours were located in the brainstem (33.3%), cerebellum (29.2%), corpus callosum (12.5%), temporal lobe (12.5%), frontal lobe (8.3%) and internal capsule (4.2%). Histology was available on nine tumours and showed seven pilocytic astrocytomas, one dysembryoplastic neuroepithelial tumour and one ganglioglioma. Three individuals developed a tumour after having at least one tumour-free scan of the affected region, and all three of these patients had pre-existing optic gliomas. Four cases showed increase in tumour volume over 20% during follow-up, and three individuals had more than one non-optic glioma.

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Prevalence and Natural History of Optic Pathway Gliomas in Neurofibromatosis 1

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Background: Optic gliomas affect about 15% of children with NF1 under the age of six years. These tumours become less frequent in later childhood and adolescence; however, their natural history and frequency in adults is poorly understood. We compared course, progression and regression in a large series of children and adults with NF1.

Methods: 1727 head MRI scans were obtained from 573 unselected individuals with NF1 at the NF outpatient department of the University Hospital Hamburg-Eppendorf between 2003 and 2014. We analyzed the clinical features of optic gliomas and focused on differences between children and adults.

Results: We identified 48 patients (8.4%) with optic gliomas. The number of scans per optic glioma patient ranged from 1 to 9; patients were followed for a median of 3.0 years. The prevalence of gliomas fell from 17% in children aged ten years or younger to 5% in the 20.0 to 29.0 year age group. The prevalence remained stable at a little less than 5% in older patients. Most patients that were symptomatic or required treatment were younger than 10 years of age. In the patient group older than 10 years, only three patients received treatment for their gliomas (two received surgery, one received chemotherapy) and two patients reported loss of vision. No other symptoms were reported in patients over than 10 years of age. Of the 48 patients with optic gliomas, 20 affected the optic nerves only, 22 affected both the optic nerves and the chiasm, and 6 affected the chiasm only (4 of these extended into the optic radiations). Of the 42 patients with tumours that affected the optic nerves, 32 affected one optic nerve and 10 affected both sides.

Conclusion: Optic gliomas are common in children with NF1 under 10 years of age, but the prevalence declines to around 5% in adults older than 20 years. Our study highlights the frequency and continuing need for surveillance of optic gliomas in adults with NF1.

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Advanced Practice Provider Use in a Neurofibromatosis Clinic: Development of a Neurology Neurofibromatosis Type I Clinic to compliment a Multidisciplinary Program at a larger tertiary care center

Stephanie Shea, MPAS, BS, PA-C, *Children's Hospital Colorado, University of Colorado School of Medicine*

Children's Hospital Colorado (CHCO) has held a quarterly Neurofibromatosis Type 1 (NF1) Multidisciplinary Clinic (MDC) since 2010. If the fall of 2012, the NF1 MDC grew significantly to a monthly clinic with the assistance of a nurse coordinator position. The MDC combines providers from neurology, oncology, neuropsychology, genetics, rehabilitation, and ophthalmology and is designed for patients with moderate to severe symptoms from NF1. Patients in the clinic require at least three of the specialties present.

As the NF1 multidisciplinary program at CHCO has grown with increased awareness, the improved provider and public knowledge about offerings in this great program has resulted in a significant increase in appropriate referrals to the clinic. The waitlist for the MDC clinic is approximately 2-4 months. As a result, two additional patient populations within NF1 were identified. The first was a population of patients with mild to moderate NF1 who required evaluation by only one to three of the specialties in MDC. This population extended the waitlist for the MDC and did not necessarily need the complex level of care provided there. The second population was a medically complex patient who was unable to wait the length of time to be seen in MDC and required triaging to specialties more rapidly.

A monthly Neurology NF1 Clinic was established with a specially trained Neurology Advanced Practice Provider (APP) and the nurse coordinator. This allowed for more appropriate care and rapid access of these subpopulations of NF1 patients. The APP provides a medical assessment, recommends testing, and is able to make referrals to appropriate specialties with the nurse coordinator facilitating education and ensuring that these recommendations are followed through. The infrastructure in place in this program has provided enhanced and coordinated care for these populations of NF1 patients. The waitlist has already reflected improvement as the Neurology NF1 clinic can get a patient seen in 1-2 months. Further progress is needed to monitor appropriateness and improve clinic flow. This clinic role is crucial in improving and developing a NF1 multidisciplinary program with in a large tertiary care center.

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Preliminary Evaluation of Pain in Schwannomatosis Patients

Monica Sheridan, BS, *Massachusetts General Hospital*

Background: The defining clinical feature of schwannomatosis is chronic pain. This patient population lacks high-quality prospective data, needed for the development of clinical trials on pain management.

Methods: In an ongoing IRB approved study, we recruited patients with schwannomatosis from the International Schwannomatosis Registry (ISR). Patients completed surveys on pain intensity (Numerical Rating Scale-11), neuropathic component of pain (ID Pain), and medication usage. Patients also completed Patient Reported Outcomes Measurement Information System (PROMIS) short forms for physical functioning, anxiety, depression, and pain interference over the past week. T-scores are reported for all PROMIS measures, for which the US general population mean=50 (SD=10). Chronic pain was defined as lasting 3 months or more, and pain intensity was assessed at its worst in the past week.

Results: 32 subjects (ages 29-76, mean age=49, 58% female) have enrolled in the study as of March 2016. Subjects had a mean age of diagnosis at 39, and reported a median of 2 surgeries to remove a schwannoma (range 1-17). 27 of 32 subjects reported chronic pain, with a mean onset at 33 years of age. The usage of narcotics, neuropathic, and/or anti-inflammatory pain medication was 50% (16), 50%, and 38% (12) respectively, with 22% (7) taking all three. Of the 31 subjects reporting pain in the last week, their mean pain intensity was 5.4/10 (SD=3.3). The mean ID-Pain score was 2.48/5 (where higher scores correlate to greater neuropathic component), with half of patients falling in the range of neuropathic pain and half in non-neuropathic pain. Pain intensity positively correlated with pain interference values (R-squared=0.65). Individuals reporting high pain intensity (NRS score $\geq 5/10$, N=18) had worse anxiety (mean T-score=57), depression (56), pain interference (63), and physical functioning (45) as compared to the US general population.

Conclusion: Despite extensive pain treatments, the quality of life of this patient population is severely impacted by pain. In our sample, over half of subjects reported high pain intensity ($\geq 5/10$), with associated decrements in anxiety, depression, pain interference, and physical functioning, representing a target population for clinical trials in pain management.

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Outpatient Treatment with Ultra Low Dose Ketamine Infusion for Neuropathic pain in patients with Neurofibromatosis

Peter Shibuya, NP, *The Jennifer and Daniel Gilbert Neurofibromatosis Institute, Children's National Medical Center/George Washington University School of Medicine*

Objectives: Ketamine is an N-methyl-D-aspartate receptor antagonist used as an anesthetic agent with less respiratory suppression properties which is ideal for pediatric anesthesia and analgesia. Ultra Low Dose Intravenous Ketamine (ULDIK) has been reported in literature as an effective therapeutic treatment among patients with nociceptive and neuropathic pain. In addition, Ketamine infusion benefits in this population may be associated with improvements in depression symptoms, frequently present in patients with chronic pain. We aim to collect data from systematic clinical observations about the effects of ULDIK as part of the treatment for chronic pain in patients with Neurofibromatosis.

Methods: Patients with diagnosis of Neurofibromatosis and severe chronic pain refractory to other interventions are evaluated at the Multidisciplinary Neurofibromatosis Pain Clinic (PS, SA, MTA) at the Children's National Health System. If clinically indicated, patient is prescribed infusion of the ULDIK. In all patients standardized evaluations pre and post treatment are being collected.

Results: We present the preliminary results of a group of 6 patients with diagnosis of NF patients with refractory chronic neuropathic pain (4 males and 2 females) with age in range from 14 to 40 years of age. 5 of the 6 patients have diagnosis of NF1 and 6th patient with NF2. All patients have evidence of plexiform neurofibromas or schwannomas and being refractory to other interventions for pain management. Individualized treatment is decided for each patient. They receive either one or two day infusions of ULDIK with dosing parameter of 0.05mg to 0.4mg/kg/hr infusions every 3 to 6 weeks. Clinical improvements have been observed in all patients receiving this treatment. Longitudinal data is being collected in these patients to better understand the impact of ULDIK in pain, mood disorders and quality of life in NF patients.

Conclusion: ULDIK may be an alternative option for treatment of severe chronic neuropathic pain in patients with Neurofibromatosis. Systematic clinical observations and larger samples are necessary to assess this intervention in NF patients.

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Funding Sources: The Jennifer and Daniel Gilbert Neurofibromatosis Institute

Isolated Gliomas of the Optic Nerve in NF1 Children; A Multi-Center Historical Cohort Study

Ben Shofty, MD, *Division of Neurosurgery, and The Gilbert Israeli NF Center, Tel Aviv Medical Center, and Tel-Aviv University, Tel-Aviv, Israel*

Isolated gliomas of the optic nerve (IONG) constitute a rare subgroup of optic pathway gliomas (OPG). Due to the rarity of this condition, and the mix-up with other types of OPG in most clinical series, little is known about these tumors. Currently, due to lack of evidence, they are managed as any other OPG, regardless of location of clinical presentation. Here, we conducted a multi-center, historical cohort study aimed at deciphering the natural history of IONG. Included were patients with clear-cut glioma of the optic nerve and no posterior (chiasmatic / hypothalamic) involvement, with more than 1 year of follow-up and at least 2 MR studies and neuro-ophthalmological exams. Twenty-two patients were included in this study. Age at diagnosis ranged between 6 months and 12 years (average 4.7 years). Follow up time was 6 years. Seven patients (31%) had bilateral disease. Twelve (54%) patients had radiological abnormality in their other optic nerve. During the follow-up period, 7 patients progressed, while 3 patients experienced some degree of spontaneous regression. Only 2 patients presented with visual decline both experienced further deterioration, for a total of 13 visually deteriorating patients. Four patients were treated with chemotherapy, out of which only 1 improved visually, and another one responded radiologically. In conclusion, IONG are dynamic tumors, with 31% in our series that were active radiologically. In addition, they may warrant closer observation, and more aggressive treatment, as 60% deteriorated visually during the follow-up period.

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Health Literacy in Adults with Neurofibromatosis

Mojtaba Talaei-Khoei, MD, *Massachusetts General Hospital (MGH)/Harvard Medical School*

Background: Health literacy (HL), the ability to access, understand and use health information to make informed health related decisions, is an important part of disease management, that has not been yet examined in patients with neurofibromatosis (NF1, NF2 and schwannomatosis). We report scores on 2 HL measures, assess for difference by NF type and assess for potential correlations among these measures.

Methods: Eighty-six adult patients with the mean of age of 44 (55% female; 87% white; 50% NF1, 41% NF2 and 9% schwannomatosis) completed an adapted version of the Functional, Communicative, and Critical Health Literacy scale (FCCHL) and the Health Literacy Assessment Using Talking Touchscreen Technology (Health LiTT). FCCHL assesses patients' perception of functional (ability to read health related information), communicative (extent to which patients extract and apply information regarding their illness), and critical HL (extent to which patients critically analyze information and used it to make decisions). Total and subscale scores of the FCCHL represent means of the composite items, scored on a 4 point Likert scale from "never" to "often", with 4 indicating the highest level of health literacy. The Health LiTT items include exercises that measure demonstrated prose literacy (e.g., understanding and use of information from texts); document literacy (e.g., ability to locate and use information from forms, tables, graphs, etc.); and quantitative literacy (e.g., ability to apply arithmetic operations using numbers embedded in printed materials).

Results: As a group, patients with NF had high scores on the Health LiTT ($M = 60.63$, $SD = 7.57$), with no patients scoring 1SD or more below general population means ($M = 50$, $SD = 10$). As a group, patients with NF had an overall FCCHL score of $2.69 \pm .49$, with a functional HL of $3.26 \pm .68$, a communicative HL of $2.26 \pm .63$ and a critical HL of $2.38 \pm .89$. There were wide ranges of scores for each of the 3 subscales of the FCCHL (.25 to 4.00) suggesting a wide variation in these 3 aspects of HL in NF population. There were no differences by NF type. There were no correlations among the Health LiTT and total score or subscales of the FCCHL except for a strong correlation between communicative and critical health literacy ($r = .520$, $P < .001$).

Conclusion: Overall patients with NF scored similarly or higher than general population norms on exercises assessing prose, document and quantitative literacy. Patients had wide variations in scores of functional, communicative and critical HL FCCHL suggesting variability in levels of *self reported* HL in this population. The general lack of correlation among the HL measures suggests that they all assess different, independent aspects of the construct. Physicians should take into account the patients' levels of various facets of HL in managing the disease and adopt flexible strategies of patient education that identify and accommodate their patients' needs and abilities accordingly.

This research was possible due to a Children's Tumor Foundation research grant to Ana-Maria Vranceanu, PhD and a Young Investigator Award to Vanessa Merker.

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Patient Reported Outcomes (PROs) in Adults with Neurofibromatosis

Mojtaba Talaei-Khoei, MD, *Massachusetts General Hospital (MGH)/Harvard Medical School*

Background: Patient reported outcomes measurement system (PROMIS) is an initiative by the National Institute of Health (NIH), which assesses patient reported outcomes (PROs) using Computer Adaptive Testing (CAT), which optimizes the administration of PROs surveys by adaptively selecting questions from a validated item banks, thus decreasing burden on respondents while increasing efficiency and precision. The NIH recommends using PROMIS to allow comparisons among various population groups and with U.S. general population. We report on the first study using PROMIS measures in patients with neurofibromatosis (NF). Specifically, we describe scores of study participants, assess for potential differences by disease type, and describe the relationships among PROs in this population.

Methods: Eighty-six adult patients with mean age of 44 (55% female; 87% white; 50% NF1, 41% NF2 and 9% schwannomatosis) completed a battery of PROMIS item banks CAT (physical function, pain interference, pain behavior, depression, anxiety, anger, fatigue, satisfaction with social roles, satisfaction with discretionary social activities) on <https://www.assessmentcenter.net>, prior to the meeting with their neurologist.

Results: Overall, scores on all measures were distributed across a broad range for each PROMIS measure (± 2 SDs). Across all PROMIS instruments, mean scores for each item banks were between 48.98 and 52.60 which is within normal limits, or less than .5 SD of US general population norms. Clinically meaningful scores (i.e., more than 1 SD impairment) were observed in 16% (physical function), 20 % (pain interference), 17% (pain behavior), 16% (anxiety), 16% (depression), 6% (anger), 13% (fatigue), 15% (satisfaction with social roles) and 5% (satisfaction with discretionary social activities) of the entire sample. All PROMIS measures were highly interrelated in bivariate analysis ($P \leq .001$). There were no significant differences in PROMIS scores by disease type (NF1, NF2 and schwannomatosis).

Conclusion: This is the first study using PROMIS CAT measures in patients with NF. Scores suggest a broad continuum of symptoms and functioning in patients with NF that is not affected by NF type, as well as interrelation among psychosocial constructs. Comparing to general population norms, information on individual PROMIS scores can be easily interpreted and used in clinical practice.

This research was possible due to a Children's Tumor Foundation research grant to Ana-Maria Vranceanu, PhD and a Young Investigator Award to Vanessa Merker.

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Predictors of Patient Satisfaction in Adults with Neurofibromatosis

Mojtaba Talaei-Khoei, MD, *Massachusetts General Hospital (MGH)/Harvard Medical School*

Background: Patient satisfaction is increasingly recognized as an important dimension of health outcome research. Patients' satisfaction with a medical visit predicts compliance with prescribed treatments, thus impacting both the effectiveness of treatment and health care costs. The level of satisfaction also predicts whether patients attend future medical appointments, and is associated with better overall health. We assessed whether health literacy and psychosocial factors are associated with satisfaction with a medical visit in adult patients with neurofibromatosis (NF).

Methods: Eighty-six adult patients (Mean age = 44; 55% female, 87% white) with NF (50% NF1, 41% NF2 and 9% schwannomatosis) completed the Functional, Communicative and Critical Health Literacy Questionnaire (FCHL), a series of the Patient Reported Outcome Measures Information System (PROMIS) psychosocial computerized adaptive tests and a demographic questionnaire before visit with a neurologist, and the Medical Interview Satisfaction Scale (MISS) after their visit.

Results: Men had higher levels of satisfaction with the medical visit compared to women ($t = -2.092$, $P = .039$). In bivariate analysis, patients with higher health literacy ($r = .293$, $P = .006$), higher satisfaction with social roles ($r = .413$, $P < .001$), higher satisfaction with discretionary social activities ($r = .405$, $P < .001$), lower pain interference ($r = -.260$, $P = .016$), fewer pain behaviors ($r = -.280$, $P = .009$), lower anxiety ($r = -.341$, $P = .001$), lower depression ($r = -.360$, $P = .001$), lower anger ($r = -.363$, $P = .001$), and less fatigue ($r = -.379$, $P = .003$), reported higher satisfaction with the medical visit. In a multivariable linear regression, only health literacy ($\beta = .223$, $t = 2.070$, $P = .042$, $\Delta R^2 = .054$) and gender ($\beta = .253$, $t = 2.435$, $P = .017$; $\Delta R^2 = .073$) were significant predictors of satisfaction with the medical visit. The entire regression model accounted for 31% of variation in satisfaction with the medical visit ($\Delta R^2 = .311$, $\Delta F = 3.384$, $P = .001$).

Conclusion: Patient satisfaction is a complex construct, influenced by both patients' psychosocial function and level of health literacy. Although psychosocial factors were individually associated with satisfaction with the medical visit, health literacy and gender emerged as the most important factors explaining patients' satisfaction. This finding reinforces the importance of understanding a patient's level of health literacy and accounting for it during the medical visit and beyond.

This research was possible due to a Children's Tumor Foundation research grant to Ana-Maria Vranceanu, PhD and a Young Investigator Award to Vanessa Merker.

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The Impact of Ras/MAPK Signaling Pathway-Targeted Therapies on Neurocognitive Functioning in NF1: Preliminary Results of Feasibility, Validity, and Monitoring of Possible Neurotoxicity using a Novel Clinical Trials Approach

Karin S Walsh, PsyD, *Children's National Health System*

Objectives: This limited site ancillary cognitive study is attached to current MEK and BRAF inhibitor clinical trials targeting plexiform neurofibromas (PN) or low grade glioma (LGG). Deregulation of the Ras/MAPK pathway is suspected in the etiology of neurocognitive deficits. Current therapeutic trials targeting PNs provide an opportunity to assess how regulating this pathway may impact neurocognitive function in NF1.

Methods: We are using a novel, computerized assessment approach (CogState) and self-report questionnaire of executive functioning (BRIEF) to document cognitive change in learning and memory following administration of a MEKi. Eligible participants are individuals with NF1, ages 4-35 years, who are on a MEKi therapeutic protocol. Participants undergo a pre-treatment evaluation and follow-up evaluations at 3 and 6 months post treatment initiation. Performance change is monitored in real-time and site PIs are informed of significant declines allowing for real-time monitoring of potential neurotoxic effects and the hypothesized improvement in working memory and visual memory.

Results: To date, 32 participants have been enrolled (age 5-27, 59% male). The majority (N=27, 84%) were enrolled on a Phase II Selumetinib trial (PBTC029B n=2; or AZD6244 n=25), with the remaining 5 patients on the Phase II Pfizer MEKi trial (PD-0325901). Thirty of the 32 patients (94%) were being treated for PNs, and 2 for LGG. Twenty-one participants have completed at least one follow-up evaluation. Current data suggests excellent feasibility of this clinical trials approach, with over 95% completion rates on the primary outcome tasks and excellent validity in our NF1 sample. There have been no significant declines in neurocognitive performance in the sample to date.

Conclusion: Preliminary data suggests that this novel approach to evaluating cognition and cognitive change in the context of a therapeutic trial of a MEKi is feasible in a wide range of patients and is a valid for the purposes of evaluating the potential effects of these drugs on cognitive function as well as in tracking potential neurotoxic effects.

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Funding for this study through the Children's Tumor Foundation, the Gilbert Neurofibromatosis Institute, and the NIH CCR Intramural Research Program

Development of Patient-reported Outcomes (PROs) to Assess Pain in Individuals with Neurofibromatosis (NF1) and Plexiform Neurofibromas (PNs) for Clinical Trial Endpoints: Preliminary Data from Qualitative Research

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

Background: Clinical trials for the treatment of PNs in children and adults with NF1 are underway. The FDA recommends including endpoints in NF1 clinical trials that assess changes in symptoms, such as pain, in addition to tumor reduction. Currently, no valid PRO measures exist that assess PN-related pain and its effect on daily functioning. The first phase of this limited-site study is to modify existing PRO pain tools using qualitative methods to ensure understanding of the concepts being assessed and to examine how the current tools work in the NF1/PN population.

Methods: Individuals with NF1, ages >5 years, and PN-related pain are eligible. The Numeric Rating Scale (NRS-11), assessing pain intensity, and Pain Interference Index (PII), measuring the impact of pain on daily functioning, were selected based on REINS consensus recommendations and accepted into the FDA Drug Development Tool Qualification Program. Investigators are conducting up to 24 focus groups (age bands: 8-11 years; 12-14 years; 15-18 years [in high school]; 18-24 years [out of high school]; 25+ years; and parents of children with NF1) and individual interviews in children 5-7 years and when groups are not feasible. Topics of investigation include length of recall period (e.g., “past 7 days”), specific tumor pain vs. overall pain, chronic vs. acute pain, areas of functioning affected by PN pain, ways to document the location of pain, specific wording of items, feasibility of daily electronic ratings from home, and the youngest age to understand the measures. Audio recording are being transcribed and thematic analysis using NVivo is being conducted until content saturation is reached.

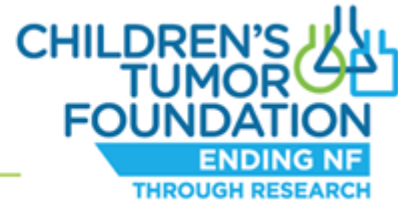
Results: To date, six focus groups (4 adult [2 male, 2 female], 1 young adult female, 1 teen male) and four child and four parent individual interviews have been completed (patient age range 5-50 years; 61% female); groups/interviews are ongoing. Discussion time has ranged from 75-120 minutes in groups and 25-75 minutes in interviews. Recurrent emerging themes include the ability to rate the pain of specific tumors and distinguish between the pains of different tumors; confusion about the meaning of “overall” pain; preference for using a figure to show the pain location; limited knowledge of PNs; generation of new items to assess pain interference; openness to daily electronic ratings from home; and difficulties in using these tools with young children.

Conclusion: This qualitative research is obtaining critical information from patients that will be used to modify existing pain tools for NF1. Preliminary data indicates that these PRO measures, which were developed for other pain populations, need to be adapted and validated to assess PN-related pain as NF clinical trial endpoints.

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Funding Support: Intramural research program of the NIH, NCI, POB and Neurofibromatosis Therapeutic Acceleration Program



NF Clinics Breakout Session

Sunday, June 19th, 5:30 - 9:00 pm, Brazos Room

** Please note that the first part of this session is for NF Clinic Network members only
For questions and to RSVP, please contact Heather Radtke at hradtke@ctf.org.*

5:30 pm NF CLINIC NETWORK MEMBERS ONLY - BY INVITATION ONLY (RSVP required)

Dinner Buffet included

Welcome	Annette Bakker, PhD, President-Chief Scientific Officer, CTF
Clinical Care Advisory Board updates	Dave Viskochil, MD, PhD, CCAB Chair, University of Utah
Low Grade and Optic Pathway Glioma Consortia	Michael Fisher, MD, Children's Hospital of Philadelphia
NFCN updates	Heather Radtke, MS, CGC, CTF
NF Guidelines/Practice Care updates	AMA/Peds: David Miller, MD, PhD Boston Children's Hospital Adult: Doug Stewart, MD National Cancer Institute
Access to Specialty NF Care through CTF	Vanessa Merker, BS, Massachusetts General Hospital Annie Dai, Harvard University

7:30 pm OPEN TO ALL NF CLINICIANS (RSVP required)

Dessert Bar and Coffee included

Volunteers in Clinics	Kelly Carpenter, parent of Travis with NF1, volunteer in Utah
NF Registry	Pamela Knight, MS, Clinical Program Director, CTF
Neurofibromatosis Clinical Trials Consortium	Roger Packer, MD, Chair of NF Consortium NFCTC Bruce Korf, MD, PI of Operations Center NFCTC
CTF Patient Advocacy	Kate Kelts, RN, CTF

International Neurofibromatosis Autism Consortium Team (INFACT)

CTF Meeting
Austin TX
June 19, 2016
6:30 p.m. – 9:00 p.m.

Organizers:

Stephanie Morris, MD

Instructor
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Department of Neurology
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John N. Constantino, MD

Blanche F. Ittleson Professor,
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Maria T. Acosta, MD

Clinical Director, Gilbert Family
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Overview and Objectives. Over the past year, we have successfully leveraged the collective expertise of six major centers with expertise in NF1 and autism to analyze the ASD phenotypic characteristics in over 600 subjects (manuscript submitted). ***The purpose of this meeting is to establish a roadmap for next-phase consortium research endeavors.***

Workshop Program:

- 6:30 p.m. Introduction and presentation**
Maria Acosta and David Gutmann (15 min)
- 6:45 p.m. Review of NF1-ASD Consortium Findings (30 min)**
Stephanie Morris
- 7:15 p.m. Genotype-Phenotype and Deep Behavioral Analyses (30 min)**
Shruti Garg, PhD
Jonathan Green, PhD (by teleconference)
- 7:45 p.m. Establishing a roadmap for next-phase collaborative studies (60 min)**
John Constantino, moderator

Scientific Questions

1. Early clinical biomarkers of social behavior in NF1
 - a. Eye tracking
 - b. Facial recognition
2. Prospective longitudinal study of ASD traits in children with NF1
3. Genotype-phenotype correlations in NF1-ASD
4. Role of background family environment on social disability in NF1
5. Impact of ADHD (and treatment of ADHD) on quantitative ASD burden
6. Role of SNPs in NF1-ASD as compared to iASD
7. Twin studies of NF1-ASD – phenotype-genotype correlation
8. Neuroimaging in NF1-ASD
 - a. UBOs
 - b. Volumetric analysis
 - c. Functional connectivity
9. Cellular markers of NF1-ASD using induced-neuronal cells

Potential Deliverables

1. Funding opportunities for consortium
 - Define sources
 - Determine scope of proposal (scientific questions)
 - Establish timeline
2. Identify lead centers for each scientific question
3. Determine what biospecimens should be collected
 - Blood for genome sequencing and SNP analysis
 - Fibroblasts for induced-neuronal cells
4. Determine which clinical variables should be collected
 - Head circumference
 - NIH diagnostic criteria
 - Hypotonia
 - Seizures
5. Establish external advisory board

- 8:45 p.m. Summary and next steps**
David Gutmann (15 min)

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Downtown Restaurant *Guide*

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E14	Ill Forks D \$\$\$	111 Lavaca St.	474-1776	3forks.com	Seafood &
G9	1886 Bakery & Café, Driskill Hotel B LD \$	604 Brazos St.	391-7066	1886cafeandbakery.com	Bakery
G13	Annie's Café and Bar LD \$	319 Congress Ave.	472-1884	anniescafebar.com	American
F9	Athenian Bar & Grill LD \$	600 Congress Ave.	474-7775	athenianbargrill.com	Mediterranean
D14	Austin Java Co. B LD \$	301 W. Willie Nelson Blvd.	481-9400	austinjava.com	Coffee House
E14	Bar Chi LD LN \$	206 Colorado St.	382-5557	barchiaustin.com	Sushi
F10	Bar Louie LD LN \$	123 W. Sixth St.	730-3050	barlouieamerica.com	American
B9	Bess Bistro LD LN \$	500 W. Sixth St.	477-2377	bessbistro.com	American
H9	BD Riley's Irish Pub B LD LN \$	204 E. Sixth St.	494-1335	bdrileys.com	Irish
G12	Blue Ribbon BBQ LD \$	120 E. Fourth St.	369-3119	brbbq.net	Barbecue
F13	Bob's Steak and Chop D \$\$\$\$	301 Lavaca Street	222-2627	bobs-steakandchop.com	Steakhouse
H10	Buffalo Billiards LD LN \$	201 E. Sixth St.	479-7665	buffalobilliards.com	American
E14	Cantina Laredo LD \$	201 W. Third St.	542-9670	cantinalaredo.com	Mexican
K10	Carmelo's LD \$	504 E. Fifth St.	477-7497	carmelosrestaurant.com	Italian
I14	Casa Chapala LD \$	101 San Jacinto Blvd.	479-1200	casachapala.com	Mexican
H13	Cedar Door B LD LN \$	201 Brazos St.	473-3712	cedardooraustin.com	American
I11	Champions Sports Bar LD \$	300 E. Fourth St.	473-0450	championsaustin.com	American
J10	Chez Nous LD \$	510 Neches St.	473-2413	cheznousaustin.com	French
F11	Chinatown LD \$	107 W. Fifth St.	637-8888	chinatown-downtown.com	Asian
D14	Coal Vines LD \$	314 W. Willie Nelson Blvd.	473-2744	coalvines.com	Pizza
F13	Congress D \$\$\$	200 Congress Ave.	827-2755	congressaustin.com	New American
M11	Easy Tiger LD LN \$	709 E. Sixth St.	614-4972	easytigeraustin.com	German
M18	El Naranjo D \$	85 Rainey St.	474-2776	elnaranjo-restaurant.com	Mexican
L9	El Sol y La Luna LD \$	600 E. Sixth St.	444-7770	elsolylalunaaustin.com	Mexican
E12	Fado Irish Pub LD LN \$	214 W. Fourth St.	457-0172	fadoirishpub.com austin	Irish American
K11	Finn & Porter D \$\$\$	500 E. Fourth St.	493-4900	finnandporter.com	Seafood & Steak
I13	Fleming's Prime Steakhouse D \$\$\$	320 E. Willie Nelson Blvd.	457-1500	flemingssteakhouse.com	Steakhouse
I13	Fogo de Chao LD \$	309 E. Third St.	472-0220	fogodechao.com	South American
F11	Frank B LD LN \$	407 Colorado St.	494-6916	hotdogscoldbeer.com	American
A10	Frank & Angie's LD \$	508 West Ave.	472-3534	hutsfrankandangies.com	Pizza
B12	Garrido's LD LN \$	360 Nueces St.	320-8226	garridosaustin.com	Mexican
A10	Haddingtons LD LN \$	601 W. Sixth St.	992-0204	haddingtonsrestaurant.com	American
E12	Halcyon B LD LN \$	218 W. Fourth St.	472-9637	halcyonaustin.com	Coffee House
F7	Hickory Street LD \$	800 Congress Ave.	477-8968	hickorystreet.com	American
C13	How Do You Roll – A Maki Sushi Bar LD \$	454 W. Willie Nelson Blvd.	320-8400	howdoyouroll.com	Asian
A10	Hut's Hamburgers LD \$	807 W. Sixth St.	472-0693	hutsfrankandangies.com	Burgers
E12	Imperia D \$\$\$	310 Colorado St.	472-6770	imperia-austin.com	Asian
I9	Iron Cactus LD LN \$	606 Trinity St.	472-9240	ironcactus.com	Mexican
K14	Iron Works LD \$	100 Red River St.	478-4855	ironworksbbq.com	Barbecue
D13	Jo's Hot Coffee B LD \$	242 W. Willie Nelson Blvd.	469-9003	joscffee.com	Coffee House
C13	La Condesa LD \$	400A W. Willie Nelson Blvd.	499-0300	lacondesaustin.com	Mexican
F12	La Traviata LD \$	314 N. Congress Ave.	479-8131	latraviata.net	Italian

MAP	RESTAURANT	ADDRESS	PHONE	WEBSITE	CUISINE
C14	Lamberts BBQ B LD LN \$	401 W. Willie Nelson Blvd.	494-1500	lambertsaustin.com	Barbecue
H13	Le Café Crepe LD \$	200 San Jacinto Blvd.	480-0084	cafecrepeofaustin.com	French
C14	Leaf LD \$	419 W. Willie Nelson Blvd.	474-5323	leafsalad.com	American
K11	Liberty Tavern B L \$	500 E. Fourth St.	493-4901	hilton.com	American
I13	Mai Thai LD \$	207 San Jacinto Blvd.	482-8244	maithaiaustin.com	Thai
C13	Malaga Tapas & Bar LD \$	440 W. Willie Nelson Blvd.	236-8020	malagatapasbar.com	Spanish
E14	Mama Fu's LD \$	100 Colorado St.	637-6774	mamafus.com	Asian
F12	Manuel's B LD \$	310 Congress Ave.	472-7555	manuel.com	Mexican
I13	Max's Wine Dive D \$	207 San Jacinto Blvd.	904-0111	maxswinedive.com austin	American
J16	Micheladas LD \$	333 E 2nd Street	320-0300	micheladasaustin.com	Mexican
L12	Moonshine LD \$	303 Red River St.	236-9599	moonshinegrill.com	American
I10	Old Pecan Street Café LD \$	504B Trinity St.	478-2491	oldpecanstreetcafe.com	American
J10	Old School Bar & Grill LD \$	401 E. Sixth St.	722-6351	oldschoolaustin.com	American
I14	P.F. Chang's China Bistro LD \$	201 San Jacinto Blvd.	457-8300	pfcchangs.com	Asian
I10	Parkside D LN \$	301 E. Sixth St.	474-9898	parkside-austin.com	American
E12	Péché D LN \$	208 W. Fourth St.	495-9669	pecheaustin.com	French
F8	Perry's Steakhouse & Grille LD \$\$\$	114 W. Seventh St.	474-6300	perryssteakhouse.com	Seafood & Steak
I14	Piranha Killer Sushi LD \$	207 San Jacinto Blvd., Ste. 202	473-8775	piranhakillersushi.com	Asian
K8	Pelon's Tex Mex LD \$	802 Red River Street	243-7874	pelonsaustin.com	Mexican
A9	Ranch 616 B LD \$	616 Nueces St.	479-7616	theranch616.com	Southwestern
G8	Roaring Fork LD \$\$\$	701 Congress Ave.	583-0000	roaringfork.com	Southwestern
F10	Ruth's Chris Steak House D \$\$\$	107 W. Sixth St.	477-7884	ruthschris-austin.com	Steakhouse
F13	Second Bar + Kitchen LD LN \$	200 Congress Ave.	827-2750	congressaustin.com	New American
L7	Stubb's B LD LN \$	801 Red River St.	480-8341	stubbasaustin.com	Barbecue
E13	Sullivan's Steak House B LD \$	300 Colorado St.	495-6504	sullivansteakhouse.com	Steakhouse
G13	Swift's Attic LD LN \$	315 Congress Ave.	482-8200	swiftsattic.com	American
H12	Taco Shack B L \$	402 Brazos St.	473-0101	tacoshack.com	Mexican
E14	Taverna Ristorante LD \$	258 W. Willie Nelson Blvd.	477-1001	tavernabylobardi.com	Italian
A10	The Original Hoffbrau LD \$	613 W. Sixth St.	472-0822	originalhoffbrausteaks.com	Steakhouse
E21	Threadgill's B LD \$	301 W. Riverside Dr.	472-9304	threadgills.com	Home Cooking
D14	Trace at the W Hotel LD LN \$\$\$	200 Lavaca St.	542-3660	traceaustin.com	American
H15	TRIO, The Four Seasons Austin B LD \$	98 San Jacinto Blvd.	685-8300	trioraustinaustin.com	New American
E12	Truluck's D \$\$\$	400 Colorado St.	482-9000	trulucks.com	Seafood & Steak
H13	Uncle Julio's LD \$	301 Brazos Street	815-2100	unclejulios.com	Mexican
H12	Vince Young Steak House D \$\$\$	301 San Jacinto Blvd.	457-8325	vinceyoungsteakhouse.com	Steakhouse
A10	Walton's Fancy & Staple LD \$	609 W. Sixth St.	542-3380	waltonsfancyandstaple.com	Bakery
G13	Willie G's D \$\$\$	401 Congress Avenue	236-9600	williegs.com	Seafood & Steak

Key B = Breakfast L = Lunch D = Dinner LN = Late Night \$ = \$5-14 \$\$ = \$15-25 \$\$\$ = \$26-50 🎵 = Music

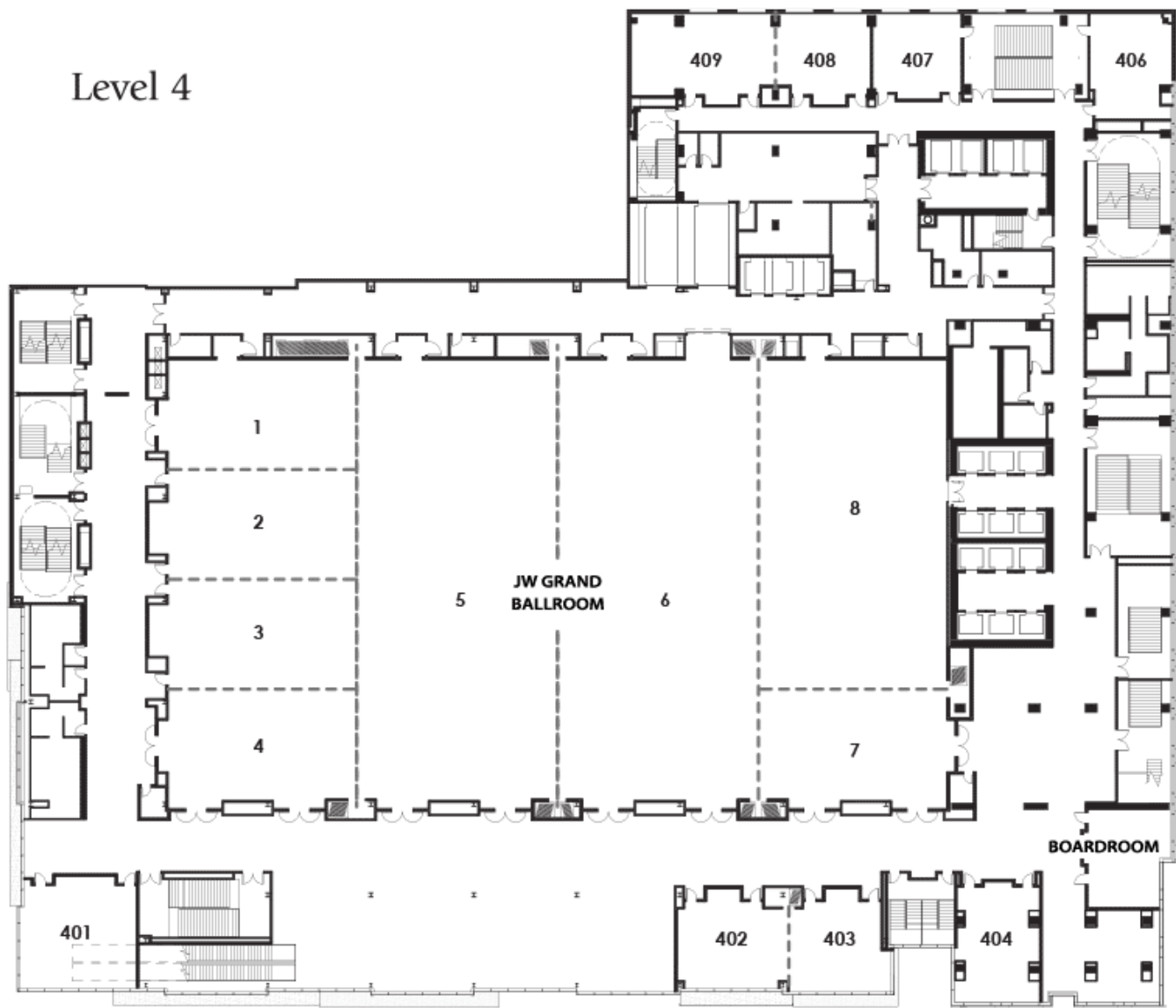
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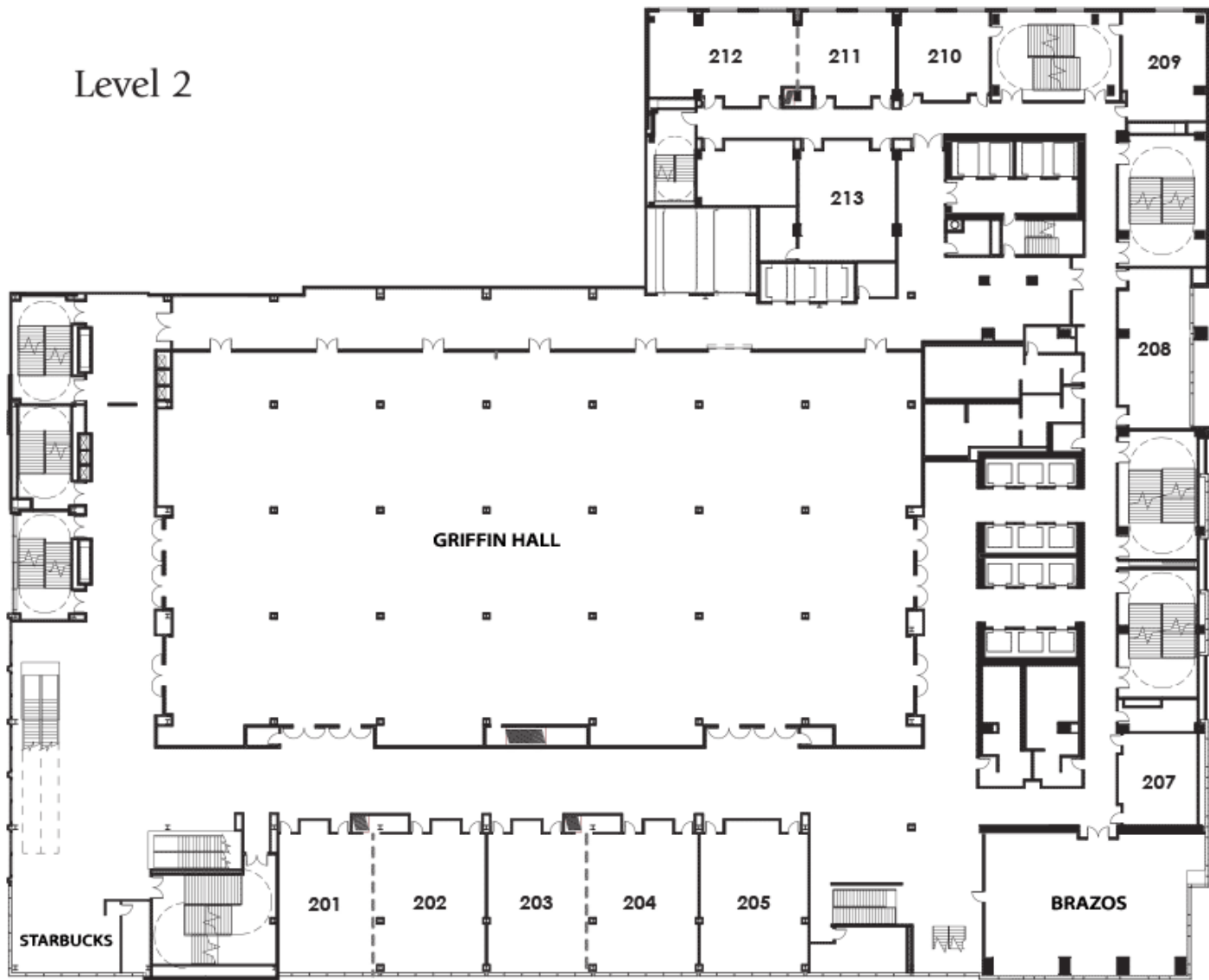
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