2017 **NF CONFERENCE**

From Process to Progress

JUNE 10-13, 2017 | RENAISSANCE WASHINGTON, DC DOWNTOWN HOTEL

Dear NF Conference Attendees:

The Children's Tumor Foundation strongly believes in collaboration—the kind that blossoms from meeting your peers, getting to know them, learning from them, and ultimately, connecting with them on a level that can only result from face-to-face interaction. Although the world of virtual communication—iPhones, Facebook, e-mail, Snapchat, Skype, Instagram (and much more)—has gained a lot of traction, I am an old-fashioned European who very much believes in authentic human connections. I am convinced that in order to build tangible, working collaborations and turn process into progress, trust is the ultimate key, and it is impossible to build trust with those you do not genuinely know.

It is because of this principle that bringing those from the NF community together to mingle, share data, exchange knowledge, share projects and dreams, and simply enjoy one another's company is *very* high on the Foundation's priority list. This is what we aim to accomplish every year with the NF Conference, and this year in Washington, D.C. will be no exception!

The Foundation is not alone in its gratitude for the annual NF Conference. At our Research Strategic Planning Retreat last fall, I was very happy to learn that a dynamic group of Board members and experts from both the NF community and other rare disease communities agreed that it should remain a top priority of the Foundation's—and it will.

Our conference is unique. Due to the heterogeneity of NF, it does not belong in one disease area, but in many, such as neurology, oncology, orthopedics, psychology, and many others. There is no other opportunity in the world for all of these diverse NF experts to meet and exchange their ideas, and to do so in an environment that encourages and cultivates these joint efforts. Although a number of collaborations in NF research began at the conference, we are particularly proud of Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) getting its start at this remarkable forum.

After having been a part the conference for over five years, I have pondered over the same interesting observation. Every year people tell me, 'this was the best conference yet.' We strive to make the conference the 'best yet' every year, and although it is indeed a costly operation, we are delighted to have the good fortune of reenergizing our NF community every year through this premier event. We *cannot* wait to see what this year has in store, and the fresh energy that it will bring!

Lastly, don't forget that next year we are crossing the Atlantic and bringing the NF Conference to Paris, France, an endeavor that is sure to give the conference the global presence it deserves. As the NF Conference itself expands, so will the amount of lives we change through our unwavering efforts to end NF around the globe. We are in D.C. today and Paris tomorrow, but we ceaselessly push the process and achieve progress as we search for the cure. Enjoy!

ANNETTE BAKKER
President and Chief Scientific Officer

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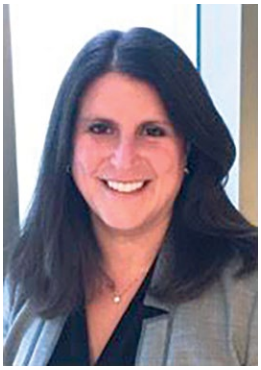
The Friedrich von Recklinghausen Award: Neurofibromatosis Tradition and Progress

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.

2017 Friedrich von Recklinghausen Award Recipient



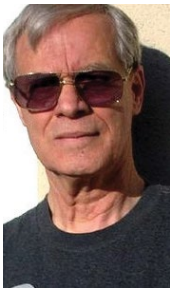
It is with great pleasure that the Children's Tumor Foundation announces the 2017 recipient of the Friedrich von Recklinghausen Award, Karen Cichowski, PhD, Professor of Medicine at Harvard Medical School and Brigham and Women's Hospital, and Chair and Scientific Director of the Neurofibromatosis Preclinical Consortium.

Dr. Cichowski is a true thought leader in the neurofibromatosis field and has a longstanding record of excellence in NF1-related research, ranging from basic discoveries to translational output. Her lab has been focusing on understanding how NF1 functions on a cellular level in sporadic tumors, and tumors that develop in NF1 patients, and has leveraged this insight to suggest new targeted cancer therapies. She has also made major contributions to the field of NF1 tumorigenesis study, neurofibromin regulation, and the PCR2 complex.

In addition, Dr. Cichowski has changed the field of NF research through her in depth studies of the RAS pathway and the discovery of a number of new negative feedback mechanisms. She showed that preclinical therapy of cancer in mice with a specific combination of targeted therapies affecting different consequences of RAS activation has a very strong therapeutic effect.

Karen Cichowski has also been at the forefront of developing new therapies for the most devastating tumors associated with NF1: malignant peripheral nerve sheath tumors (MPNSTs). Her work has led to multiple clinical trials that are ongoing or under development, and has provided clarity on the biology of these aggressive tumors. This work has been fundamental to moving the field forward, and the paradigm of pre-clinical studies in her lab has provided an incredible framework for thinking about the partnership between basic and clinical research, which is a fundamental goal of the Children's Tumor Foundation.

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
David Viskochil, MD, PhD
University of Utah

2017 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 initiated the Excellence in Team Science Award in 2016, and we are thrilled to present this award again in 2017.

Launched in 2014, **Synodos for NF2** is a multi-year initiative that has assembled a team of researchers from twelve world-class labs and medical centers. This comprehensive, collaborative model brought together experts in basic, translational, and clinical research, who have joined forces to share information, free of bureaucratic obstacles and institutional competition.

The most common medical issues were identified by NF2 patients, proposals were sought from the entire research community, and strategies that have a higher probability of finding solutions were designed. The brightest minds in the NF field are now working on these solutions using a real-time data sharing platform developed through our partnership with Sage Bionetworks. Synodos for NF2 aims to deliver multiple new and advanced cell and animal models to accelerate drug screening, new target pathways, and an increased understanding of response and resistance to treatment. Eventually this work will result in new clinical trials for NF2, and effective combination therapies.

We are pleased to announce the 2017 Excellence in Team Science Award goes to Synodos for NF2 and each of the labs and clinics that make up the Synodos for NF2 Consortium.

Jaishri Blakeley Clinic
Johns Hopkins University

Long-Sheng Chang Lab
The Ohio State University College of Medicine

Wade Clapp Lab
Indiana University School of Medicine

Cristina Fernandez-Valle Lab
University of Central Florida

Marc Ferrer Lab
National Institutes of Health

James Gusella Lab
Massachusetts General Hospital and Harvard Medical School

Stephen Haggarty Lab
Massachusetts General Hospital and Harvard Medical School

Gary Johnson Lab
University of North Carolina

Helen Morrison Lab
Fritz-Lipmann-Institute

Scott Plotkin Clinic
Massachusetts General Hospital and Harvard Medical School

Vijaya Ramesh Lab
Massachusetts General Hospital and Harvard Medical School

Anat Stemmer-Rachamimov Lab
Massachusetts General Hospital and Harvard Medical School

D. Bradley Welling Clinic
Massachusetts General Hospital and Harvard Medical School

Abhishek Pratap and Robert Allaway
Sage Bionetworks

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

The Medical College of Wisconsin designates this live activity for a maximum of 20.25 *AMA PRA Category 1 Credits™.* Physicians should claim only the credit commensurate with the extent of their participation in the activity.

The Medical College of Wisconsin designates this activity for up to 20.25 hours of participation for continuing education for allied health professionals.

Questions? Contact Heather Radtke - hradtke@ctf.org

FOUNDATION STAFF

Children's Tumor Foundation

Annette Bakker, PhD	President and Chief Scientific Officer	abakker@ctf.org
Reid Horovitz	Chief Operating and Chief Financial Officer	rhorovitz@ctf.org

Research and Medical Programs

Salvatore La Rosa, PhD	Vice President, Research and Development	slarosa@ctf.org
Maria Carela	Grant Manager	mcarela@ctf.org
Vidya Browder, PhD	Basic Science Manager	vbrowder@ctf.org
Kate Kelts	Patient Support Coordinator	kkelts@ctf.org
Pamela Knight	Director, Clinical Program	pknight@ctf.org
Patrice Pancza	Research Program Director	ppanzca@ctf.org
Heather Radtke	NF Clinic and Symposium Coordinator	hradtke@ctf.org
Traceann Rose	Director, Patient Engagement	trose@ctf.org
Sarah Rosenberg	Senior Executive Assistant; Manager of Special Projects & Board Affairs	srosenberg@ctf.org

Development

Allison Cote	Regional Development Manager	acote@ctf.org
Emily Crabtree	Director, Development Operations	ecrabtree@ctf.org
Angela Dumadag	Regional Development Manager	adumadag@ctf.org
Angela Earle	Director, Development Innovations & Kids Program	aearle@ctf.org
Adam Gracia	Regional Development Manager	agracia@ctf.org
Channell Hogan	Regional Development Manager	chogan@ctf.org
Lolita Jerido	Regional Development Manager	ljeriod@ctf.org
Kelly Mills	Regional Development Manager	kmills@ctf.org
Julie Pantoliano	Regional Development Manager	jpantoliano@ctf.org
Kristine Poirier	Senior Director, Development	kpoirier@ctf.org
Kim Robinson	Regional Development Manager	krobinson@ctf.org

Major Gifts & Major Events

Melissa Sosa-Longo	Vice President, Major Gifts	msosa-longo@@ctf.org
Rebecca De Ornelas	National Manager, Special Events	rdeornelas@ctf.org
Mary Vetting	Stewardship Manager	mvetting@@ctf.org

Finance and Administration

Catherine Blessing	Director of Human Resources	cblessing@ctf.org
Monique Boucher	Gift Processing Manager	mboucher@ctf.org
Sarah Bourne	Director, Finance	sbourne@ctf.org
Albert Diaz	Director of IT and Salesforce Administration	adiaz@ctf.org
Margaret Flaccamio	Fulfillment Coordinator	mflaccamio@ctf.org
Keena Hutchinson	Gift Processing Coordinator	khutchinson@ctf.org
Morgan Kellogg	Gift Processing Assistant	mkellogg@ctf.org
Latisha Maxwell	Gift Processing Coordinator	lmaxwell@ctf.org
Danielle Meyer	Technical Support, National Programs	dmeyer@ctf.org
Carey Milligan	Accountant	cmilligan@ctf.org
Rosa Amelia Perez	Project Administrator	rperez@ctf.org
Connie Sorman	Senior Manager, Volunteer Engagement	csorman@ctf.org

Marketing and Communications

Simon Vukelj	Vice President, Marketing and Communications	svukelij@ctf.org
Rebecca Harris	Public Relations Manager	rharris@ctf.org
Alissa Marks	Marketing Manager	amarks@ctf.org
Susanne Preinfalk	Design Director	spreinfalk@ctf.org
Vanessa Younger	Communications Senior Manager	vyounger@ctf.org

Children's Tumor Foundation National Programs

NF WALK

The Children's Tumor Foundation NF Walk program helps fund research, raises awareness, and supports individuals and families living with neurofibromatosis. NF Walk brings together families, friends, and neighbors for memorable community events. Whether you have NF, know someone who does, or just want to show your support, come and join an NF Walk in your community and take steps to end NF.

NF ENDURANCE

The Children's Tumor Foundation NF Endurance Team gives participants the opportunity to run, bike, or swim in endurance events across the country. By joining our team you can raise awareness and research funds to find a cure for neurofibromatosis, while receiving training from experienced coaches, entry into a variety of races, fundraising support, and inspiration from our NF Heroes.

DO-IT-YOURSELF

Make a difference in your community in the fight to end NF by creating your own fundraiser for the Children's Tumor Foundation. With the help of our regional staff, you can plan an event around your interests and talents to help fund NF research.

KIDS PROGRAM

With our Kids Program offerings, young children and teens can join in the mission of the Children's Tumor Foundation to make a difference in the lives of millions living with NF. Schools and youth programs can host events through our Classrooms for a Cure program, kids can create their own fundraisers as part of our Do-it-Yourself (DIY) Kids program, or young walkers or athletes can take part in one of our nationwide NF Walk or NF Endurance events.

Schedule At-A-Glance

	TIME		EVENT	LOCATION
SATURDAY JUNE 10	8:00 AM	12:00 PM	**Satellite Educational Symposium**	Grand Ballroom Central/South
	12:00 PM	12:45 PM	Lunch on Your Own	
	12:45 PM	1:00 PM	**OPENING REMARKS**	Grand Ballroom Central/South
	1:00 PM	2:00 PM	**KEYNOTE 1:** Stem Cells in Silence, Action and Cancer	Grand Ballroom Central/South
	2:00 PM	2:15 PM	Break	Foyer Congressional Ballroom
	2:15 PM	4:15 PM	**SESSION 1:** Stem Cells and NF Tumor Biology	Grand Ballroom Central/South
	4:15 PM	4:25 PM	Poster Advertisements - Basic Science	Grand Ballroom Central/South
	4:30 PM	6:00 PM	Poster Session - Basic Science	Congressional Ballroom A/B
	6:30 PM	10:00 PM	**Welcome Dinner and von Recklinghausen Award Presentation**	National Press Club
SUNDAY JUNE 11	7:30 AM	9:00 AM	Clinic Coordinators' Breakfast (by invitation only)	City Tap House
	7:30 AM	9:00 AM	Breakfast	Foyer Grand Ballroom Central/South
	9:00 AM	10:00 AM	**KEYNOTE 2:** Identifying Tumor Vulnerabilities through Integrated Analysis	Grand Ballroom Central/South
	10:00 AM	10:30 AM	Break	Foyer Grand Ballroom Central/South
	10:30 AM	11:00 AM	**Plenary Talk:** Mind the Research Science - Data Science Gap	Grand Ballroom Central/South
	11:00 AM	12:30 PM	**SESSION 2:** Genes, Genotypes and Phenotypes	Grand Ballroom Central/Southr
	12:30 PM	1:30 PM	Clinical Care Advisory Board Lunch (closed meeting)	TBA
	12:30 PM	1:30 PM	Lunch	Foyer Grand Ballroom Central/South
	1:30 PM	3:30 PM	**SESSION 3:** Non-Tumor Manifestations of NF	Grand Ballroom Central/South
	3:30 PM	3:40 PM	Poster Advertisements - Clinical Science	Grand Ballroom Central/South
	3:40 PM	5:10 PM	Poster Session - Clinical Science	Congressional Ballroom A/B
	5:30 PM	8:30 PM	Dinner on Your Own	
	5:30 PM	6:30 PM	"Speed-Dating": Pre and Post-Doc Mentoring Session	Congressional Ballroom C
MONDAY JUNE 12	7:00 AM	8:30 AM	Breakfast	Foyer Grand Ballroom Central/South
	8:30 AM	9:30 AM	**CONCURRENT PLATFORM SESSION - 4A CLINICAL SCIENCE**	Grand Ballroom Central/South
	8:30 AM	9:30 AM	**CONCURRENT PLATFORM SESSION - 4B BASIC SCIENCE**	Congressional Ballroom C
	9:30 AM	12:35 PM	**SESSION 5:** NF Tumors	Grand Ballroom Central/South
	12:35 PM	2:00 PM	Lunch and Mentoring	Foyer Grand Ballroom Central/South
	2:00 PM	4:00 PM	**CONCURRENT SESSION 6A:** Learning, Memory and Behavior through the Ages in NF	Grand Ballroom Central/South
	2:00 PM	4:00 PM	**CONCURRENT SESSION 6B:** New Advances in NF2 and Schwannomatosis	Congressional Ballroom C
	4:00 PM	10:30 PM	Free Time and Dinner on Your Own	
	4:15 PM	6:35 PM	INFACT Working Group Meeting	Congressional Ballroom C
	4:15 PM	7:15 PM	All Clinics Meeting (Please RSVP to Heather Radtke - hradtke@ctf.org)	Mount Vernon, Meeting Room Level
TUESDAY JUNE 13	7:30 AM	9:00 AM	Breakfast	Foyer Grand Ballroom Central/South
	9:00 AM	10:00 AM	Top Oral Poster Presentations and Awards	Grand Ballroom South
	10:00 AM	11:00 AM	**KEYNOTE 3:** Peripheral Nerve Homeostasis and Repair - Impaired Signaling Circuits in Neurofibromatosis Type 2 (NF2)	Grand Ballroom South
	11:00 AM	11:15 AM	Break	
	11:15 AM	1:15 PM	**SESSION 7:** Signaling in Neurofibromatosis	Grand Ballroom South
	1:15 PM	1:35 PM	**SESSION 8:** Development of New Therapies for NF1 And NF2: A Model Strategy for Orphan Diseases	Grand Ballroom South
	1:35 PM	1:50 PM	Closing Remarks	Grand Ballroom South

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 10th – 13th in Congressional A/B.
- Posters can be set up Saturday morning, starting 7am; your poster should be on display for the duration of the Conference.
- The Basic Science poster session (these posters will carry odd numbers) will be held on Saturday, June 10th, from 4:30pm – 6:00pm. Please stand by your poster during this time. Refreshments will be served.
- The Clinical Science poster session (these posters will carry even numbers) will be held on Sunday, June 11th, from 3:40pm – 5:10pm. Please stand by your poster during this time. Refreshments will be served.
- A jury will be judging posters during each session and will select the top three 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

Saturday · June 10, 2017

8:00 AM	**12:00 PM**	**SATELLITE EDUCATIONAL SYMPOSIUM** Session Co-Chairs: Robert Listernick, MD, *Ann and Robert Lurie Children's Hospital;* Michael Fisher, MD, *Children's Hospital of Philadelphia;* Jaishri Blakeley, MD, *Johns Hopkins University Medical Center*	Grand Ballroom Central/South
8:00 AM	9:50 AM	**NF1**	
		Skeletal Abnormalities	
8:00 AM	8:25 AM	**Neurofibromatosis Type 1 and Scoliosis** Matthew Oetgen, MD, Division Chief, Orthopaedic Surgery and Sports Medicine, *Children's National Medical Center*	
8:25 AM	8:50 AM	**Tibial Dysplasia** B. Stephens Richards, MD, Professor of Orthopaedic Surgery, *Texas Scottish Rite Hospital for Children*	
8:50 AM	9:00 AM	**Q&A**	
		Neurovascular Disease	
9:00 AM	9:20 AM	**Pediatric** Tena Rosser, MD, Associate Professor of Clinical Neurology, *University of Southern California*	
9:20 AM	9:40 AM	**Adult** Paul Holmes, MD, Consultant Neurologist, *Guy's & St. Thomas' NHS Foundation Trust, London, UK*	
9:40 AM	9:50 AM	**Q&A**	
9:50 AM	10:10 AM	**BREAK**	
10:10 AM	11:00 AM	**Basic Science for Clinicians**	
10:10 AM	10:30 AM	**From GEMMs to PDXs to Avatars, Mouse Models in Discovery and Translational Research** Karlyne Reilly, PhD, *Rare Tumors Initiative, Center for Cancer Research, NCI, Bethesda, MD*	
10:30 AM	10:50 AM	**Signaling Pathways in NF2 - A Primer** Cristina Fernandez-Valle, PhD, *University of Central Florida*	
10:50 AM	11:00 AM	**Q&A**	
11:00 AM	11:55 AM	**NF2**	
11:00 AM	11:20 AM	**Neuro-ophthalmologic Manifestations of NF2** Grant Liu, MD, Professor of Neurology and Ophthalmology, *University of Pennsylvania*	
11:20 AM	11:45 AM	**Neurofibromatosis Type 2 in Childhood** Scott Plotkin, MD, PhD, *Massachusetts General Hospital/Harvard Medical School*	
11:45 AM	11:55 AM	**Q&A**	
12:00 PM	**12:45 PM**	**Lunch on Your Own**	
12:45 PM	**1:00 PM**	**OPENING REMARKS** Conference Co-Chairs: Rosalie Ferner, MD, Professor of Neurology, *Guy's and St. Thomas' NHS Foundation Trust, and King's College London;* Lawrence Sherman, PhD, Professor, *Division of Neuroscience, Oregon National Primate Research Center and Department of Cell, Developmental and Cancer Biology, Oregon Health & Science University;* Annette Bakker, PhD, *Children's Tumor Foundation President and CSO*	Grand Ballroom Central/South
1:00 PM	**2:00 PM**	**KEYNOTE SPEECH 1: Stem Cells in Silence, Action and Cancer** Elaine Fuchs, PhD, *Rebecca C. Lancefield Professor of Mammalian Cell Biology and Development, Rockefeller University*	Grand Ballroom Central/South
2:00 PM	2:15 PM	**BREAK**	Foyer Congressional Ballroom

AGENDA

Saturday · June 10, 2017

2:15 PM	**4:15 PM**	**SESSION 1: STEM CELLS AND NF TUMOR BIOLOGY** Session Co-Chairs: Matthias Karajannis, MD, MS, *Memorial Sloan Kettering Cancer Center;* Peter deBlank, MD, MSCE, *Cincinnati Children's Hospital Medical Center*	Grand Ballroom Central/South
2:15 PM	2:50 PM	**Perspectives Talk: The Cellular and Molecular Pathogenesis of NF1 Optic Gliomas** David Gutmann, MD, PhD, *Washington University*	
2:50 PM	3:15 PM	**Roots of Neurofibromas** Juha Peltonen, MD, PhD, *University of Turku, Finland*	
3:15 PM	3:35 PM	**The Role of MEK Inhibitors for the Prevention and Treatment of OPG in an NF1-Deficient Mouse Model** Miriam Bornhorst, MD, *Children's National Medical Center*	
3:35 PM	3:55 PM	**Platform: Differentiation of Plexiform Neurofibroma (PNF)-derived NF1(+/-) and NF1 (-/-) iPS Cells into Schwann Cells Mimicking Primary PNF Cells: Closing the Circle** Eduard Serra, PhD, *The Institute for Health Science Research Germans Trias i Pujol (IGTP) - Program of Predictive and Personalized Medicine of Cancer (PMPPC)*	
3:55 PM	4:15 PM	**Platform: Cancer Stem Cells as the Target for MPNST Tumorigenesis and Relapse** Daochun Sun, PhD, *Brain Center, Memorial Sloan Kettering Cancer Center*	
4:15 PM	**4:25 PM**	**Poster Advertisements - Basic Science**	Grand Ballroom Central/South
4:30 PM	**6:00 PM**	**Poster Session - Basic Science** Refreshments will be served.	Congressional Ballroom A/B
6:30 PM	**10:00 PM**	**Welcome Dinner, von Recklinghausen and Team Science Award Presentation** Keynote Speaker: Greg Simon, JD, *Executive Director, Biden Foundation, former Executive Director, White House Cancer Moonshot Initiative*	National Press Club 529 14th St. NW, 13th Floor Washington, DC 20045 202-662-7500

Sunday · June 11, 2017

7:30 AM	**9:00 AM**	**Clinic Coordinators' Breakfast** (by invitation only) Please RSVP to Heather Radtke - hradtke@ctf.org	City Tap House 901 9th Street, NW (meet in hotel lobby @ 7:15am)
7:30 AM	**9:00 AM**	**Breakfast**	Foyer Grand Ballroom Central/South
9:00 AM	**10:00 AM**	**KEYNOTE SPEECH 2: Identifying Tumor Vulnerabilities through Integrated Analysis** Michael Dyer, PhD, *Richard C. Shadyac Endowed Chair in Pediatric Cancer Research, St. Jude's Children's Research Center*	Grand Ballroom Central/South
10:00 AM	10:30 AM	**BREAK**	Foyer Grand Ballroom Central/South
10:30 AM	**11:00 AM**	**Plenary Talk: Mind the Research Science - Data Science Gap** Shasha Jumbe, PhD, *Senior Program Officer, Quantitative Sciences, Bill & Melinda Gates Foundation*	Grand Ballroom Central/South
11:00 AM	**12:30 PM**	**SESSION 2: GENES, GENOTYPES AND PHENOTYPES** Session Chairs: Ludwine Messiaen, PhD, *University of Alabama at Birmingham;* Miriam Smith, PhD, *University of Manchester, UK*	Grand Ballroom Central/South
11:00 AM	11:30 AM	**Perspectives Talk: Towards Genome-Guided Therapy for NF1** Bruce Korf, MD, PhD, *University of Alabama at Birmingham*	
11:30 AM	11:50 AM	**Mutations in Schwannomatosis** Laura Papi, MD, *University of Florence, Italy*	
11:50 AM	12:10 PM	**Large NF1 Deletions: Underlying Mutational Mechanisms and Genotype-Phenotype Relationships** Hildegard Kehrer-Sawatzki, PhD, *University of Ulm, Germany*	

AGENDA

Sunday · June 11, 2017

12:10 PM	12:30 PM	**Platform: Genotype-phenotype correlation in NF1 patients: evidence for a more severe phenotype associated with missense mutations affecting NF1 codons 844-848** Magdalena Koczkowska, PhD, *Department of Genetics, University of Alabama at Birmingham*	
12:30 PM	**1:30 PM**	**Clinical Care Advisory Board Lunch** (closed meeting)	TBA
12:30 PM	**1:30 PM**	**Lunch**	Foyer Grand Ballroom Central/South
1:30 PM	**3:30 PM**	**SESSION 3: NON-TUMOR MANIFESTATIONS OF NF** Session Co-Chairs: Aaron Schindeler, PhD, *Children's Hospital at Westmead, AUS;* Nicole Ullrich, MD, PhD, *Boston Children's Hospital, Harvard University*	Grand Ballroom Central/South
1:30 PM	1:50 PM	**A Dietary Intervention for NF1-Associated Muscle Weakness** Aaron Schindeler, PhD, *Children's Hospital at Westmead, AUS*	
1:50 PM	2:10 PM	**Investigating Double Inactivation of NF1 in Cases of Scoliosis** David Stevenson, MD, *Stanford University*	
2:10 PM	2:30 PM	**Neuropathies** Helen Morrison, PhD, *Leibniz Research Institute on Aging, Fritz Lipmann Institute, Germany*	
2:30 PM	2:50 PM	**Platform: The Reduced Osteogenic Differentiation Potential of NF1-Deficient Osteoprogenitors is EGFR-Independent** Seyedmohammad Ebrahim Tahaei, PhD Candidate, *Pharmacology Department, Vanderbilt University*	
2:50 PM	3:10 PM	**Platform: Clinically Significant Neuropathy in NF2 - Frequency, Clinical and Neurophysiological Characteristics in 33 of 175 Patients Attending a National NF2 Service** Victoria Williams, MD, *Neurofibromatosis Centre, Department of Neurology, Guy's and St. Thomas' NHS Foundation Trust, Guy's Hospital, London, UK*	
3:10 PM	3:30 PM	**Platform: Resting Metabolic Rate and Adiposity Assessment in Individuals with Neurofibromatosis Type 1: Comparing Gold Standard to Conventional Methods** Juliana Souza, MD, PhD, *Neurofibromatosis Outpatient Reference Center, Federal University of Minas Gerais, Brazil*	
3:30 PM	**3:40 PM**	**Poster Advertisements - Clinical**	Grand Ballroom Central/South
3:40 PM	**5:10 PM**	**Poster Session - Clinical Science** Refreshments will be served.	Congressional Ballroom A/B
5:30 PM	**8:30 PM**	**Dinner on Your Own**	
5:30 PM	**6:30 PM**	**"Speed-Dating": Pre and Post-Doc Mentoring Session** Refreshments will be served.	Congressional Ballroom C

Monday · June 12, 2017

7:00 AM	**8:30 AM**	**Breakfast**	Foyer Grand Ballroom Central/South
8:30 AM	**9:30 AM**	**CONCURRENT PLATFORM SESSION 4A: CLINICAL SCIENCE** Session Chair: D. Wade Clapp, MD, *Indiana University School of Medicine*	Grand Ballroom Central/South
8:30 AM	8:50 AM	**Platform: Peripheral Neuropathy - Expanding the Clinical Phenotype of Schwannomatosis** Matthew Evans, MRB MD, *Neurofibromatosis Centre, Department of Neurology, Guy's and St. Thomas' NHS Foundation Trust, Guy's Hospital, London, UK*	
8:50 AM	9:10 AM	**Platform: Multiple Cancers in Neurofibromatosis Type 1** Roope A. Kallionpää, MSc (pharm), *University of Turku, Finland*	
9:10 AM	9:30 AM	**Platform: NF1-Associated Dystrophic Scoliosis: Standardization of Diagnostic Criteria and Prediction Using Single-Nucleotide Polymorphism Markers** Christopher L. Moertel, MD, *University of Minnesota Masonic Children's Hospital*	

AGENDA

Monday · June 12, 2017

8:30 AM	**9:30 AM**	**CONCURRENT PLATFORM SESSION 4B: BASIC SCIENCE** Session Chair: Yuan Zhu, PhD, *Children's National Medical Center*	Congressional Ballroom C
8:30 AM	8:50 AM	**Platform: Exploiting Novel Vulnerabilities in NF1-Associated Tumorigenesis: A Small Molecule Screen Identifies Compounds Capable of Selectively Killing NF1-Deficient Human Schwann Cells** Kyle B. Williams, PhD, *Department of Pediatrics, Masonic Cancer Center, University of Minnesota, Twin Cities*	
8:50 AM	9:10 AM	**Platform: Nonsense Suppression Rescues Lethal Phenotype in Systemic Induced NF1 Adult Mouse Harboring Patient-Specific Mutation** Ashley N. Turner, MS, *Department of Genetics, University of Alabama at Birmingham*	
9:10 AM	9:30 AM	**Platform: Immunotherapy for MPNST** Thomas De Raedt, PhD, *Harvard Medical School*	
9:30 AM	**12:35 PM**	**SESSION 5: NF TUMORS** Session Co-Chairs: Scott Plotkin, MD, PhD, *Massachusetts General Hospital/Harvard University;* Brigitte Widemann, MD, *National Cancer Institute*	Grand Ballroom Central/South
9:30 AM	9:40 AM	**MPNST State of the Science: Outlining a Research Agenda for the Future** AeRang Kim, MD, PhD, *Children's National Medical Center*	
9:40 AM	9:50 AM	**Introducing ANNUBP - Atypical Neurofibromatous Neoplasms of Uncertain Biologic Potential; Summary of Consensus NIH Workshop on Neurofibromas, Atypical Neurofibromas and MPNST in NF1 Patients** Anat Stemmer-Rachamimov, MD, *Harvard University*	
9:50 AM	10:15 AM	**Defining Cutaneous Neurofibromas** Pierre Wolkenstein, MD, PhD, *Hôpital Henri-Mondor, Universite de Paris EST Creteil;* Jaishri Blakeley, MD, *Johns Hopkins University Hospital*	
10:15 AM	10:30 AM	**BREAK**	
10:30 AM	10:50 AM	**Drug Screening in Synodos for NF2** James Gusella, PhD, *Harvard University*	
10:50 AM	11:10 AM	**Genomic Landscape of Schwannomas** Gelareh Zadeh, MD, *University of Toronto*	
11:10 AM	11:30 AM	**Targeting the cMET Signaling in NF2 Schwannoma Models** Lei Xu, MD, PhD, *Massachusetts General Hospital and Harvard University*	
11:30 AM	11:50 AM	**NF Mouse Models** Nancy Ratner, PhD, *Cincinnati Children's Hospital Medical Center*	
11:50 AM	12:05 PM	**Platform: A Phase II Prospective Study of Selumetinib in Children with Recurrent or Refractory NF1-Associated Low-Grade Glioma (LGG): A Pediatric Brain Tumor Consortium (PBTC) Study** Roger Packer, MD, *Children's National Health System, Washington, DC*	
12:05 PM	12:20 PM	**Platform: NF106: A Phase 2 NF Consortium Trial of the MEK Inhibitor PD-0325901 in Adolescents and Adults with NF1-Related Plexiform Neurofibromas** Brian Weiss, MD, *Cincinnati Children's Hospital Medical Center*	
12:20 PM	12:35 PM	**Platform: A Swine Model of Neurofibromatosis Type 1** Adrienne L. Watson, PhD, *Recombinetics, Inc.*	
12:35 PM	**2:00 PM**	**Lunch and Mentoring**	Foyer Grand Ballroom Central/South Location of Mentoring Tables TBA

AGENDA

Monday · June 12, 2017

2:00 PM	**4:00 PM**	**CONCURRENT SESSION 6A: LEARNING, MEMORY AND BEHAVIOR THROUGH THE AGES IN NF** Session Co-Chairs: Ype Elgersma, PhD, *Erasmus University Medical Center, Netherlands;* Eric Legius, MD, PhD, *University of Leuven, Belgium*	Grand Ballroom Central/South
2:00 PM	2:30 PM	**Perspectives Talk: Cognition in NF through all Ages** Eric Legius, MD, PhD, *University of Leuven, Belgium*	
2:30 PM	2:50 PM	**Cognition and Behavior in Neurofibromatosis Type 1: Recent Advances** Jonathan Payne, PsyD, *Murdoch Children's Research Hospital, Victoria, Australia*	
2:50 PM	3:10 PM	**Traits and Symptoms of Autism Spectrum Disorder in Neurofibromatosis Type 1** John Constantino, MD, *Washington University, St. Louis*	
3:10 PM	3:30 PM	**Platform: Neurofibromin Deficiency Alters Brain-Wide Intrinsic Functional Organization of the Developing Brain** Ben Shofty, MD, *Tel Aviv Medical Center*	
3:30 PM	3:50 PM	**Platform: Habituation Learning in Drosophila - a High-Throughput Platform to Identify Drugs that Ameliorate Cognitive and Behavioral Problems in NF1 and Other Rasopathies** Michaela Fenckova, PhD, *Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands*	
2:00 PM	**4:00 PM**	**CONCURRENT SESSION 6B: NEW ADVANCES IN NF2 AND SCHWANNOMATOSIS** Session Co-Chairs: Marco Giovannini, MD, PhD, *UCLA;* Gareth Evans, MD, *St. Mary's Hospital, University of Manchester, UK*	Congressional Ballroom C
2:00 PM	2:20 PM	**Characterization of SMARCB1/NF2 Mice** Jeremie Vitte, PhD, *UCLA*	
2:20 PM	2:40 PM	**Prenatal Smo Activitation in Meningeal Cells is Necessary to Promote Meningothelial Meningioma of the Skull Base** Michel Kalamarides, MD, PhD, *Hôpital de la Pitié-Salpétrière, Paris*	
2:40 PM	3:00 PM	**Pain Phenotypes and Mechanisms Linked to Mouse and Human SMARCB/1 Mutant Schwann Cells** Steven Matsumoto, PhD, *Oregon Health and Science University*	
3:00 PM	3:20 PM	**Platform: YAP/TAZ-mediated Metabolic Reprogramming is Required for Growth and Survival of NF2-Deficient Tumor Cells in Vitro and in Vivo** Shannon White, BS, *Lombardi Comprehensive Cancer Center, Georgetown University Medical Center*	
3:20 PM	3:40 PM	**Platform: Co-Targeting mTORC1/2 and EPH Receptor Pathways as a Therapeutic Potential for NF2-Deficient Meningiomas** Roberta Beauchamp, BA, *Massachusetts General Hospital*	
3:40 PM	4:00 PM	**Platform: Combined Inhibition of NEDD8-Activating Enzyme and mTOR Suppresses NF2 Loss-Driven Tumorigenesis** Filippo Giancotti, MD, PhD, *MD Anderson Cancer Center*	
4:00 PM	**10:30 PM**	**Free Time and Dinner on Your Own**	
4:15 PM	**6:30 PM**	**INFACT Meeting**	Congressional Ballroom C
4:15 PM	**7:15 PM**	**All Clinics Meeting** Please RSVP to Heather Radtke - hradtke@ctf.org	Mount Vernon, Meeting Room Level

AGENDA

Tuesday · June 13, 2017

7:30 AM	**9:00 AM**	**Breakfast**	Foyer Grand Ballroom Central/South
9:00 AM	**10:00 AM**	**Top Oral Poster Presentations and Awards**	Grand Ballroom South
10:00 AM	**11:00 AM**	**KEYNOTE SPEECH 3: Peripheral Nerve Homeostasis and Repair – Impaired Signaling Circuits in Neurofibromatosis Type 2 (NF2)** Helen Morrison, PhD, *Leibniz Institute on Aging, Fritz Lipmann Institute, Germany*	Grand Ballroom South
11:00 AM	11:15 AM	**BREAK**	
11:15 AM	**1:15 PM**	**SESSION 7: SIGNALING IN NEUROFIBROMATOSIS** Session Co-Chairs: Karlyne Reilly, PhD, *National Cancer Institute;* Alison Lloyd, PhD, *University College London, UK*	Grand Ballroom South
11:15 AM	11:35 AM	**Role of NF1 in Reward, Motor Learning and Opioid Actions in the Striatum** Kirill Martemyanov, PhD, *Scripps Institute*	
11:35 AM	11:55 AM	**Opposing Effects of Activating Mutations on Developmental Ras Signaling and Morphogenesis** Rebecca Burdine, PhD, *Princeton University*	
11:55 AM	12:15 PM	**Platform: The Molecular and Immunologic Landscape of Cutaneous Neurofibroma** Robert Allaway, PhD, *Sage Bionetworks*	
12:15 PM	12:35 PM	**Shutting Your TRAP to Kill Neurofibromas? The Oncogenic Role of the Mitochondrial Chaperone TRAP1 in NF1** Andrea Rasola, PhD, *University of Padova, Italy*	
12:35 PM	12:55 PM	**Tipping the Redox Balance in the Fight against NF2** Chunling Yi, PhD, *Lombardi Comprehensive Cancer Center, Georgetown University Medical School*	
12:55 PM	1:15 PM	**Merlin and the Hippo Signalling Pathway in the Nucleus** Joseph Kissil, PhD, *Scripps Research Institute*	
1:15 PM	**1:35 PM**	**SESSION 8: DEVELOPMENT OF NEW THERAPIES FOR NF1 AND NF2: A MODEL STRATEGY FOR ORPHAN DISEASES** D. Wade Clapp, MD, *Indiana University School of Medicine*	Grand Ballroom South
1:35 PM	**1:50 PM**	**CLOSING REMARKS**	Grand Ballroom South

2017 NF Conference Co-Chairs

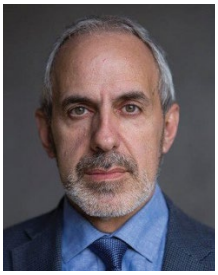
Rosalie Ferner, MD, *Professor of Neurology, Guy's and St. Thomas' NHS Foundation Trust and King's College, London*

Rosalie Ferner is Professor of Neurology at Guy's and St. Thomas' NHS Foundation Trust and King's College London, and chair of the UK Neuro Foundation medical advisory board. She was the 2016 recipient of the European Theodor Schwann award for contributions to neurofibromatosis. She is the national lead for the multi-disciplinary Complex NF1 service, funded by NHS England. She was the London lead for the national NF2 service from 2010-2014. Her interests include defining the clinical phenotype of the neurofibromatoses, the diagnosis of malignant peripheral nerve sheath tumours in NF1 using positron emission tomography computerised tomography, and the development of robust clinical and patient centred outcome measures for monitoring therapy.

Larry S. Sherman, PhD, *Professor, Division of Neuroscience, Oregon National Primate Research Center, Department of Cell, Developmental and Cancer Biology, Oregon Health and Sciences University*

Larry S. Sherman is a Professor in the Division of Neuroscience at the Oregon National Primate Research Center and in the Department of Cell, Developmental and Cancer Biology at the Oregon Health & Science University. He is also President of the Oregon Chapter of the Society for Neuroscience. Over the past twenty years he has been focused on studying nervous system development and disease, with a focus on Schwann cell and oligodendrocyte biology. His work includes studies on NF1, NF2 and schwannomatosis, including current studies on the mechanisms underlying schwannomatosis pain. He has served on a number of national and international scientific review panels including the Neurofibromatosis Congressionally Directed Medical Research Program.

2017 NF Conference Keynote Speakers



Gregory C. Simon, JD, *Director, Biden Cancer Initiative*

Greg Simon served as the Executive Director of the White House Cancer Moonshot Task Force, a position created by President Barack Obama and for which he was chosen by Vice President Joe Biden in March 2016. Over the past nine months Greg and his team helped launch over seventy innovative collaborations. Greg returned to the White House after serving as Vice President Al Gore's Chief Domestic Policy Advisor between 1993 and 1997. Previously, Greg was the CEO of Poliwogg, a financial services company creating unique capital market opportunities in healthcare and life sciences. In addition, Greg was Senior Vice President for Worldwide Policy and Patient Engagement at Pfizer, co-founded with Michael Milken, FasterCures/ The Center for Accelerating Medical solutions, and, with Leon and Debra Black, co-founded the Melanoma Research Alliance. Greg is a cancer survivor, and has recently been successfully treated for chronic lymphocytic leukemia.

Elaine Fuchs, PhD, *Rebecca C. Lancefield Professor of Mammalian Cell Biology and Development, Rockefeller University*

Elaine Fuchs is renowned for her research in skin biology, its stem cells and associated genetic disorders, including cancers. She received her Ph.D. in Biochemistry from Princeton. After postdoctoral research at MIT, she joined the faculty at University of Chicago. In 2002, she relocated to Rockefeller University, where she is the Rebecca C. Lancefield professor of Mammalian Cell Biology and Development. She has been an Investigator of the Howard Hughes Medical Institute since 1988. Fuchs' awards and honors include the Richard Lounsbery Award from the National Academy of Sciences, National Medal of Science, L'Oreal-UNESCO Award, Albany Prize in Medicine, March of Dimes Prize in Developmental Biology, Pasarow Award in Cancer, Pezcoller Award in Cancer Research, EB Wilson Award, and in 2017 will receive the Vanderbilt Prize. Fuchs is an elected member of the National Academy of Sciences, National Academy of Medicine (formerly Institute of Medicine), American Philosophical Society and European Molecular Biology Organization (foreign member). She holds honorary doctorates from NYU School of Medicine, University of Illinois, Albany Medical College and Harvard University. She is past-President of American Society for Cell Biology, The International Society for Stem Cell Research and the Harvey Society, and serves on the NYAS Board of Governors and NAM/IOM Council. She's trained >25 graduate students and 100 postdocs, most now at academic universities and medical schools. Her lecture will focus on her latest work in the field of skin stem cells, how these tissue stem cells replenish dying cells within the skin, how they become mobilized to repair wounds, and how they acquire mutations that will ultimately lead to skin cancers.

BIOS

2017 NF Conference Keynote Speakers

Michael Dyer, PhD, *Investigator, HHMI*
Richard C. Shadyac Endowed Chair in Pediatric Cancer Research
Chair/Member, Department of Developmental Neurobiology
Co-Leader, Developmental Biology and Solid Tumor Program
Head, Division of Developmental Biology
St. Jude's Children's Research Hospital

Michael Dyer received his bachelor's degree with honors from UCLA in Microbiology and Molecular Genetics. For his doctoral training, Dr. Dyer went to Harvard University where he studied globin gene switching during hematopoiesis with Dr. Margaret Baron. After completing his degree, he moved to Harvard Medical School for a postdoctoral fellowship with Dr. Connie Cepko. In Connie's lab he became interested in the regulation of retinal progenitor cell proliferation during neurogenesis. In 2002, Dr. Dyer was recruited to the department of Developmental Neurobiology at St. Jude Children's Research Hospital. At St. Jude, he expanded his interest in neural progenitor cell proliferation to include cancer biology, evolutionary biology and stem cell biology. In 2005, he was promoted to Associate Member and in 2008 he was promoted to Member at St. Jude. He has received numerous awards since joining the faculty at St. Jude including being named a Pew Scholar, the Cogan Award Recipient and a Howard Hughes Medical Institute Early Career Scientist. In 2009, Dr. Dyer was named co-leader of the Solid Tumor Program in the NCI designated comprehensive cancer center at St. Jude Children's Research Hospital and in 2011 he was named head of the Division of Developmental Biology. In 2013 he became an investigator of the Howard Hughes Medical Institute and in 2014 he was named the Richard C. Shadyac Endowed Chair in Pediatric Cancer Research. In 2016 he was named the Chair of the Developmental Neurobiology Department.

Shasha Jumbe, PhD, *Bill & Melinda Gates Foundation*

Dr. N. L'ntshotsholé "Shasha" Jumbe has developed and applied data science problem solving techniques in infectious diseases, autoimmune diseases, oncology, and is now focused on the maternal and child health global challenge. He developed the NLME growth deceleration model and is leading the healthy birth growth and development knowledge integration (HBGDki) big data effort at the Bill & Melinda Gates Foundation.

Helen Morrison, PhD, *Group Leader, Leibniz-Institute of Age Research - Fritz Lipmann Institute, Jena, Germany*

Helen Morrison, Ph.D. is a group leader at the Leibniz-Institute of Age Research - Fritz Lipmann Institute (FLI) in Jena, Germany. She has served on several national and international review panels and has authored numerous peer-reviewed manuscripts. Her interest is the nature of cell communication, and the mis-wiring of signalling pathways in disease and in the ageing process. Specifically, her focus is on age dependent signalling impairments underlying nervous system maintenance and regeneration, and in disease mechanisms for disorders of myelinating cells and brain tumours. Dr. Morrison has extensive experience in NF2 research including dissecting NF2 signalling pathways.

Speaker Abstracts

OPTIONAL SATELLITE SESSION: Educational Symposium

Chairs: Robert Listernick, MD, *Ann and Robert Lurie Children's Hospital;* Michael Fisher, MD, *Children's Hospital of Philadelphia;* Jaishri Blakeley, MD, *Johns Hopkins University Medical Center*

Neurofibromatosis Type 1 and Scoliosis

Satellite Session: Saturday, June 10, 8:00am – 8:25am

Matthew Oentgen, MD, MBA, *Children's National Medical Center*

Spinal deformity associated with neurofibromatosis type 1 is a common finding in children and adolescents. The prevalence of this manifestation has been reported to be about 50%. While there are certainly a number of typical phenotypic changes that are associated with scoliosis in the setting of NF 1, the exact etiology of the spinal deformity is not known.

In general, scoliosis associated with NF 1 presents in two varieties. The first is called non-dystrophic scoliosis. This is more common and typically less severe then the second variety, dystrophic scoliosis. While both forms of scoliosis associated with NF 1 can occur, there is also a well described, but poorly understood association between these two manifestations, wherein non-dystrophic deformities can change into dystrophic deformities through a process called modulation.

Evaluation of spinal deformity in children with NF 1 is key, as early recognition and frequent re-examination allows for clear identification of progressive deformity or modulation, two key elements in determining the treatment of the scoliosis.

Posterior spinal fusion is the standard of care for deformities that show progression in magnitude. Current surgical techniques allow for safe and effective spinal deformity correction and successful long-term outcomes.

Tibial Dysplasia in Neurofibromatosis

Satellite Session: Saturday, June 10, 8:25am – 8:50am

B. Stephens Richards, MD, *Texas Scottish Rite Hospital for Children, Univ. Texas Southwestern, Dallas TX*

Patients with NF1 have an increased risk of bone complications, including tibial dysplasia. This dysplasia is a congenital deficiency which makes the tibial bone prone to fracture followed by non-union of the fracture site (known as tibial pseudarthrosis). As many as 5% of NF1 patients may experience this condition. Fractures can occur before the age of one year, or anytime during the growing years leading to significant problems in those affected. A specific bi-allelic abnormality in the NF1 gene has been found in the tissues at the fracture site. Rarely will this fracture heal with conservative management because of the deficient bone formation and increased bone resorption that occurs at the fracture site. As such, successful treatment to achieve healing is very challenging. Cast immobilization is the initial means used in an effort to gain union in this fracture but, realistically, surgical intervention is almost always needed once a fracture has occurred.

The most common surgical treatment includes rigid immobilization of the fracture ends using an intramedullary rod followed by the addition of autogenous bone graft obtained from the patient's iliac crest region. Other forms of treatment have included the use of external fixation to immobilize the bone and, in few cases of this disorder, a vascularized fibular graft is interposed in the tibia region after the diseased portion of the bone is removed. Occasionally, if several surgical attempts to achieve healing have not been successful, an amputation through the fracture site followed by a below-knee prosthesis is needed.

In recent years, additional biological materials have been added to the surgical treatment in an effort to supplement the autogenous bone grafting. Bone morphogenetic protein (rhBMP-2) is a protein useful in enhancing bone formation. Because this product has not yet gained FDA clearance for routine use in children, it must be used in a physician-directed manner. There have been several reports in the medical literature suggesting a beneficial effect of this material. To gain a better understanding of the effect of rhBMP-2 in this condition, a multicenter NF1 Consortium therapeutic trial is currently underway.

Cerebral Vasculopathy in the Pediatric NF1 Population

Satellite Session: Saturday, June 10, 9:00am – 9:20am

Tena Rosser, MD, *Associate Professor of Clinical Neurology, University of Southern California*

Cerebral vasculopathy is a significant but under-recognized complication of NF1 in childhood. Neurofibromin, the protein product of the NF1 gene, is expressed in the endothelium of blood vessel walls. It has been hypothesized that the loss of neurofibromin expression in these cells results in vascular smooth muscle proliferation. In NF1, the arterial system is more commonly affected than the venous system. Vessels of all sizes may be involved with the underlying pathology typically demonstrating hyperplasia of the intimal wall with luminal narrowing. While the literature is sparse and primarily based on retrospective studies, the prevalence of NF1-associated cerebral arteriopathy in childhood has been reported to be 2.5 - 7.4%. A variety of vascular lesions have been reported in children with NF1 including occlusion, aneurysms, ectasia, stenosis, fistula, arteriovenous malformation and rupture with Moyamoya syndrome being the most common and well-known. Many children with NF1-associated vasculopathy remain asymptomatic but arteriopathy can be progressive and affected children are at risk for strokes resulting in focal neurologic deficits. Early identification and close monitoring for cerebral vasculopathy can lead to medical interventions such as anti-platelet therapy and surgical revascularization procedures which may limit the morbidity and improve the long-term outcome associated with these lesions. However, there is little consensus on the use of screening MRI and MRA imaging in asymptomatic children with NF1 or on the management of pediatric NF1-associated vasculopathy. This talk will address our current knowledge of the pathophysiology, prevalence, neuroimaging findings and intervention options for pediatric NF1-associated vasculopathy.

Neurovascular Disease in Adults with NF1

Satellite Session: Saturday, June 10, 9:20am – 9:40am

Paul Holmes, MD, *Consultant Neurologist, Guy's and St. Thomas' Hospital, London, UK*

Neurofibromatosis type 1 (NF1) is an autosomal dominant tumour suppressor syndrome with a birth incidence of about 1 in 3000, making it the most common neurocutaneous disorder, and among the most common neurogenetic disorders.

Along with a predisposition to certain types of neoplasms, NF1 is additionally associated with an incompletely understood vasculopathy. Vascular abnormalities seen include a moyamoya arteriopathy, an increased frequency of incidental intracerebral aneurysms and stenotic or ectatic cerebral vessels. There appears to be an increased risk of ischaemic and hemorrhagic stroke in affected individuals.

In our population of NF1 patients, we have also seen an increased rate of small vessel intracranial cerebrovascular disease. NF1 predisposes to pheochromocytoma and renal artery stenosis, both of which cause secondary hypertension, a major risk factor for stroke and silent small vessel cerebrovascular change. Somewhat surprisingly, we found more incidental aneurysms than anticipated. Conversely, an autopsy study of 25 subjects did not demonstrate a significant increase in intracranial aneurysm in NF1 patients.

In my talk, I hope to expand on the possible pathophysiology of the vasculopathy in NF1, illustrate the types of vascular pathologies we have seen and discuss their investigation and management in the clinical setting.

From GEMMs to PDXs to Avatars, Mouse Models in Discovery and Translational Research

Satellite Session: Saturday, June 10, 10:10am – 10:30am

Karlyne Reilly, PhD, *Rare Tumors Initiative, Center for Cancer Research, NCI, Bethesda, MD*

Mouse models have been a critical tool in NF1 research for over 2 decades. Sophisticated mouse models of NF1 tumors are beginning to guide clinical trials in a predictive way, and much of what we understand about the importance of heterogeneity in NF1 tumors can from studies in mice. Given the complexity of NF1 tumors, there is still plenty that can be learned from developing new NF1 mouse models. This presentation will give an overview of mouse modeling, the types of mouse models that can be used for different types of research, and limitations and pitfalls of using mice to study human diseases.

Signalling Pathways in NF2: A Primer

Satellite Session: Saturday, June 10, 10:30am – 10:50am

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

The neurofibromatosis type 2 gene located on chromsomome 22q12.2 encodes the merlin tumor suppressor. Loss of merlin function in Schwann cells and arachnoidal cells leads to schwannoma and meningioma formation respectively. Merlin is increasingly recognized to function as an adaptor protein and thus able to modulate activity of signaling pathways regulating cell morphology, proliferation and survival. This presentation will highlight key merlin-dependent signaling cascades that contain druggable targets such as EGFR, MEK, FAK, PAK, and YAP, signaling.

Neuro-ophthalmologic Manifestations of NF2

Satellite Session: Saturday, June 10, 11:00am – 11:20am

Grant Liu, MD, *University of Pennsylvania, Children's Hospital of Philadelphia*

Neuro-ophthalmic manifestations of NF2 include posterior subcapsular cataracts, epiretinal membranes, and astrocytic hamartomas of the retina. In addition, ocular motor nerves can be affected by schwannomas, and facial palsies following acoustic neuroma surgery can lead to corneal exposure. Anatomy of the eye and the ocular motor nerves will be reviewed.

Neurofibromatosis Type 2 in Childhood

Satellite Session: Saturday, June 10, 11:20am – 11:45am

Scott Plotkin, MD, PhD, *Massachusetts General Hospital/Harvard Medical School*

Neurofibromatosis type 2 (NF2) is an autosomal neurogenetic syndrome predisposing affected individuals to schwannomas and meningiomas. Diagnosis of NF2 in children is challenging as they may not have a family history of NF2 or features commonly associated with the condition (such as bilateral vestibular schwannomas). A pediatric presentation of NF2 occurs in about 20% of individuals. Unlike adults with NF2 who typically present with dysfunction of the eighth cranial nerve, children with NF2 commonly present with neurologic symptoms related to an isolated schwannoma or meningioma (seizure, myelopathy), dermatologic manifestations (limited cafe-au-lait macules, cutaneous schwannoma), or ophthalmologic manifestations (cataract, strabismus, amblyopia). Children with NF2 typically have a more severe phenotype marked by heavy tumor burden and progressive neurologic dysfunction. Management in a dedicated NF2 center is desirable to optimize outcomes. Routine surveillance includes MRI scans of the brain and spinal cord, ophthalmology, and audiology with additional testing of any abnormalities on history or examination. Clinical management of progressive vestibular schwannomas, meningiomas, and ependymomas is most commonly surgical. Surgical resection of vestibular schwannoma in expert centers is associated with hearing loss in about 50% of children. Post-operative neurologic deficits may occur and lead to impaired quality of life. The role of radiation is limited for children with NF2 given the risk of adverse outcomes for this age group. However, radiation may be useful for progressive lesions that are not amenable to surgery or other modalities. Clinical trials of bevacizumab, lapatinib, and everolimus for treatment of NF2-related tumors have been published. Conclusions about drug efficacy is limited at this time since pediatric patients represent a small percentage of participants in these trials. Clinical trials designed to prevent tumor formation or growth would be helpful in this population.

KEYNOTE 1: Stem Cells in Silence, Action and Cancer

Saturday, June 10, 1:00pm – 2:00pm

Elaine Fuchs, PhD, *Howard Hughes Medical Institute, The Rockefeller University, New York, NY*

Adult tissue stem cells have the ability to self-renew long term and differentiate into one or more tissues. Many stem cells are used sparingly to replenish cells during normal homeostasis. However, even stem cells that are quiescent must be able to respond quickly to injury in order to fuel rapid tissue regeneration. How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of cancers.

The skin is an excellent model system to understand how stem cells transition between quiescence and tissue regeneration. We are particularly interested in how stem cells become mobilized during normal tissue regeneration and wound repair, and how the normal process of stem cell activation goes awry in cancer. We've identified and characterized at a molecular level an important stem cell niche within the hair follicle, and using single cell sequencing, we've illuminated how dynamic niche-stem cell interactions choreograph tissue regeneration. We've also mapped the chromatin landscape of these stem cells while they reside quiescently in this niche, and elucidated how this changes when the stem cells become activated to proliferate and progress along their lineages during hair growth, and how the cells are able to contribute to regeneration of the epidermis in wound repair. We've discovered that when these stem cells are removed from their niche and placed in culture, they undergo marked chromatin remodeling with strong parallels to the mobilization of stem cells during a wound-response.

Hair follicle stem cells are known to be a source of squamous cell carcinomas (SCCs), which as a class, are one of the most common and life-threatening cancers world-wide. We've applied our knowledge of normal hair follicle stem cells to explore the tumor-initiating stem cells of SCCs, and study the relation between normal stem cells and malignant ones. These studies have shed light on the age-old adage that cancer is a wound that never heals. We've devised a method to mark, track and transcriptionally profile these tumor-initiating SCC cells in vivo, within the tumor, and seek the roots of how a small subset of stem cells within the cancer are able to evade chemotherapy. Our findings are now paving the way for new clinical strategies to treating this deadly cancer.

SESSION 1: Stem Cells and NF Tumor Biology

Chairs: Matthias Karajannis, MD, MS, *Memorial Sloan Kettering Cancer Center;* Peter deBlank, MD, MSCE, *Cincinnati Children's Hospital Medical Center*

Perspectives: The Cellular and Molecular Pathogenesis of *NF1* Optic Gliomas

Session 1: Saturday, June 10, 2:15pm – 2:50pm

David Gutmann, MD, PhD, *Donald O. Schnuck Family Professor, Vice Chair for Research Affairs, Department of Neurology; Director, Neurofibromatosis Center; Washington University School of Medicine, St. Louis MO*

The most common brain tumor arising in children with neurofibromatosis type 1 (NF1) is the optic pathway glioma. These tumors can arise anywhere along the extent of the optic pathway, but most commonly involve the optic nerves and chiasm. When symptomatic, children with these low-grade gliomas present with reduced visual acuity. Due to their location, these tumors are rarely biopsied or removed, limiting our ability to define the cellular and molecular determinants that underlie disease pathogenesis in humans. For this reason, we have developed numerous *NF1* genetically-engineered mouse models of optic glioma over the past 15 years, and used these strains to identify new treatments that inhibit tumor growth and attenuate further vision loss, as well as to dissect the impact of the germline *NF1* gene mutation, cooperating genetic mutations, sex, the cell of origin, and the timing of somatic *NF1* loss on optic glioma formation and progression. Moreover, we have identified a population of optic glioma stem cells which provide a tractable platform for *in vitro* and *in vivo* translational research investigations. In this presentation, recent advances in these areas will be highlighted.

Presenting on behalf of the members of the Gutmann laboratory

Roots of Neurofibromas

Session 1: Saturday, June 10, 2:50pm – 3:15pm

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

The dermal neurofibromas are composed of a variety of cell types displaying different gene expression profiles and ultrastructural characteristics. These cells are embedded in a voluminous extracellular matrix which gives neurofibromas their characteristic rubbery consistency. Even though cutaneous neurofibromas never turn malignant and do not pose a threat to life, they create the main disease burden in most adult patients with NF1. The understanding of the development of neurofibromas is the key in attempts to control the proliferation of cells and the deposition of extracellular connective tissue.

The traditional view sees the development of neurofibromas as a process of disruption of small nerve tributaries of the skin and subsequent proliferation of the resident cells. More recent studies utilizing gene-manipulated mice and cells cultured from human neurofibromas suggest that multipotent precursor cells may play a crucial role in the tumor initiation and in the development of neurofibromas with their astonishingly numerous cellular phenotypes. Also e.g. mast cells are recruited to neurofibromas. A second hit on the NF1 gene has been detected in a subpopulation of tumor Schwann cells (NF1-/-), but not in other cell types in human neurofibromas.

Our recent studies have concentrated on co-cultures of NF1-/- Schwann cells and neurofibroma-derived multipotent precursor cells, NFPs. The results reveal complex phenomena and interactions between the two cell types, and in part help to elucidate the origin of neurofibromas. Being only partially understood, the origin and growth of neurofibromas apparently involve somatic mutations of the NF1 gene, and endocrine, paracrine, and juxtacrine regulation of cellular differentiation and matrix gene expression.

Full List of Authors: Eeva-Mari Jouhilahti PhD, Paula Pennanen MSc, Roope Kallionpää MSc, Jaakko Pullinen MD, University of Turku; Sirkku Peltonen MD PhD, University of Turku and Turku University Hospital.

Grants: Academy of Finland, Cancer Society of Finland, Finnish Cultural Foundation, Turku University Foundation, Turku University Hospital, Emil Aaltonen Foundation

Short Term MEK-inhibitor Treatment Prevents Optic Nerve Glia Pathology in an NF1-deficient Mouse Model

Session 1: Saturday, June 10, 3:15pm – 3:35pm

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

Up to 20% of patients with neurofibromatosis 1 (NF1) are diagnosed with optic pathway gliomas (OPGs) before 7 years of age, suggesting these tumors are formed early in development. During optic nerve (ON) development, two distinct glial populations ($Olig2^+$ oligodendrocytes and $GFAP^+$ astrocytes) arise from different progenitor cells to populate the nerve. The goal of study is to characterize differences in the glial cell populations that precede OPG development, and develop a targeted treatment that can improve/prevent these abnormalities.

Using a well-established *NF1*$^{-/-}$ mouse model (hGFAPcre;*Nf1*flox/flox) that develops OPGs around postnatal day 60 (P60), we characterized the glial cell populations ($Olig2^+$ and $GFAP^+$ cells) and their proliferation rate ($Ki67^+$) during early postnatal development. The *NF1*$^{-/-}$ mice have increased numbers of both glial populations ($GFAP^+$ and $Olig2^+$ cells) throughout early postnatal development. Interestingly, we also observed an early presence of $Olig2^+$ cells in the *NF1*$^{-/-}$ proximal (near the eye) nerve, suggesting that an abnormal population of $Olig2^+$ cells is present very early during ON development. Using Ki67 as a marker for proliferation, we see an increase of proliferation at P0.5 but no other time point. Following treatment with a MEKi, the mutant nerve glial phenotype was rescued to the control level at P5.

These results provide support that the events preceding OPG development occur very early during ON development. Treatment with a MEKi during this critical time frame can improve the glial abnormalities seen in the *NF1*$^{-/-}$ mouse model, suggesting that early MEKi therapy, when used during a specific developmental time window, could potentially be used to prevent OPGs in NF1.

Platform: Differentiation of Plexiform Neurofibroma (PNF)-derived *NF1*(+/-) and *NF1*(-/-) iPS Cells into Schwann Cells Mimicking Primary PNF Cells: Closing the Circle

Session 1: Saturday, June 10, 3:35pm – 3:55pm

Eduard Serra, PhD, *The Institute for Health Science Research Germans Trias i Pujol (IGTP) - Program of Predictive and Personalized Medicine of Cancer (PMPPC)*

We have generated several *NF1*(+/-) and *NF1*(-/-) iPS cell lines directly from plexiform neurofibromas (PNFs) that developed in different NF1 patients. Despite the high proliferation capacity of iPS cells, *NF1*(-/-) iPSC exhibited around 15% increase in proliferation rate compared to WT iPS cells, an increase not shown by *NF1*(+/-) iPS cells. Using control embryonic stem (ES) and iPS cell lines we adopted and set up a robust protocol for generating Neural Crest Stem Cells (NCSC) that was later applied to PNF-derived iPSC. Expression of specific neural crest markers was detected in control cell lines and in PNF-derived iPS cells, both by immunofluorescence (p75, AP2, Sox10) as well as by FACS analysis (p75) and RT-qPCR. We were able to expand and cryo-preserve batches of NCSC while maintaining their NCSC identity. FACS analysis revealed a higher degree of heterogeneity regarding p75 expression in the PNF-derived NCSC cells compared to controls. We then set up conditions to successfully differentiate NCSC towards the Schwann cell (SC) lineage, up to SCs able to express myelin. Different steps of *in vitro* differentiation were characterized, observing expression patterns along time that recapitulated those of *in vivo* differentiation during embryo development. SC commitment was monitored by expression analysis of specific SC markers such as s100, p75, myelin basic protein (MBP) and others, using different techniques. Along with SC differentiation, control NCSC progressively slowed the proliferation rate, while *NF1*(-/-) NCSC remarkably maintained a high proliferation capacity. Moreover, *NF1*(-/-) differentiating cells, in addition to being able to grow in monolayer, had a high tendency to form large spheres in culture plates, visible at nude eye, that did not require attachment. Cells within these spheres expressed p75 and S100 levels as primary PNF-derived cells. We have developed a non-perishable cell culture model useful for PNF research, closing the circle that goes from primary PNF SCs, through iPSC, NCSC and finally again to SC resembling PNF primary SCs.

Meritxell Carrió PhD, IGTP, Yvonne Richaud BS, Center For Regenerative Medicine in Barcelona (CMRB), Ernest Terribas PhD, IGTP, Helena Mazuelas MS IGTP, Bernat Gel, PhD, IGTP, Senda Jimenez-Delgado PhD, CMRB, Imma Rosas, IGTP, Josep Biayna PhD, IGTP, Leen Vendredy BS, IGTP , Elisabeth Castellanos PhD, IGTP, Ignacio Blanco MD PhD Germans Trias i Pujol Hospital (HUGTiP), Conxi Lázaro PhD, Catalan Institute of Oncology (ICO), Ángel Raya MD, PhD, CMRB

Funding: Neurofibromatosis Therapeutic Acceleration Program at Johns Hopkins (NTAP)

Platform: Cancer Stem Cell as the Target for MPNST Tumorigenesis and Relapse

Session 1: Saturday, June 10, 3:55pm – 4:15pm

Daochun Sun, PhD, *Brain Center, Memorial Sloan Kettering Cancer Center, New York, NY*

Malignant peripheral nerve sheath tumor (MPNST) is a type of soft tissue sarcoma that arises from the neural crest lineage and commonly associated with nerve trunks. Surgical removal is the major treatment conjugated with chemo/radiation therapy when applicable. However, the complete tumor clearance is limited by the resectability, especially when associated with large peripheral nerve. The local recurrence of MPNST is 40∼45% with high morbidity.

We previously harnessed a specific transgene, using the components of rat endogenous *Nestin* promoter and enhancer, to find a subset of glioblastoma (GBM) cells that are relatively quiescent and show cancer stem cell properties. This transgene has herpes simplex virus thymidine kinase gene (*TK*) and green fluorescent protein (*Nes-TK-GFP*), and labels a small quiescent cell population in MPNSTs from both *cisNF1*$^{+/-}$*;Trp53*$^{+/-}$ (*cisNP*) spontaneous model and skin progenitor (SKP) based allograft model. Ganciclovir chow treatment kills the dividing cells expressing the transgene and significantly decreases the tumor growth in both models. We also found that the transgene labeled GFP positive cells enrich in the sciatic nerve region compared to the distal tumor mass in MPNST allografts. Tumor primary culture from the sciatic nerve vicinity forms more and significantly larger spheres *in vitro* than the distal tumor mass. The BrdU and EdU sequential labeling assay after chemotherapy regimens in MPNST allografts shows that the GFP positive cells can continuously repopulate the tumor in a hierarchical organization. These results indicate that the transgene labeled cells may serve as CSCs to propagate tumor growth and relapse after the chemotherapy, which provides a novel therapeutic strategy to MPNST.

Author List: Zilai Wang PhD, Elsa Vera PhD, Sameer Farouk Sait MD and Luis Parada PhD, Memorial Sloan Kettering Cancer Center

Funding: Department of Defense Congressionally Directed Medical Research Programs

KEYNOTE 2: Identifying Tumor Vulnerabilities Through Integrated Analysis

Sunday, June 11, 9:00am – 10:00am

Michael A. Dyer, PhD, *Investigator, HHMI; Richard C. Shadyac Endowed Chair in Pediatric Cancer Research; Member, Department of Developmental Neurobiology; Co-Leader, Developmental Biology and Solid Tumor Program; Head, Division of Developmental Biology, St. Jude Children's Research Hospital, Memphis, TN*

Personalized cancer therapy based on the somatic mutations identified in patient tumors is becoming an increasingly emphasized approach to improve outcomes of patients with cancer. There are also examples of therapeutic vulnerabilities in cancer that result from changes in gene expression that are a direct or indirect result of tumor specific epigenetic perturbations. These genomic and epigenomic changes are ultimately manifestations in the tumor proteome and phosphoproteome. In this study, we integrated genomic, epigenomic and proteomic data for rhabdomyosarcoma (RMS) to identify therapeutic vulnerabilities. RMS was selected for this analysis because RAS pathway mutations in rhabdomyosarcoma (RMS) are the most common potentially actionable lesions across pediatric solid tumors. The epigenomic data was useful for identifying deregulated developmental pathways in RMS including the WNT, HH, BMP, adenyl cyclase, p38/MAPK and PI3K pathways. Perturbations in those 6 myogenic signal transduction pathways were also evident in the proteome and phosphoteome data. In addition, the proteomic/phosphoproteomic data revealed that the cell cycle checkpoint, unfolded protein response and RB/E2F pathways were deregulated in RMS relative to normal muscle. Recent success targeting CDK4/6 and MEK in adult cancers with RAS mutations led us to test the value of these targets in RMS tumors. We also targeted the unfolded protein response pathway and cell cycle checkpoint pathway using molecular targeted therapeutics in orthotopic patient derived xenografts. Taken together, these data demonstrate the value of integrating epigenomic and proteomic data to identify tumor vulnerabilities that extend beyond somatic mutations identified in the genome.

PLENARY TALK: Mind the Research Science - Data Science Gap

Sunday, June 11, 10:30am – 11:00am

Shasha Jumbe, PhD, *Senior Program Officer, Quantitative Sciences, Bill & Melinda Gates Foundation*

Data scientists approach their work very differently from research scientists. These differences — in preferred methods and tools, but also in underlying assumptions about the purpose of data analysis and what it can reasonably achieve — can lead to miscommunication and misunderstanding, and if unresolved, can create significant barriers to effective collaboration. This collaboration challenge is exacerbated in large-scale, distributed collaborations like the Bill & Melinda Gates Foundation Healthy Birth, Growth and Development (HBGD) Knowledge Integration Initiative (HBGDki), due to the complexity of the problem being addressed and the many different disciplines necessarily involved to quantify and integrate interdisciplinary frame of reference toward a common conceptual framework to eradicate childhood stunting and improve childhood development outcomes. This discussion bares all along the HBGDki data to discovery and decisions journey, and communication lessons learned — about how to form and ask the right questions about data, about analysis techniques, about results and about quantifying how and what we are learning.

SESSION 2: Genes, Genotypes and Phenotypes

Chairs: Ludwine Messiaen, PhD, *University of Alabama at Birmingham;* Miriam Smith, PhD, *University of Manchester, UK*

Perspectives: Towards Genome-Guided Therapy for NF1

Session 2: Sunday, June 11, 11:00am – 11:30am

Bruce R. Korf, MD, PhD, *Department of Genetics, University of Alabama at Birmingham, Birmingham, AL*

Most efforts to date aimed at development of medical treatments for NF1 have focused on use of small molecules to block Ras signaling, intercellular communications, or tumor angiogenesis. These are appropriate targets given what is known about the pathogenesis of various lesions associated with NF1. An alternative approach, which has been used with success in other genetic disorders, such as cystic fibrosis, spinal muscular atrophy, or Duchenne muscular dystrophy, is to restore function to the mutated gene or gene product. More than 3,000 distinct pathogenic *NF1* mutations have been characterized, but these can be grouped into classes, such as whole gene deletion, missense mutations, frameshifts, nonsense mutations, splicing mutations, and intragenic deletions or insertions. Although no individual approach would successfully treat all patients by targeting specific mutations, it is possible that a group of different drugs could be developed that would target either specific mutations or specific classes of mutations. Our group has been developing reagents to enable screening and testing of compounds, including cell lines and animal models that include human *NF1* gene mutations. We have focused so far on nonsense read through drugs, but also are now looking at use of oligonucleotides to induce exon skipping and to screen for compounds that might correct the effects of missense mutations. These approaches may offer a useful adjunct to other treatment modalities based on blocking the Ras or other signaling pathways, and represent a potential “precision medicine” approach to treatment of NF1.

Mutations in Schwannomatosis

Session 2: Sunday, June 11, 11:30am – 11:50am

Laura Papi, MD, *University of Florence, Italy*

Schwannomatosis is primarily characterized by the development of multiple non-vestibular, non-intradermal schwannomas. Signs and symptoms of the condition vary based on the size, location and number of schwannomas and may include pain, numbness, tingling, and/or weakness. Inherited forms of the disorder account for only 15 percent of all cases. Constitutional inactivating variants in two genes, *SMARCB1* and *LZTR1* have been reported, but in many cases the exact underlying genetic cause is still unknown. 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 schwannomatosis. Furthermore, I will review the clinical phenotypes associated with mutations in *SMARCB1* and *LZTR1* versus the one in schwannomatosis due to unknown genetic defect.

Large *NF1* Deletions: Underlying Mutational Mechanisms and Genotype-Phenotype Relationships

Session 2: Sunday, June 11, 11:50am – 12:10pm

Hildegard Kehrer-Sawatzki, PhD, *Institute of Human Genetics, University of Ulm, Germany*

The most frequent recurring mutations in neurofibromatosis type 1 (NF1) are microdeletions encompassing the *NF1* gene and its flanking regions. The majority of these deletions, termed type-1, encompass 1.4-Mb are mediated by nonallelic homologous recombination. These deletions are associated with the loss of 14 protein-coding genes and four microRNA genes. Patients with germline type-1 *NF1* microdeletions frequently exhibit dysmorphic facial features, overgrowth/tall-for-age stature, significant delay in cognitive development, large hands and feet, hyperflexibility of joints and muscular hypotonia. These patients also have significantly more cardiovascular anomalies and often exhibit increased numbers of subcutaneous, plexiform and spinal neurofibromas as compared with the general NF1 population. Further, an extremely high burden of internal neurofibromas, characterized by >3000 ml tumour volume, is significantly more frequent in non-mosaic *NF1* microdeletion patients than in NF1 patients lacking such deletions. *NF1* microdeletion patients also have an increased risk of malignant peripheral nerve sheath tumours (MPNSTs); their lifetime MPNST risk is considerably higher than that of NF1 patients with intragenic *NF1* mutations. Co-deletion of the *SUZ12* gene in addition to *NF1* further increases the MPNST risk in *NF1* microdeletion patients. Although numerous studies have described the *NF1* microdeletion-associated phenotype, comprehensive analyses are still missing that would answer several open questions, e.g., whether pre-pubertal onset of growth of multiple cutaneous neurofibromas is significantly more prevalent in children with *NF1* microdeletions as compared to children with intragenic *NF1* mutations. In addition, a comparative analysis including a large number of age-matched adult patients is urgently required in order to ascertain whether high numbers of cutaneous neurofibromas (N > 1000) occur significantly more often in patients with *NF1* microdeletions than in patients with intragenic mutations. Furthermore, the influence of co-deleted genes on the phenotype in patients with non-mosaic *NF1* microdeletions is not well understood. The less frequent but recurrent type-3 *NF1* deletions spanning only 1.0-Mb are of great interest in this regard. Analyses of a larger number of patients with type-3 *NF1* microdeletions would be necessary to determine the influence of the *RNF135* gene on the overgrowth and dysmorphic facial features. Although the deletion of *SUZ12* may well predispose patients with *NF1* microdeletions to malignancy, the reasons for the disproportionately higher frequency of benign plexiform, subcutaneous and spinal neurofibromas in patients with *NF1* microdeletions is still unclear.

In my talk, I will summarize the current knowledge about the mechanisms underlying *NF1* microdeletions and the associated genotype-phenotype relationships.

Platform: Genotype-Phenotype Correlation in NF1 Patients: Evidence for a More Severe Phenotype Associated with Missense Mutations Affecting *NF1* Codons 844-848

Session 2: Sunday, June 11, 12:10pm – 12:30pm

Magdalena Koczkowska, PhD, *Department of Genetics, University of Alabama at Birmingham*

Neurofibromatosis type 1 (NF1) is characterized by a highly heterogeneous clinical presentation, both inter- and intrafamilial. Due to the complexity of the NF1 phenotype, its strong age-dependency and the wide *NF1* allelic heterogeneity, only three clinically relevant genotype-phenotype correlations have so far been reported; namely, individuals with a constitutional *NF1* microdeletion present with a large number of neurofibromas at an earlier age, dysmorphic features and developmental delay, whereas p.Met992del and p.Arg1809 are associated with a milder/distinct phenotype without externally visible cutaneous or plexiform neurofibromas.

We identified 148 patients (119 unrelated probands and 29 affected relatives) carrying a constitutional missense mutation affecting one of five neighboring codons Leu844, Cys845, Ala846, Leu847 and Gly848 in the *NF1* gene. None of these variants have been reported in control populations (1000Genomes, Exome Variant Server, ExAC). These recurrent missense mutations were identified in ~0.8% of unrelated *NF1*-positive probands in the UAB cohort. We compared the studied group with the cohort of individuals with missense mutations at p.Arg1809 (Rojnueangnit et al., 2015) and p.Met992del (Upadhyaya et al., 2007), as well as with cohorts of "classic" NF1 patients. Patients harboring a missense mutation affecting codons 844-848 tend to exhibit a more severe phenotype with a significantly higher incidence of plexiform and/or spinal neurofibromas, symptomatic optic pathway gliomas and bone abnormalities.

Our results clearly demonstrate that *NF1* missense mutations are not solely associated with a mild phenotype, but may have a more severe presentation. A novel genotype-phenotype correlation at the *NF1* AA844-848 exists and may be valuable in the management and genetic counseling of a significant number of NF1 patients.

References: Rojnueangnit et al., Hum Mutat. 2015; 36:1052-63; Upahyaya et al., Am J Hum Genet. 2007; 80: 140-51.

Author List: Magdalena Koczkowska[1], Yunjia Chen[1], Tom Callens[1], Jaqueisa S. Reaves[1], Alicia Gomes[1], Meng-Chang Hsiao[1], Angela Sharp[1], Bruce R. Korf[1], Eric Legius[2], Ludwine M. Messiaen[1] and collaborating physicians*

[1] Department of Genetics, University of Alabama at Birmingham, AL, USA; [2] Department of Human Genetics, KU Leuven, Leuven, Belgium; * the full list of collaborating physicians will be provided during the conference

Funding: Children's Tumor Foundation, Issac and Sadie Fuchs Genotype-Phenotype Study

SESSION 3: Non-Tumor Manifestations of NF

Chairs: Aaron Schindeler, PhD, *Children's Hospital at Westmead, AUS;* Nicole Ullrich, MD, PhD, *Boston Children's Hospital, Harvard University*

Dietary Intervention for NF1 Muscle Weakness

Session 3: Sunday, June 11, 1:30pm – 1:50pm

Aaron Schindeler, PhD, *Children's Hospital at Westmead, AUS*

Children with NF1 can present with reduced muscle size, global muscle weakness, and impaired motor control, which can have a significant impact on quality of life. Analysis of genetic mouse models of NF1 has shown evidence for a metabolic myopathy, which we now report is recapitulated in muscle biopsies from n=6 individuals with NF1. Moreover, abnormal intramyocellular lipid accumulation was inversely correlated with neurofibromin expression by western analysis. Lipidomics analysis of $NF1^{null}$ muscle from $NF1_{MyoD}^{-/-}$ mice indicated a significant increase in neutral lipids and cholesterol esters containing long-chain fatty acids. The $NF1_{Prx1}^{-/-}$ mice lacking *NF1* in mesenchymal tissues of the limb were found to recapitulate myopathic features seen in the $NF1_{MyoD}^{-/-}$ mouse muscle and *NF1* human muscle biopsies. These mice were placed from weaning on a modified diet enriched for short- and medium-chain fatty acids and supplemented with L-carnitine. Following 8 weeks of dietary intervention, $NF1_{Prx1}^{-/-}$ mice showed a 45% increase in maximal grip strength, and a 71% reduction in intramyocellular lipid compared with littermates fed standard chow. These data provide evidence for a lipid storage myopathy underlying muscle weakness in NF1, and proof of principle that dietary modification can ameliorate this condition.

Aaron Schindeler [1,2§], Matthew A Summers [1,2], Thusitha W T Rupasinghe [5], Emily R Vasiljevski [1,2], Frances J Evesson [3], Kathy Mikulec [1], Lauren Peacock [1], Kate G Quinlan [4], Sandra T Cooper [2,3], Ute Roessner [5], David A Stevenson [6], David G Little [1,2]

[1] Orthopaedic Research & Biotechnology, The Children's Hospital at Westmead, Westmead, NSW, Australia.
[2] Discipline of Paediatrics & Child Heath, Faculty of Medicine, University of Sydney, Camperdown, NSW, Australia
[3] Institute for Neuroscience and Muscle Research, The Children's Hospital Westmead, Sydney, NSW, Australia
[4] School of Biotechnology and Biomolecular Sciences, University of New South Wales, NSW, Australia.
[5] Metabolomics Australia, University of Melbourne, VIC, Australia.
[6] Division of Medical Genetics, Stanford University, Stanford, CA, USA.

Second Hits in NF1 Scoliosis

Session 3: Sunday, June 11, 1:50pm – 2:10pm

David Stevenson, MD, *Stanford University*

The etiology of dystrophic scoliosis in NF1 is not fully understood. Somatic mutations in *NF1* have been shown in tibial pseudarthrosis tissue, providing rationale for similar processes in other NF1-associated skeletal maladies such as dystrophic scoliosis. This is supported by conditional *Nf1* mouse models where double inactivation of *NF1* in osteoprogenitor cells can lead to skeletal phenotypes reminiscent of humans with NF1. Hypotheses for dystrophic scoliosis in NF1 include biomechanical forces of an adjacent plexiform neurofibroma, intrinsic bony dysplasia from somatic mutation of osteoprogenitor cells, and potential paracrine effects.

In order to investigate the pathophysiology of dystrophic scoliosis in NF1, we obtained tissues from individuals with a clinical diagnosis of NF1 and dystrophic scoliosis. Samples from the vertebral region during surgical procedures with matched peripheral blood of individuals with NF1 were obtained and DNA extracted. Next generation sequencing (NGS) of various vertebral sections as well as a peripheral blood sample were performed using a Rasopathy gene panel inclusive of the NF1 gene. Variants were compared between affected tissue and the germline blood data. In addition, the NGS allele frequencies were used to detect somatic loss of heterozygosity (LOH).

Blood samples identified the *NF1* germline mutations in the 3 cases. Two of the individuals demonstrated an alleleic imbalance inclusive of *NF1* for the NGS data, indicating a somatic deletion or copy neutral LOH of the NF1 gene. The percent of the germline mutation in the vertebral tissue samples was increased. Microarray analysis verified somatic (copy neutral) LOH in these two vertebral samples.

These results support that dystrophic scoliosis in NF1 is due to a primary bone dysplasia and/or paracrine effect of cells with complete inactivation of *NF1,* rather than mechanical forces from large plexiform neurofibromas. Our data do show mosaicism in the samples in which DNA was obtained suggesting a mixed cellular popluation. Although a somatic *NF1* mutation was documented in the resected tissue, the cellular origin and timing of the somatic event is still in question. It is possible that there is a paracrine effect from array of different cell types with complete loss of *NF1* adjacent to bone structures, but our data in combination with conditional *NF1* mouse models showing segmental vertebral fusion anomalies and distorted vertebral bone microarchitecture, suggest that somatic *NF1* second hits of osteoprogenitor lineages are also key in the development of dystrophic scoliosis.

Neuropathies

Session 3: Sunday, June 11, 2:10pm – 2:30pm

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

This talk will focus on the occurrence of neurofibromatosis-associated neuropathies. Neurofibromatosis (NF) comprises of a group of diseases - NF1, NF2 and schwannomatosis - they are distinct genetic conditions with related clinical features predisposing individuals to glial-cell-derived tumours. Independent of tumour compression, neurofibromatosis-associated neuropathies frequently occur increasing the overall neurologic disability of these patients. However, while neuropathies are a common and clinically important feature in NF the underlying pathogenesis of these non-tumour manifestations is not entirely known. I will summarize the latest basic research findings, as well as clinical observations, in respect to neurofibromatosis-associated neuropathies. I will then focus in detail on NF2-associated neuropathies. I will show that merlin haploinsufficiency in neurons not only contributes to atrophic damaged axons leading to neuropathic symptom.

Helen Morrison[1], Alexander Schulz[2], Victor-Felix Mautner[3] and Said Farschtschi[3]

[1]Leibniz Institute on Aging, Fritz Lipmann Institute, 07745 Jena, Germany
[2]Department of Genetics and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT 06510, USA
[3]Department of Neurology, University Medical Center Hamburg-Eppendorf, University of Hamburg, Martinistrasse 52, 20246 Hamburg, Germany

Platform: The Reduced Osteogenic Differentiation Potential of NF1-Deficient Osteoprogenitors is EGFR-Independent

Session 3: Sunday, June 11, 2:30pm – 2:50pm

Seyedmohammad Ebrahim Tahaei, PhD Candidate, *Pharmacology Department, Vanderbilt University*

Recalcitrant bone healing following fracture (pseudarthrosis, PA) is one of the most problematic pediatric skeletal complications associated with NF1. The etiology of this condition is still unclear; thus, pharmacological options for clinical management are limited to off-label use of BMP. Multiple studies have shown that *NF1*-deficient osteoprogenitors are characterized by a reduced response to osteogenic cues. Recent expression profiling of cells cultured from the pseudarthrotic sites of children with NF1 PA (shown to harbor somatic double hit mutations in *NF1*) compared to cells cultured from iliac crest (haploinsufficient for *NF1* mutations) revealed that *EREG* and *EGFR,* encoding epiregulin and its receptor Epidermal Growth Factor Receptor, respectively, were significantly over-expressed in NF1 PA cells. Because EGFR stimulation is known to inhibit osteogenic differentiation, we hypothesized that chronic EGFR stimulation in *NF1*-deficient skeletal progenitors contributed to their reduced osteogenic differentiation potential. In this study, we confirmed using single-cell RNA sequencing of human bone-derived cells that presence of a *NF1* second hit somatic mutation is associated with increased *EREG* expression, whereas *TGFβ1* expression was unchanged. We then show that this molecular signature is conserved in mouse bone marrow stromal cells deficient for *NF1;* however, inhibiting EGFR signaling using Poziotinib or AG-1478, or blocking the epiregulin ligand, did not correct the differentiation defect of *NF1*-deficient bone marrow stromal cells. From a translational point of view, these results suggest that available pharmacological strategies aimed at inhibiting EGFR signaling are unlikely to ameliorate the osteogenic deficiency of NF1 PA osteoprogenitors and are unlikely to promote bone union in children with NF1 PA. These results also emphasize the need for more efforts to identify the mechanism whereby *NF1* deficiency inhibits osteogenic differentiation and bone repair.

Seyedmohammad Ebrhaim Tahaei, Ph.D. candidate, Vanderbilt University; Greig Couasnay, Ph.D., Baylor College of Medicine; Nandina Paria, Ph.D., Texas Scottish Rite Hospital for Children; Jinghua Gu, Ph.D., Baylor Scott & White Research Institute; Benjamin F. Lemoine, M.S., Baylor Scott & White Research Institute; Xuan Wang, Ph.D., Baylor Scott & White Research Institute; Jonathan J. Rios, Ph.D., Texas Scottish Rite Hospital for Children, and Florent Elefteriou, Ph.D., Baylor College of Medicine.

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Platform: Clinically Significant Neuropathy in NF2 - Frequency, Clinical and Neurophysiological Characteristics in 33 of 175 Patients Attending a National NF2 Service

Session 3: Sunday, June 11, 2:50pm – 3:10pm

Victoria Williams, MD, *Neurofibromatosis Centre, Department of Neurology, Guy's and St. Thomas' NHS Foundation Trust, Guy's Hospital, London, UK*

Background: Generalised axonal neuropathy has previously been described in neurofibromatosis type 2 (NF2)[12], but there are no published large systematic studies. Neuropathy is of clinical importance as the signs may overlap with other NF2 pathology, for example cauda equina lesions, and affect assessment and management.

Aim: To identify clinically important generalised peripheral neuropathy in people with NF2.

Method and Results: We reviewed the patient records of 175 sequential patients attending the Guy's Hospital Specialist NF2 clinic between 2010 and 2016. 80 were female, 95 male, median age 39 years, age range 8-80 (19 < 18 years old). 85 patients had nerve conduction studies (NCS) because of clinical suspicion of peripheral nerve pathology; 46 female, 39 male, median age 38 years, age range 10-41.

43/85 NCS (51%) were abnormal, 9 of these showed evidence of focal nerve or plexus pathology, one was consistent with myotonic dystrophy (subsequently confirmed genetically) and were excluded from the study.

There were 33 individuals with NCS-confirmed generalised axonal polyneuropathy, representing 19% of all 175 patients seen in the clinic. 27 individuals had distal sensorimotor symptoms suggestive of neuropathy, 6 were asymptomatic but investigated due to absent reflexes. On NCS, 13 were purely sensory, 20 were sensory and motor. 15 were female, 18 male, age range at diagnosis was 18-74 years, median 43. 9/33 (27%) had a severe mutation. MRI evidence of lumbosacral root pathology was twice as common in the neuropathy group.

Discussion: In our National Centre, seeing adults and children with NF2, 19% of 175 cases had clinically significant generalised axonal polyneuropathy. This was only seen in adults and seems to be more common in patients with higher disease burden, more severe genotype and MRI evidence of lumbosacral schwannomas. It is important to distinguish neuropathy from cauda equina lesions in order to inform treatment decisions.

[1] Brain (2002) 125 (5): 996-1004, [2] Neuropathology 2010; 30, 515–52

Full List Authors: Christopher Curry MD, Shazia Afridi MD, Rosalie Ferner MD Neurofibromatosis Centre, Department of Neurology, Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London, UK.

Platform: Resting Metabolic Rate in Neurofibromatosis Type 1: Indirect Calorimetry Versus Predictive Equations

Session 3: Sunday, June 11, 3:10pm – 3:30pm

Rezende Nilton, MD, PhD, Juliana Souza, MD, PhD presenting, *Neurofibromatosis Outpatient Reference Center, Federal University of Minas Gerais, Brazil*

Introduction: Indirect calorimetry (IC) is the gold standard method to assess individual resting metabolic rate (RMR). However, due to its high cost and time demand, predictive equations are largely used to estimate energy requirements, which may vary according to different body compositions and health status. In this regard, neurofibromatosis type 1 (NF1) patients are a special group to be assessed.

Aim: To measure RMR, using IC, in adults with NF1 and verify the most appropriate predictive equation to estimate ER.

Methods: 26 individuals with NF1, 14 male, 12 female, aged over 18 years underwent nutritional assessment, including weight, height, body mass index (BMI). Body composition was measured by dual energy x-ray absorptiometry (DXA). RMR was measured by IC (mRMR) and predicted by eight different equations (pRMR): Harris-Benedict, FAO/WHO (only weight), FAO/WHO (weight and height), Schofield, Henry and Rees, Cunningham(1980), Cunningham(1991), Mifflin-St.Jeor. Statistical analysis were carried out by Kolmogorov-Smirnov Test, Student's *t* test, Pearson correlation and Bland and Altman plots.

Results: The mean age was 34.3 ± 6.1 years of age. The mean mRMR was 1633.9 ± 471.1 kcal, and the pRMR ranged from 1244.6 ± 239.9 kcal to 1519.9 ± 271.1. There was a positive correlation between mRMR and weight ($r=0.864$; $P<0.001$), muscle mass ($r=0.885$; $P<0.001$), bone mass ($r=0.772$; $P<0.001$), height ($r=0.653$; $P<0.001$) and fat mass ($r=0.514$; $P=0.007$). There was no correlation with body fat percentage ($r=-0.124$; $P=0.546$). The best RMR predictive equation for individuals with NF1 was the FAO/WHO equation (which encompasses weight and height) with the smaller difference (although significant; $P=0.041$), better median of adequacy (92.0%) and greater percentage of accuracy (46.2%). The next best equations were the FAO/WHO including only weight and the Schofield. All the eight equations tested underestimated the RMR.

Conclusion: This study showed that all the eight predictive equations evaluated underestimated RMR in NF1 individuals (with large differences and low accuracy when compared to a gold standard method). Further studies should investigate more suitable methods to determine the energy requirements for this population.

Full List Authors: Souza MLR, MSc; Jansen AK, PhD; Vilela DLS; Martins AS, PhD; Correia MITD, PhD MD; Souza JF, PhD MD; Rodrigues LOC, PhD MD; Rezende NA, PhD MD. *(Federal University of Minas Gerais, Brazil)*

Granting agencies: CAPES and FAPEMIG [APQ-00928-11]

Platform: Assessment of Adiposity in Neurofibromatosis Type 1 By Dual Energy X-ray Absorptiometry Compared to Conventional Methods

Session 3: Sunday, June 11, 3:10pm – 3:30pm

Rezende Nilton, MD, PhD, Juliana Souza, MD, PhD presenting, *Neurofibromatosis Outpatient Reference Center, Federal University of Minas Gerais, Brazil*

Introduction: A simpler, non-invasive and cost-effective method for measuring adiposity would be useful in the care of individuals with neurofibromatosis type 1 (NF1). Dual energy X-ray absorptiometry (DXA) is considered the reference method for this assessment but it is not widely accessible in daily clinical practice.

Aim: To evaluate body composition in individuals with NF1, using DXA, compared to bioelectrical impedance analysis (BIA) and skinfold thickness (ST) predictive equations.

Methods: 26 individuals with NF1 (14 male) aged > 18 years, were submitted to nutritional assessment, including weight, height, body mass index (BMI). Body composition was measured by DXA (Hologic Discovery W). Body fat percentage (BF%) was also predicted using five ST-equations and four BIA-equations. Statistical analyses used: Kolmogorov-Smirnov, T of Student, Pearson correlation and Bland and Altman plots.

Results: The mean age and BMI were 34.3 ± 6.1 years and 23.9 ± 4.8 kg/m2, respectively, with no difference between males and females ($P=0.287$ and $P=0.207$). Using DXA, the BF% was 26.6 ± 7.3 and 37.4 ± 7.2 % for men and women, respectively. The best predictor of BF% was Sun et al. BIA-equation, with a smaller difference compared to DXA ($P=0.664$), better median of adequacy (101.0%) and accuracy of 46.2%. The next best equation was Kyle et al., also using BIA data. For males, Kyle et al. and Lohman (both BIA-equations) were the best predictors (78.6 and 64.3%, respectively). For females, all nine equations showed lower differences compared to DXA ($P<0.001$ for each equation), and Kyle et al. equation had greater percentage of accuracy (50%). Between ST-equations, Durnin and Womersley showed smaller difference, greater median of adequacy and percentage of adequacy (compared to DXA), even when stratified by gender.

Conclusion: This study showed that BIA-equations present better adequacy and accuracy compared to SK-equations. Nevertheless, these equations should be used with caution in this population due to the differences observed when compared to DXA. Validation or NF1-specific equations should be performed and tested by further studies.

Full List Authors: Souza MLR, MSc; Jansen AK, PhD; Vilela DLS; Kakehasi AM, PHD MD; Martins AS, PhD; Souza JF, PhD MD; Rodrigues LOC, PhD MD; Rezende NA, PhD MD. *(Federal University of Minas Gerais, Brazil)*

Granting agencies: CAPES and FAPEMIG [APQ-00928-11]

CONCURRENT SESSION 4A: CLINICAL SCIENCE PLATFORM PRESENTATIONS

Chair: D. Wade Clapp, MD, *Indiana University School of Medicine*

Platform: Peripheral Neuropathy – Expanding the Clinical Phenotype of Schwannomatosis

Session 4A: Monday, June 12, 8:30am – 8:50am

Matthew Evans, MRB MD, *Neurofibromatosis Centre, Department of Neurology, Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London, UK*

Introduction: Schwannomatosis is a rare inherited disorder. As more individuals are identified, the reported phenotype continues to expand. Although length-dependent peripheral neuropathy is a reported feature of NF1 and NF2, it has not been previously described in schwannomatosis. This study aims to identify clinically significant neuropathy in patients who fulfil the diagnostic criteria for schwannomatosis.

Methods: We undertook a retrospective review of the clinical records of 60 patients with schwannomatosis, identifying mutation, neurological symptoms/deficit, pain, neuroimaging and neurophysiology results. Common causes of axonal sensorimotor neuropathies were identified.

Results: Seventeen patients had neurological deficit, and had undergone peripheral neurophysiology. One patient who had been treated for tuberculosis was excluded. Four had clinical and neurophysiological findings in keeping with a predominantly length-dependent, sensory, axonal, peripheral neuropathy. One patient in the neuropathy group, and two in the non-neuropathy group had a *SMARCB1* mutation, and additionally one in the non-neuropathy group had an *LZTR1* mutation. No other mutations were identified. Comparison of the neuropathy (n=4) and non-neuropathy (n=12) groups revealed no significant difference in patient age (median age 61.5y; range 46-70y in the neuropathy group vs 52y; range 39-73y). The ratio of male:female was 2:2 in the neuropathy group, vs 7:4 in the non-neuropathy group. Disease burden was 3/4 in the neuropathy vs 3/12 in the non neuropathy group. Self reported pain was similar between groups.

Conclusion: Peripheral neuropathy occurred in 6.7% of patients with schwannomatosis. The phenotype is in keeping with that seen in patients with NF1 and NF2. Patients with neuropathy tend to have larger disease burden compared to those without neuropathy. This is the first report of peripheral neuropathy associated with schwannomatosis. Larger studies are needed to more clearly characterise the phenotype.

Wood S, RN[1], Inniss R, RN[1], Evans DGR, MD[2], Ferner RE MD[1].
[1] Neurofibromatosis Centre, Department of Neurology, Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London, UK.
[2] Department of Genetic Medicine. Manchester Academic Health Science Centre, Manchester NHS Foundation Trust, Manchester, UK

Platform: Multiple Cancers in Neurofibromatosis Type 1

Session 4A: Monday, June 12, 8:50am – 9:10am

Roope A. Kallionpää, MSc (pharm), *University of Turku, Finland*

Background: Neurofibromatosis type 1 (NF1) is a monogenic cancer predisposition syndrome associated with e.g. tumors of the central and peripheral nervous systems. Several reports have mentioned a number of cases where one NF1 patient has been diagnosed with multiple cancers. However, the incidence of multiple cancers in NF1 has not been systematically studied. The current study was designed to characterize the incidence of multiple cancers in NF1 patients.

Methods: The Finnish NF1 cohort was cross-linked with the Finnish Cancer Registry. Patients were followed up from the diagnosis of the first cancer or the first NF1-related hospital visit in 1987-2011, whichever occurred later. The follow-up ended at the diagnosis of the subsequent cancer, death, emigration or December 31st, 2014. Standardized incidence ratios (SIRs) were stratified by the type of the previous cancer and compared between the NF1 cohort and the Finnish population. Summary estimates were calculated by combining the observed and expected numbers of different cancer types.

Results: A total of 42 second or third cancers were observed among 1476 NF1 patients. The SIR for multiple cancer was 4.43 among NF1 patients and 1.38 in the Finnish population ($P < 0.001$ for difference). The cumulative risk of NF1 patients for having a second cancer within 10 years from the diagnosis of the first cancer was 6.2%, 2.8% for males and 8.7% for females. Cancers most often followed by another malignancy were those of the central nervous system (SIR 5.94 in NF1 *vs* 1.42 in population) and breast (SIR 3.69 *vs* 1.48), and malignant peripheral nerve sheath tumors (MPNSTs) (SIR 6.84 *vs* 1.57).

Conclusions: NF1 patients with a history of malignancy and especially those with a prior MPNST or cancer of the central nervous system should be followed up closely for the timely detection of new malignancies.

Author list: Roope A. Kallionpää MSc (pharm), University of Turku; Matti Rantanen MSc, Finnish Cancer Registry; Heli Ylä-Outinen MD, PhD, University of Turku; Elina Uusitalo MSc, University of Turku; Minna Pöyhönen MD, PhD, University of Helsinki and Helsinki University Central Hospital; Janne Pitkäniemi PhD, Finnish Cancer Registry; Sirkku Peltonen MD, PhD, University of Turku and Turku University Hospital; Juha Peltonen MD, PhD, University of Turku

Funding: Cancer Society of Finland, Turku University Foundation, Turku Doctoral Programme of Molecular Medicine, Emil Aaltonen Foundation

Platform: NF1 Associated Dystrophic Scoliosis: Standardization of Diagnostic Criteria and Prediction Using Single-Nucleotide Polymorphism Markers

Session 4A: Monday, June 12, 9:10am – 9:30am

Christopher L. Moertel, MD, *University of Minnesota Masonic Children's Hospital*

Scoliosis in patients with NF1 can manifest as dystrophic or non-dystrophic. Dystrophic scoliosis is rapidly progressive, making treatment challenging. In the first part of our study, we evaluated inter-observer reliability of eight radiographic characteristics of dystrophic modulation in NF1. Of the 122 cases available for analysis, the readers concurred with the assessment of dystrophic scoliosis with a sensitivity of 75% (310/415 reads). Similarly, the readers correctly assessed non-dystrophic scoliosis for specificity of 73%(142/195). Positive predictive value 85% and negative predictive value was 57%. Among readers, the sensitivity ranged from 61% to 83% and the specificity from 67% to 90%. For the 8 radiographic characteristics individually, sensitivity ranges from 18% for spindling to 76% for rotation, and the specificity ranges from 69% for wedging to 93% for atypical location. All 8 characteristics are strongly associated with dystrophic scoliosis ($p<0.002$). The association is strongest for atypical location ($RR=4.45$) and weakest, (still significant) for scalloping ($RR=1.9$).

The second part of our study evaluated whether genetic markers associated with curve progression in adolescent idiopathic scoliosis (AIS) patients are predictive of dystrophic scoliosis in patients with NF1. Patients with and without dystrophic scoliosis were recruited from medical centers and through individual solicitation. Cheek swabs were sent for genotyping with 53 single-nucleotide polymorphism (SNP) markers associated with curve progression in AIS. The test results are represented as a numerical score for curve progression (Scoliscore™). Scores were compared between dystrophic and non-dystrophic scoliosis using the Mann-Whitney test. The logistic regression modeled the association between Scoliscore and probability of being dystrophic. 57 NF1 patients with clinical and radiographically confirmed scoliosis were included: 29 dystrophic and 28 non-dystrophic. Average age was 22 years. Scoliscores were significantly higher among dystrophic than non-dystrophic cases (median 35 vs. 15, $p< 0.05$). Regression analysis showed that risk scores predicted dystrophic scoliosis in NF1 patients. A Scoliscore >123 or greater yielded a likelihood of dystrophic scoliosis of > 80%.

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Co-authors: David W. Polly, University of Minnesota; Ann M. Brearley, University of Minnesota; Alvin H. Crawford, University of Cincinnati; Daniel J. Sucato, Texas Sottish Rite Hospital (UT Southwestern); Leah Carreon, University of Louisville; A. Noelle Larson, Mayo Clinic; David Stevenson, Lucile Packard Children's Hospital Stanford; Michael Vitale, Columbia University; and Charles Ledonio, University of Minnesota.

CONCURRENT SESSION 4B: BASIC SCIENCE PLATFORM PRESENTATIONS

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

Platform: Exploiting Novel Vulnerabilities in NF1-Associated Tumorigenesis: A Small Molecule Screen Identifies Compounds Capable Of Selectively Killing NF1 Deficient Human Schwann Cells

Session 4B: Monday, June 12, 8:30am – 8:50am

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

Treatment options for both plexiform neurofibromas and MPNSTs are limited, relying mostly on surgical resection and broad-spectrum chemotherapy. Finding new molecular targets for therapeutics effective against both benign tumors and MPNSTs is critical for improved patient outcomes and quality of life. The genetic basis of NF1 syndrome makes it a top candidate for using synthetic lethal genetic and therapeutic approaches to uncover unique variabilities in *NF1* deficient cells.

Given that both plexiform neurofibromas and MPNSTs arise within the Schwann cell linage, we have developed a drug discovery pipeline to identify targeted therapeutics for treatment of NF1-related neoplasia, including MPNSTs. Using CRISPR/Cas9, we have created immortalized human Schwann cell lines that are deficient for the *NF1* gene, and pairing these with isogenic wild-type yields an outstanding tool for identifying synthetic lethal interactions. We have performed extensive phenotypic characterization of these cell lines, as well as transcriptional profiling using RNA-seq. The *NF1* deficient cells exhibit increased oncogenic phenotypes, including increased anchorage independent growth, higher basal levels of Ras-GTP, and form tumors in xenograft models.

These isogenic cell lines are currently being utilized for several synthetic lethal screens for therapeutic and target identification specific to cells lacking *NF1.* These include: **1**. A large-scale screen (~12,000 compounds) for drugs that preferentially kill *NF1* deficient cells. **2**. Synthetic lethal screens using genome-wide RNAi and CRISPR/Cas9 approaches to knockdown/out expression of additional genes.

Our primary small molecule screening efforts have resulted in identification of ~650 compounds found to effectively kill or inhibit growth of the *NF1* deficient cells. We have conducted follow-up dose response experiments with these, on both *NF1* deficient and proficient pairs of cells, to determine relative IC_{50} concentrations and identify those selectively lethal against *NF1* deficient cells. To date, we have identified ~20 compounds showing selective lethality to the *NF1* deficient cells. Examples from these hits represent diverse classes of drugs, and include SN-38 (a strong topoisomerase I inhibitor) and monensin (an antibiotic reported as a potent inhibitor to Wnt-induced transcription). We plan on moving ~5 of our top performing candidate compounds into our rapidly progressing genetically engineered mouse models of PNSTs to assess efficacy in a preclinical disease model.

Other Authors: Rory L. Williams B.S.[1], Sam A Finnerty B.S.[1], Adrienne L. Watson Ph.D.[2], Sue Rathe Ph.D.[1], Jon Hawkinson Ph.D.[1], Gunda Georg Ph.D.[1], Christopher L. Moertel MD[1], David A. Largaespada Ph.D[1], [1]University of Minnesota, [2]Recombinetics Inc.

Funding Support: Children's Tumor Foundation, NF1 Synodos, K.B.W. is supported by Children's Cancer Research Fund Emerging Scientist Award and 5T32CA009138-40

Platform: Nonsense Suppression Rescues Lethal Phenotype in Systemic Induced *NF1* Adult Mouse Harboring Patient-Specific Mutation

Session 4B: Monday, June 12, 8:50am – 9:10am

Ashley N Turner, MS, *Department of Genetics, University of Alabama at Birmingham*

Mutations in the *NF1* gene that result in either a change or loss of neurofibromin protein function(s) can manifest in numerous tissues. The nature of the underlying genetic mutation impacts the specific neurofibromatosis type 1 (NF1) disease phenotype. Our lab is currently creating and characterizing mice with recurrent nonsense mutations found in NF1 patients to recapitulate the premature termination codons (PTCs). These mice provide models for therapeutic intervention studies (e.g., nonsense suppression therapy (NST) that enhances the insertion of an amino acid at a PTC and allows translation to proceed to produce a full-length protein). One of these models is a novel NF1 mouse line carrying a recurrent nonsense mutation found in NF1 patients at exon 18 (c. 2041 C>T; p. Arg681*, $NF1^{Arg681*}$) that can be combined with a conditional knockout allele ($NF1^{4Flox}$). In addition, we established an "acute" conditional knockout model using a tamoxifen-inducible $CAGG^{Cre\text{-}ER}$ recombination system to gain a better understanding of the role of *NF1* in the adult mouse and to develop more rapid methods of assessing neurofibromin function in response to treatment. Following inactivation of floxed *NF1* allele(s), adult $NF1^{4F/4F}$; $CAGG^{Cre\text{-}ER}$ mice lose function of *NF1* systemically and are not able to survive beyond 12 days with animals showing severe damage to multiple tissues throughout the body. During this acute crisis, mice continue to consume chow and absorb calories comparable to littermate-paired controls; however they are not able to maintain body mass or body temperature, in particular total body fat. They also experience periods of torpor. Similarly, mice harboring the PTC $NF1^{Arg681*}$ allele along with a single floxed *NF1* allele ($NF1^{Arg681*/4F}$; $CAGG^{Cre\text{-}ER}$) fail to survive more than 12 days following inactivation of the floxed allele. Nonsense suppressor treatment with gentamicin and amlexanox rescued the lethal phenotype in two out of seven $NF1^{Arg681*/4F}$; $CAGG^{Cre\text{-}ER}$ mice. Studies with other nonsense suppressor drugs are underway. This model allows rapid testing of nonsense suppressor drugs and the identification of *NF1* sensitive cells and tissues in the adult for a more robust readout of restoration of *NF1* activity. This study provides evidence that neurofibromin is essential for survival and nonsense suppression therapy appears to rescue neurofibromin to levels necessary to rescue lethality.

Stephanie N. Brosius, MD, PhD, Children's Hospital of Philadelphia; Maria S. Johnson, PhD, UAB; Timothy R. Nagy, PhD, UAB; Trent R. Schoeb, DVM, PhD, UAB; Bruce R. Korf, MD, PhD, UAB; Robert A. Kesterson, PhD, UAB.

Funding Support: UAB Neurofibromatosis Program

Platform: Immunotherapy for MPNST

Session 4B: Monday, June 12, 9:10am – 9:30am

Thomas De Raedt, PhD, *Harvard Medical School*

One of the most promising developments in recent years is the successful application of immunotherapy in the clinic. Observed clinical responses, with for example PD1-antibody checkpoint blockade, can be spectacular and durable. Unfortunately our data shows that PD1 checkpoint blockade, as a single agent, is ineffective in a genetically engineered mouse model for MPNST. In the clinic favorable responses to PD1 therapy are only observed in a small proportion of the patient population and are often dependent on a favorable immune microenvironment in the tumor. One approach to improve the response to immunotherapy is the identification of drugs that result in a more favorable immune microenvironment, enhancing the probability of immune checkpoint blockade to work.

Combining MEK and BRD4 inhibitors potently kills MPNST cells by synergistically inhibiting the RAS transcriptional output (De Raedt et al. *Nature* 2014). Surprisingly, upon combined inhibition of MEK and BRD4, we observe a rapid (5 days) influx of CTLs (CD8 positive T-cells) in the tumor. Interestingly MEK and BRD4 inhibitors alter a number of, for CD8 T-cells, key ligands on the surface of tumor cells. For example, we see a downregulation of the immune inhibitory ligands PDL1, PDL2 and of NTE5. We also observed a cell intrinsic inhibition of regulatory T-cells by BRD4 and MEK inhibition. These dysfunctional regulatory T-cells in turn fail to inhibit CD8 T-cells. All of these factors help to reshape the immune microenvironment and enhance the likelihood for immunotherapy to work. Excitingly, because of more favorable immune microenvironment, adding the anti-PD1 antibody to our MEKi/BRD4i therapy significantly enhanced tumor regression in our MPNST model.

De Raedt T, Juneja V, McGuire K, Emmerson C, Gavin A, Sharpe A, Cichowski K
Harvard Medical School, Boston, 02115 MA, USA

SESSION 5: NF Tumors

Chairs: Scott Plotkin, MD, PhD, *Massachusetts General Hospital/Harvard University;* Brigitte Widemann, MD, *National Cancer Institute*

MPNST State of the Science: Outlining a Research Agenda for the Future

Session 5: Monday, June 12, 9:30am – 9:40am

AeRang Kim, MD, PhD, *Children's National Medical Center*

In 2002, an international consensus meeting on NF-1 associated MPNST provided guidance for the diagnosis and management of MPNST and identified research priorities. While the clinical outcome of MPNST has not changed over the past 15 years, there has been substantial advances in understanding MPNST natural history, biology and pathogenesis, genomics, and preclinical models. In October 2016, a second international meeting was held to establish short and long term research priorities and address how these advances can be translated to improve outcomes for patients with or at risk for MPNST in the future. The conference was divided into five working groups: 1) Diagnosis, imaging, and primary management, 2) Pathology, 3) Genomics and biomarkers, 4) Preclinical models, and 5) Clinical trials methodology. The proposed recommendations and research priorities will be discussed.

Introducing ANNUBP – Atypical Neurofibromatous Neoplasms of Uncertain Biologic Potential; Summary of Consensus NIH Workshop on Neurofibromas, Atypical Neurofibromas and MPNST in NF1 Patients

Session 5: Monday, June 12, 9:40am – 9:50am

Anat Stemmer-Rachamimov, MD, *Harvard Medical School*

Malignant transformation of neurofibroma to MPNST occurs in 8-15% of NF1 patients during their lifetime. The prediction of transformation, typically from plexiform neurofibroma, may be clinically and histologically challenging.

This is a summary of recommendations for histological diagnosis of a consensus meeting in NIH in October 2016, where a group of soft tissue pathologists, neuropathologists and NF1 neurologists, reviewed clinical and histological features and formed an outline for the workup and diagnosis of the histopathologic changes in the spectrum of transformation from neurofibroma to MPNST.

Atypical features in a neurofibroma include nuclear atypia, increased cellularity, mitoses and loss of neurofibroma architecture. Currently such tumors are diagnosed inconsistently as atypical neurofibroma or low-grade MPNST. Most MPNSTs arising from neurofibromas are high-grade sarcomas and pose little diagnostic difficulty, although rare non-necrotic tumors with 3-9 mitoses/10 HPFs can be recognized as low-grade variants.

Nuclear atypia alone is generally insignificant. However, with atypia, loss of neurofibroma architecture, high cellularity, and/or mitotic activity $>1/50$ but $<3/10$ high power fields, the findings are worrisome for malignancy.

We propose the term "atypical neurofibromatous neoplasms of uncertain biologic potential (ANNUBP)" for lesions displaying at least two of the above features. This diagnosis should prompt additional sampling, clinical correlation, and possibly, expert pathology consultation.

Immunohistochemistry can be helpful in the diagnosis: while neurofibromas contain numerous S100 protein/SOX10-positive Schwann cells and CD34-positive fibroblasts, both components are reduced or absent in MPNST. Loss of p16/CDKN2A expression, elevated Ki67 labeling, and nuclear p53 positivity are also features of MPNST that can to some degree already occur in ANNUBP. Complete loss of trimethylated histone 3 lysine 27 (H3K27me3) expression is potentially more reliable, being immunohistochemically detectable in about half of MPNSTs.

Correlated clinicopathologic, radiologic, and genetic features may result in better understanding of the transformation process and improved treatment options for the patients.

Defining 'Cutaneous Neurofibromas'

Session 5: Monday, June 12, 9:50am – 10:15am

Pierre Wolkenstein, MD, PhD, *Hôpital Henri-Mondor, Universite de Paris EST Creteil;* Jaishri Blakeley, MD, *Johns Hopkins University Hospital*

Cutaneous neurofibromas are the main concern of patients with neurofibromatosis 1. They contribute to the visibility of the disease and have a strong impact upon quality of life.

A simple terminology should be adopted by the community of professionals to define their features and to differentiate neurofibromas found in the skin.

In this talk we will discuss the different skin neurofibromas, mainly cutaneous and subcutaneous ones, and factors associated with their development. Finally, we will propose a clinico-pathological classification.

Drug Screening in Synodos for NF2

Session 5: Monday, June 12, 10:30am – 10:50am

James F. Gusella, PhD, *Harvard Medical School - on behalf of the NF2 Synodos Consortium*

Under the sponsorship of the Children's Tumor Foundation, the NF2 Synodos Consortium comprises a group of collaborating laboratories in the U.S. and Europe cooperating in the development, implementation, and, importantly, continuous improvement of a pipeline for drug testing for NF2-associated tumors. The foundation of the strategy is to screen drugs against the normal cells that are the actual targets for tumor formation, Schwann cells for schwannoma and arachnoidal cells for meningioma, in direct comparison with their merlin-deficient counterparts, and to test candidates *in vivo* in the mouse, either in a genetically-modified model (GEM) or in one carrying a human tumor xenograft. While we primed the pipeline with a set of drugs chosen for their potential relevance to NF2 biology, we have also applied whole genome transcriptomics and active kinome assessment to characterize the cells and models and their responses to drug perturbation. Our goals are 1) to use these functional measures to optimize the pipeline for further drug screens, making all data available to the research community to catalyze further NF2-relevant research; and 2) to directly implicate additional drug targets, either by their difference in expression/activation between merlin-expressing and merlin-deficient target cells or by their adaptive response to drug treatment. While our overall intent is to establish a robust, genetically accurate system that can be used into the future for drug screening directly relevant to NF2-associated tumors, we also aim to use this platform in the near-term to identify one or more compounds that can be tested in a human NF2 trial and are likely to have clinical benefit.

Genomic Landscape of Schwannomas

Session 5: Monday, June 12, 10:50am – 11:10am

Gelareh Zadeh, MD, PhD, *University of Toronto*

We performed an integrative genomic analysis to determine the somatic landscape of sporadic and NF2-associated schwannomas. Exome sequence analysis with validation by targeted DNA-sequencing of 125 samples uncovered, in addition to expected *NF2* disruption, recurrent mutations in *ARID1A, ARID1B* and *DDR1,* along with other novel mutations. Genome-wide methylation profiling identified four molecular subgroups, each with unique molecular signatures. RNA sequence analysis identified an in-frame *SH3PXD2A-HTRA1* fusion in 12/125 (10%) cases, shown to arise from a balanced inversion on chromosome 10q. Expression of the *SH3PXD2A-HTRA1* fusion resulted in elevated phosphorylated-ERK, increased proliferation, increased invasion, in vivo transformation and increased tumorgenesis. Targeting ERK using MEK inhibition in vitro was effective in fusion-positive schwann cells, suggesting a possible therapeutic approach for this subset of tumors. RNA pathway analysis indicates a distinct inflammatory and immune pathway.

Targeting the cMET Signaling in NF2 Schwannoma Models

Session 5: Monday, June 12, 11:10am – 11:30am

Lei Xu, MD, PhD, *Massachusetts General Hospital and Harvard Medical School*

HGF/cMET pathway is activated in schwannomas resistant to radiation therapy in a preclinical model of schwannoma. Previous studies show that in bevacizumab-treated NF2 patients, hearing responses are inversely associated with baseline plasma HGF level. Therefore, we investigated whether combining cMET blockade with radiation therapy could achieve a better tumor control and improve hearing. Our study reported: i) cMET-targeted therapy enhances radiation efficacy in schwannoma preclinical model, ii) the hearing changes in response to treatment in mouse model of schwannoma-induced hearing loss, and iii) cMET blockade decreased patient-derived vestibular schwannoma growth in brain slice model.

Platform: A Phase II Prospective Study of Selumetinib in Children with Recurrent or Refractory NF-1 Associated Low-Grade Glioma (LGG): A Pediatric Brain Tumor Consortium (PBTC) Study

Session 5: Monday, June 12, 11:50am – 12:05pm

Jason R. Fangusaro, *Children's National Health System, Washington, DC*
Roger Packer, MD, *Children's National Health System, Washington, DC, presenting*

Approximately 15-20% of children with NF 1 will develop tumors within the central nervous system (CNS), most often involving the optic pathway. When sampled, these tumors are most commonly pilocytic astrocytomas or other low grade gliomas (LGG), WHO Grade I and II. It is now understood that multiple key pathways are involved with the development of CNS tumors in children with NF-1, including the Ras/mitogen-activated protein kinase (MAPK) pathway and the Akt/mammalian target of rapamycin (mTOR) pathway. A greater understanding of the Ras-MAPK signaling cascade in pediatric low-grade glioma (LGG) and neurofibromatosis type 1 paired with the availability of potent selective inhibitors have enhanced the ability to target this pathway with therapeutic intent. The Pediatric Brain Tumor Consortium (PBTC) conducted a multi-institutional phase II study (NCT01089101) evaluating selumetinib (AZD6244, ARRY-142886), a MEK I/II inhibitor, in children with recurrent and refractory LGG assigned to 6 strata and treated at the Recommended Phase 2 dose of 25 mg/m2/dose orally given twice daily for up to two years. Here we present the data from Stratum 3 which included those children with NF 1 associated LGG. Eligibility required either radiographic evidence of refractory/progressive tumor or a decline in vision as assessed by visual acuity. Tissue for tumor molecular evaluation was not required. Ten of 25 (40%) patients achieved a partial response (PR) with a 2-year progression-free survival (PFS) of 96+/-4%. Only one patient progressed while on treatment. All radiographic responses were confirmed centrally. Careful evaluation of the visual acuity assessments is still ongoing. The most common toxicities were grade 1/2 CPK elevation, diarrhea, hypoalbuminemia, elevated AST and rash. Rare grade 3/4 toxicities included elevated CPK, rash, neutropenia, emesis and paronychia. Selumetinib was effective in treating children with NF 1 associated recurrent and progressive LGG. Larger prospective studies specifically incorporating validated functional and visual outcomes are necessary to determine the future, specific role of this agent in treating children with NF 1 associated LGG.

Jason R. Fangusaro, Arzu Onar-Thomas, Tina Young-Poussaint, Shengjie Wu, Azra H Ligon, Neal Ian Lindeman, Anuradha Banerjee, Roger Packer ,Lindsay B. Kilburn, Ian Pollack, Regina Jakacki, Ibrahim A. Qaddoumi, Paul Graham Fisher, Girish Dhall, Patricia Ann Baxter, Susan G. Kreissman, L. Austin Doyle, Malcolm A. Smith, Ira J. Dunkel, Maryam Fouladi

Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; St. Jude Children's Research Hospital, Memphis, TN; Children's Hospital Boston, Boston, MA; Brigham and Women's Hospital, Boston, MA; University of California, San Francisco, San Francisco, CA; Children's National Health System, Washington, DC; Pittsburgh Children's Hospital, Pittsburgh, PA; AstraZeneca, Brookeville, MD; Stanford University, Palo Alto, CA; Children's Hospital Los Angeles, Los Angeles, CA; Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX; Duke University Medical Center, Durham, NC; Greenbaum Cancer Center, Baltimore, MD; Cancer Therapy Evaluation Program, National Cancer Institute, Washington, DC; Memorial Sloan-Kettering Cancer Center, New York, NY; Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Platform: NF106: A Phase 2 NF Consortium Trial of the MEK Inhibitor PD-0325901 in Adolescents and Adults with NF1-Related Plexiform Neurofibromas

Session 5: Monday, June 12, 12:05pm – 12:20pm

Brian Weiss, MD, *Cincinnati Children's Hospital Medical Center*

Background: Plexiform neurofibromas (PNs) can cause significant disfigurement, compression of vital structures, neurologic dysfunction, and pain. Until recently, the only effective management strategy was surgical resection; however, new evidence suggests that inhibition of the MEK pathway can lead to significant PN shrinkage in a majority of childhood NF1-related PN (NF-PN). We set out to test the efficacy of the MEK inhibitor, PD0325901, in adolescents and adults with NF- PN.

Methods: This phase II open label study evaluated adolescents (≥ 16 years of age) and adults with NF1 and symptomatic or growing PNs treated with PD-0325901. The primary aim of the study was to assess quantitative radiographic response in a target lesion. Subjects received PD-0325901 by mouth twice daily at 2 mg/m^2/dose (maximum dose of 4 mg bid). Each course was 4 weeks, and subjects received drug on a 3 week on/1 week off schedule. Response was defined as at least 20% decrease in tumor volume from baseline.

Results: NF106 enrolled 19 subjects (7M;12F) in 2 stages with a median age of 24 years (range 16-39 years). The mean volume of target PN was 797.8 mL. Eight subjects (42.1%; 95% CI: 20%,67%) had response to PD0325901 as defined by 20% shrinkage reached by cycle 12. PD0325901 was well tolerated with the most common dose limiting toxicity being acneiform rash in 3/19 patients (16%). Five subjects (26.3%) developed Grade 3 toxicities, and for four of those subjects (21%), the Grade 3 toxicity was pain. No subjects developed Grade 4 or higher toxicities. Five subjects (26.3%) had a dose reduction for toxicity: one for Grade 3 abdominal and back pain, the others for Grade 1-2 nausea, rash, or fatigue.

Conclusions: This study demonstrated that PD0325901 causes PN shrinkage in 42% of adolescent and adult subjects with NF-PN. Treatment with PD0325901 is well tolerated at this dose.

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Brian Weiss MD, Cincinnati Children's Hospital Medical Center (CCHMC); Scott Plotkin MD PhD Massachusetts General Hospital; Brigitte Widemann MD, NCI-POB; James Tonsgard MD, University of Chicago; Jaishri Blakely MD, Johns Hopkins Medical Center; Jeffrey Allen MD, New York University; Elizabeth Schorry MD, CCHMC; Bruce Korf MD PhD, University of Alabama-Birmingham (UAB); Tena Rosser MD, Children's Hospital Los Angeles; Stewart Goldman MD, Ann & Robert H. Children's Lurie Hospital of Chicago; Alexander (Sander) Vinks PhD, Pharm D, FCP, CCHMC; Gary Cutter PhD, UAB; Eva Dombi MD, NCI-POB; Nancy Ratner PhD, CCHMC; Roger Packer MD, Children's National Hospital; Michael Fisher MD, Children's Hospital of Philadelphia

Platform: A Swine Model of Neurofibromatosis Type I

Session 5: Monday, June 12, 12:20pm – 12:35pm

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

We have developed a neurofibromatosis type 1 (NF1) mini pig that harbors a known human *NF1* premature termination codon, resulting in a pig model that highly replicates the broad spectrum of disease that develops in human patients. We have applied criteria used for diagnosing NF1 in patients in our model and show that the NF1 mini pig meets the diagnostic criteria for NF1, displaying café au lait spots, dermal neurofibromas, and RAS hyperactivation. Further, the NF1 boars are fertile and the mutant allele is transmitted at a Mendelian rate. These animals are enrolled in an ongoing, longitudinal study where they are phenotypically characterized for the diagnostic criteria of NF1, peripheral nerve hyperplasia, 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 also 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. To date, we have observed 100% penetrance of café au lait spots, a phenotype that has never been demonstrated in any other animal model. The NF1 minipig develops skin lesions over time that appear to histologically resemble human dermal neurofibromas. Additionally, we have 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 currently conducting pharmacological studies in our NF1 swine to look at the pharmacokinetics and pharmacodynamics of currently used drugs in the treatment of NF1 such as Gabapentin, as well as a variety of MEK inhibitors (PD0325901, Selumetinib, and Trametinib) which are currently being tested in clinical trials for NF1. We envision this large animal model of NF1 will become a standard in the evaluation of the safety and efficacy of new drugs prior to Phase I clinical trials and aid in the discovery of effective treatments and cures for patients with NF1. Further, an NF1 minipig may enable researchers to better understand the biological and genetic mechanisms underlying this complex disease, facilitate early detection of NF1-related tumors, identify biomarkers, discover novel drug targets, and test new drugs and combination therapies for safety and efficacy.

Full list of authors: Adrienne L. Watson PhD, Recombinetics Inc., Sara Isakson DVM, University of Minnesota, Alex Coutts BS, Recombinetics Inc., Kyle Williams PhD, University of Minnesota, Rory Williams BS, University of Minnesota, Daniel F. Carlson PhD, Recombinetics Inc., Christopher L. Moertel MD, University of Minnesota, Scott C. Fahrenkrug, Recombinetics Inc., David A. Largaespada PhD, University of Minnesota.

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CONCURRENT SESSION 6A: Learning, Memory and Behavior through the Ages in NF

Chairs: Ype Elgersma, PhD, *Erasmus University Medical Center, Netherlands;* Eric Legius, MD, PhD, *University of Leuven, Belgium*

Cognition in NF Through All Ages

Session 6A: Monday, June 12, 2:00pm – 2:30pm

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

Neurofibromatosis type 1 affects learning, memory and behavior during life, not only in humans but also in animals. Most studies focus on school aged children but similar findings are also present in young infants, adults and at older age. Elderly people with NF1 are underrepresented in virtually all studies and evolution of learning and especially memory is of great importance in this age category. There is a large variability in learning disabilities among individuals with NF1 and the reason for this variability is not very well understood.

Cognition and Behavior in Neurofibromatosis Type 1: Recent Advances

Session 6A: Monday, June 12, 2:30pm – 2:50pm

Jonathan M. Payne, PsyD, *Murdoch Children's Research Institute, The Royal Children's Hospital; Department of Paediatrics, The University of Melbourne, Australia*

Children with neurofibromatosis type 1 (NF1) are at significant risk of cognitive and behavioral deficits and there is a pressing need to identify effective treatments. Recent negative clinical trials of statin medications indicate we need to continue refining our understanding of the disease mechanisms underlying the clinical phenotype. Clinical trial design can also be advanced by (1) identifying markers at the brain-behavior interface that can distinguish children with NF1, monitor progression and predict likely outcome or treatment response, and (2) improving our understanding of the clinical phenotype, so that intervention studies target meaningful outcomes for the patient and their family. This talk will provide an update of our recent neuropsychological studies aimed at refining our understanding of the NF1 clinical phenotype and identifying neurobiological correlates of impairment. We will show that children with NF1 demonstrate various social cognitive vulnerabilities including reduced social information processing, theory of mind and abnormal visual scanning of faces. We will also report on results of recent neuroimaging studies that suggest attention and inhibitory control deficits in NF1 are related to abnormal neural networks. Finally, a new multisite study that we are currently enrolling for, which aims to characterize the interplay between neurobiological markers, social cognitive outcomes and neurodevelopmental symptoms in NF1, will also be presented.

Traits and Symptoms of Autism Spectrum Disorder in Neurofibromatosis Type 1

Session 6A: Monday, June 12, 2:50pm – 3:10pm

John N. Constantino, MD, *Washington University, St. Louis*

In this presentation, results of the ascertainment of autistic traits and symptoms in a large, international multi-site study of individuals with NF1 will be described, and contextualized with respect to new advances in understanding of the early development of social impairments in familial autistic syndromes. These syndromes are believed to arise from disparate combinations of specific and non-specific neurodevelopmental liabilities that are strongly genetically influenced. Implications for a next wave of scientific discovery exploring NF1 as a quantitative trait locus for autistic social impairment will be discussed, as will opportunities for early intervention, and clinical approaches to the appraisal and treatment of behavioral "comorbidity".

Platform: Neurofibromin Deficiency Alters Brain-Wide Intrinsic Functional Organization of the Developing Brain

Session 6A: Monday, June 12, 3:10pm – 3:30pm

Ben Shofty, MD[1,2,3], *Tel Aviv Medical Center*

Children with NF1 display multiple structural and functional changes in the central nervous system, such as white matter alterations, and a unique profile of neuropsychological cognitive abnormalities. Assessment of resting state networks (RSNs) can reveal differences in the functional architecture of the developing brain in response to neurofibromin deficiency resulting from NF1 mutation. Here, we focused on resting-state functional connectivity between the subcortical striatum and cortical networks differentiated as primary (e.g., visual, somatomotor) versus association (e.g., ventral attention, default). Eighteen children with NF1 who had resting-state fMRI scans were group-matched (age, gender and head movement) with 18 typically developing children (TDC) from the ABIDE repository. Coherent slow fluctuations in the fMRI signal across the entire brain were used to interrogate the pattern of functional connectivity of cortical-subcortical structures. Assessment of RSNs was done using a previously established automated clustering algorithm. NF1 children demonstrated abnormal organization of association networks, particularly, deficient long-distance functional connectivity. Examining the contribution of the striatum revealed that corticostriatal functional connectivity was altered, with NF1 children demonstrating diminished functional connectivity between striatum and the ventral attention network, as well as the posterior cingulate area, which is associated with the default network. By contrast, somatomotor functional connectivity with the striatum was increased. Functional connectivity of the visual network with the striatum did not differ in the NF1 group. These findings suggest that, much like in animal studies, the striatum plays a major role in NF1 cognitive pathogenesis. In addition, the "immature" pattern of deficient long distance functional connectivity suggests that NF1-associated myelin abnormalities may also play a significant role in the disrupted formation of RSNs.

Gil Zur, MD[1], Francisco X Castellanos, MD[4], Liat Ben-Sira MD[2], Roger J. Packer, MD[5], L.Gilbert Vezina[5], MD, Shlomi Constantini, MD, MSc[2,3], Maria T. Acosta, MD[5] and Itamar Kahn, PhD[1]

[1]Rappaport Faculty of Medicine, Technion
[2]The Gilbert Israeli NF Center, Tel Aviv Medical Center
[3]Department of Neurosurgery, Tel Aviv Medical Center
[4]NYU Langone Medical Center
[5]Gilbert Family Neurofibromatosis Institute, Children's National Health System

Platform: Habituation Learning in *Drosophila* - A High-Throughput Platform to Identify Drugs that Ameliorate Cognitive and Behavioral Problems in NF1 and Other Rasopathies

Session 6A: Monday, June 12, 3:30pm – 3:50pm

Michaela Fenckova, PhD, *Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center (Radboudumc), Nijmegen, The Netherlands*

Habituation, the ability to suppress a reaction to repeated nonthreatening stimuli, is one of the most fundamental forms of learning. It serves as a neuronal mechanism to filter out irrelevant information and represents a prerequisite for higher-order cognitive functioning. Habituation defects have been reported in a number of cognitive and behavior disorders, including autism spectrum disorder (ASD). To determine the genetic basis of habituation and its relevance for human disease, we used genes implicated in human cognitive disorders as a window and high-throughput light-off jump habituation in the fruit fly *Drosophila* as readout.

We identified orthologs of >100 genes implicated in intellectual disability (ID) that control habituation learning in *Drosophila.* These genes characterize ID disorders with co-morbid autism spectrum disorder (ASD), and highlight specific ASD related behavioral anomalies. They converge on increased Ras-MAPK signaling, the molecular mechanism underlying cognitive impairments in NF1 and other Rasopathies. Habituation defects associated with NF1 and increased Ras-MAPK signaling originate from inhibitory, GABAergic neurons and can be partially corrected with lamotrigine, a novel pharmacological treatment that successfully restored the learning defects in the NF1 mouse model[1] and that is currently being tested in a first clinical trial (NF1-EXCEL).

Our work shows that habituation is a suitable readout to study cognitive deficits in NF1. The unique advantage of *Drosophila* light-off jump habituation is the high efficiency of the assay that allows us to test drugs in high-throughput. Moreover, based on the fundamental importance and our results in *Drosophila,* we hypothesize that defective habituation is an underlying mechanism for cognitive and behavioral problems in NF1. To test this hypothesis and to facilitate translation of drugs identified in *Drosophila* to the clinic, we also aim to implement highly similar, objective and quantitative habituation measures in NF1 clinical research. Together, this work can open completely new avenues for improved translational research and treatment of cognitive and behavioral deficits in NF1 and other Rasopathies.

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Full List Authors: Lenke Asztalos, Aktogen Ltd.; Pavel Cizek, Radboudumc; Euginia L. Singgih, Radboudumc; Laura E.R. Blok, Radboudumc; Jeffrey C. Glennon, Radboudumc; Joanna IntHout, Radboudumc; Christiane Zweier, Friedrich-Alexander-Universität Erlangen-Nürnberg; Evan E. Eichler, University of Washington School of Medicine; Raphael A. Bernier, University of Washington; Sarah Lippe, Mother and child university hospital center Sainte-Justine; Zoltan Asztalos, Aktogen Ltd.; Annette Schenck, Radboudumc.

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1. Omrani et al. HCN channels are a novel therapeutic target for cognitive dysfunction in Neurofibromatosis type1. Mol. Psychiatry 1–11 (2015). doi:10.1038/mp.2015.48

SESSION 6B: New Advances in NF2 and Schwannomatosis

Chairs: Marco Giovannini, MD, PhD, *UCLA;* Gareth Evans, MD, *St. Mary's Hospital, University of Manchester, UK*

Natural History of Tumor Development in Mice Carrying *SMARCB1* and/or NF2 Inactivation

Session 6B: Monday, June 12, 2:00pm – 2:20pm

Jeremie Vitte, PhD, *UCLA*

Germline mutations of the *SMARCB1* gene predispose to two distinct tumor syndromes: rhabdoid tumor predisposition syndrome, with malignant pediatric tumors mostly developing in brain and kidney, and familial schwannomatosis, with adulthood benign tumors involving cranial and peripheral nerves. The mechanisms by which *SMARCB1* germline mutations predispose to rhabdoid tumors versus schwannomas are still unknown. To understand the origin of these two types of *SMARCB1*-associated tumors, we generated different tissue- and developmental stage-specific conditional knockout mice carrying *Smarcb1* and/or *NF2* deletion. Early loss of *SMARCB1* in neural crest cells was necessary to initiate tumorigenesis in the cranial nerves and meninges with typical histological features and molecular profiles of human rhabdoid tumors. By inducing *Smarcb1* loss at later developmental stage in addition to biallelic *NF2* gene inactivation, we generated the first mouse model developing schwannomas with the same underlying gene mutations found in schwannomatosis patients.

Smo Activation in Meningeal Cells to Promote Meningioma Development at the Skull Base

Session 6B: Monday, June 12, 2:20pm – 2:40pm

Michel Kalamarides, MD, PhD, *Hôpital de la Pitié-Salpétrière, Paris*

Recent whole genome analysis of meningiomas identified somatic activating mutations of Smoothened (SMO), a component of the embryonic Sonic hedgehog signaling pathway, in 3% to 5% of grade I meningiomas. Herein, we have characterized a large cohort of human *SMO*-mutant meningothelial meningiomas, mostly located at the anterior skull base. Using genetically engineered mouse models, we defined a restricted developmental window during which conditional activation of *SMO* in Prostaglandin D2-synthase-positive mesoderm-derived meningeal layer of the skull base results in meningioma formation. This prenatal period is concomitant with the role of *SMO* and SHH signaling in the formation of meninges, strongly supporting the hypothesis of a developmental origin for *SMO*-activated meningiomas. Finally, we provide preclinical *in vitro* evidence of the efficacy of the SMO-inhibitor Sonidegib, supporting further preclinical and clinical evaluation of targeted treatment for refractory *SMO*-mutant meningiomas.

Julien Boetto*[1,2], Matthieu Peyre*[1,2,3], Franck Bielle[1,2,4], Michel Kalamarides[1,2,3]
1 – Université Paris 6 - Pierre et Marie Curie, Paris, F-75013, France
2 – CRICM INSERM U1127 CNRS UMR 7225, Institut du Cerveau et de la Moelle Epinière, Paris, F-75013, France
3 – Department of Neurosurgery, AP-HP, Hôpital Pitié-Salpêtrière, Paris, F-75013, France
4 – Department of Neuropathology, AP-HP, Hôpital Pitié Salpétrière, Paris, F-75013, France

Loss of *SMARCB1* in Schwann Cells Leads to Increased Expression of Pain Mediators and Pain Sensitivity: A Model For Schwannomatosis Pain

Session 6B: Monday, June 12, 2:40pm – 3:00pm

Steven Matsumoto, PhD, *Oregon Health and Science University*

A significant number of schwannomatosis patients 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 *SMARCB1* gene (also called *INI1, BAF47* and *SNF5*). We found that inducible conditional disruption of the *SMARCB1* 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 *SMARCB1* in Schwann cells demonstrate behavioral phenotypes consistent with increased pain sensitivity. We find that dorsal root ganglion (DRG) neurons from mice with Schwann cell-targeted disruption of *SMARCB1* 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 the DRG neurons of our mice with Schwann cell-targeted *SMARCB1* mutations. Wild type DRG cells grown in *SMARCB1*-null Schwann cell conditioned media 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 *SMARCB1*-null Schwann cell conditioned media or conditioned media from schwannoma cells derived from schwannomatosis patients expressed elevated levels of TRPV1, TRPA1 and CGRP as indicated by immunocytochemistry. Collectively, these data indicate that loss of *SMARCB1* 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.

Steven Matsumoto[1], Fatima Banine[1], Scott Foster[1], Brian Hammond[1], Cristina Fernandez-Valle[2], and Larry S. Sherman[1]

[1]Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA and [2]Department of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA

Platform: YAP/TAZ-mediated Metabolic Reprogramming is Required for Growth and Survival of NF2-Deficient Tumor Cells *in Vitro* and *in Vivo*

Session 6B: Monday, June 12, 3:00pm – 3:20pm

Shannon White, BS, *Lombardi Comprehensive Cancer Center, Georgetown University Medical Center*

Neurofibromatosis type 2 is a disease caused by genetic loss of the tumor suppressor *NF2*/Merlin, which functions upstream of the Hippo pathway as an negative regulator of transcriptional coactivators, YAP and TAZ. In an effort to further understand the characteristics of *NF2*-deficient tumors, we have investigated the metabolic profiles of multiple *NF2*-deficient human and mouse tumor cell lines and their dependency on YAP/TAZ signaling. Our study shows that *NF2*-loss-induced upregulation of YAP/TAZ activity shifts the cell's energetics and enhances their ability to adapt to metabolic changes in the environment. Furthermore, we show that *NF2*-deficient cells rely on YAP/TAZ to prevent aberrant ROS accumulation and oxidative stress-induced cell death under nutrient-deprived conditions. Our results have uncovered a previously unappreciated role for *NF2*/Merlin in regulating the cell metabolism, and have revealed new metabolic vulnerabilities that could potentially be exploited to selectively kill *NF2*-mutant tumor cells.

Author List: Chunling Yi, PhD, Georgetown University

Funding: AdvocureNF2 and The V Foundation for Cancer Research

Platform: Co-Targeting mTORC1/2 and EPH Receptor Pathways as a Therapeutic Potential for NF2-Deficient Meningiomas

Session 6B: Monday, June 12, 3:20pm – 3:40pm

Roberta L. Beauchamp, BA, *Massachusetts General Hospital*

Meningiomas (MN), which arise from the arachnoidal layer of the meninges, are the most common primary intracranial tumor in adults. Neurofibromatosis 2 (NF2)-associated MN and ~50-60% of sporadic MN show bi-allelic *NF2* inactivation and loss of the tumor suppressor protein merlin. These tumors are non-responsive to conventional chemotherapies, and thus MN are generally treated with surgery or radiation. We previously showed aberrant activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling upon NF2 loss, and resulting clinical trials using rapalogs (RAD001/everolimus) for NF2 were mixed, showing tumor growth stabilization but no shrinkage. More recently, we demonstrated that NF2 loss also activates a distinct mTORC2-SGK1-NDRG1 signaling axis, and treatment of NF2-null MNs using dual mTORC1/mTORC2 inhibitor AZD2014 decreased proliferation with greater efficacy than rapamycin. This also led to clinical trials with AZD2014 for MN, which are ongoing. To understand additional dysregulated pathways in NF2 tumor cells, particularly druggable kinases that may lead to improved therapeutic strategies, we performed a large-scale kinome screen using multiplex inhibitor beads coupled with mass spectrometry (MIB/MS) on our isogenic CRISPR/Cas9-modified human arachnoidal cells (ACs), NF2-expressing vs NF2-null. In NF2-null ACs, MIB/MS identified elevation of several kinases, including members of the erythropoietin-producing hepatocellular (EPH) receptor tyrosine kinase (RTK) family EPHA2, EPHA4 and EPHB1, as well as c-KIT. We validated increased expression/activation and observed *1)* robust pEPHA2(S897), pEPHB1(Y594) and downstream pSrc/SFK(Y416) in NF2-null ACs and MN cells by immunoblotting, and *2)* increased *EPHA2* and *EPHA4* transcription in NF2-null ACs. EPHA2 is often overexpressed/upregulated in human cancers, and our mechanistic studies revealed that phosphorylation of oncogenic EPHA2(S897) upon NF2 loss was MEK/MAPK-dependent. Treatment with the multi-kinase inhibitor dasatinib significantly inhibited pEPHA2, pEPHB1, c-KIT and Src/SFK in NF2-null ACs and MN cells with minimal effect on mTORC1/2 signaling. Conversely, AZD2014 strongly blocked mTORC1/mTORC2 pathways with no effect on EPH-RTK or Src/SFK targets. This activation of independent downstream pathways upon NF2 loss led us to test effects of combined AZD2014+dasatinib on cell viability/proliferation. Using a 6x6 dose matrix screening approach, we found that combined AZD2014+dasatinib treatment inhibited proliferation rate and exhibited synergy in NF2-null ACs and primary MNs. A follow-up MIB/MS screen on AZD2014- and dasatinib-treated NF2-null cells confirmed effective inhibition of respective kinase targets as well as co-targeting of relevant kinases when combined. Taken together, in addition to mTORC1/2, we show for the first time that EPH-RTK, SFK and c-KIT pathways are upregulated upon NF2 loss, supporting a potential therapeutic opportunity to co-target mTORC1/2 and EPH-RTK/SFK pathways as a novel, more effective strategy for NF2-deficient meningioma.

Full List Authors: Tim Stuhlmiller PhD, University of North Carolina; Patrick DeSouza BS, Massachusetts General Hospital; Steve Angus PhD, University of North Carolina; Luke Witt BS, Massachusetts General Hospital; Justin T. Jordan MD, MPH, Massachusetts General Hospital; Anat Stemmer-Rachamimov MD, Massachusetts General Hospital; James Gusella PhD, Massachusetts General Hospital; Scott Plotkin MD, Massachusetts General Hospital; Stephen Haggarty PhD, Massachusetts General Hospital; Gary Johnson PhD, University of North Carolina and Vijaya Ramesh PhD, Massachusetts General Hospital

Granting Agencies: Children's Tumor Foundation-Synodos, Neurofibromatosis Northeast, National Institutes of Health

Platform: Combined Inhibition of NEDD8-Activating Enzyme and mTOR Suppresses *NF2* Loss-Driven Tumorigenesis

Session 6B: Monday, June 12, 3:40pm – 4:00pm

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

Inactivation of NF2/Merlin causes the autosomal dominant cancer predisposition syndrome familial neurofibromatosis type 2 (NF2) and contributes to the development of malignant pleural mesothelioma (MPM). In order to develop a targeted therapy for NF2-mutant tumors, we have exploited the recent realization that Merlin loss drives tumorigenesis by activating the E3 ubiquitin ligase CRL4DCAF1 – thereby inhibiting the Hippo pathway component Lats. Here, we show that MLN4924 – a NEDD8 activating enzyme (NAE) inhibitor – suppresses CRL4DCAF1 and attenuates activation of YAP in NF2-mutant tumor cells. Additionally, MLN4924 sensitizes MPM to traditional chemotherapy, presumably as a result of collateral inhibition of cullin-RING ubiquitin ligases (CRLs) involved in DNA repair. However, even in combination with chemotherapy, MLN4924 does not exhibit significant preclinical activity. Further analysis revealed that depletion of DCAF1 or treatment with MLN4924 does not affect mTOR hyperactivation in NF2-mutant tumor cells, suggesting that loss of Merlin activates mTOR independently of CRL4DCAF1. Intriguingly, combining MLN4924 with the mTOR/PI3K inhibitor GDC-0980 suppresses the growth of NF2-mutant tumor cells in vitro as well as in mouse and patient-derived xenografts. These results provide preclinical rationale for the use of NAE inhibitors in combination with mTOR/PI3K inhibitors in NF2-mutant tumors.

Jonathan Cooper[1,2]*, Lu Zhou[3], Milica Pavlovic[1,4], Virginia Ojeda[1], Kamalika Moulick[1], Elisa de Stanchina[5], J.T. Poirier[6], Marjorie Zauderer[6,7], Charles M. Rudin[6], Matthias A. Karajannis[8], C.O. Hanemann[3], and Filippo G. Giancotti[4]*

[1]Cell Biology Program and Center for Metastasis Research, Sloan Kettering Institute for Cancer Research, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; [2]Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medical College, New York, NY 10065, USA; [3]Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth, UK; [4]Department of Cancer Biology and David H. Koch Center for Applied Research of Genitourinary Cancers, UT MD Anderson Cancer Center, Houston, TX 77230, USA; [5]Antitumor Assessment Core Facility, Sloan Kettering Institute for Cancer Research, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; [6]Thoracic Oncology Service and Druckenmiller Center for Lung Cancer Research, Memorial Hospital, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA; [7]Weill Cornell Medical College, New York, NY 10065, USA; [8]NYU Cancer Institute and Department of Pediatrics, NYU Langone Medical Center, New York, NY 10016, USA.

Correspondence: FGGiancotti@MDAnderson.org or Cooper.Jonathan@gene.com

KEYNOTE 3: Peripheral Nerve Homeostasis and Repair – Impaired Signaling Circuits in Neurofibromatosis Type 2 (NF2)

Tuesday, June 13, 10:00am – 11:00am

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

This talk will address the cellular and molecular pathways used to ensure effective peripheral nerve homeostasis and repair and discuss the signaling impairments contributing to the neurofibromatosis type 2 (NF2) tumor syndrome. I will include investigations on the Schwann cell differentiation state; research on the Schwann cell and axonal interactions. I aim to demonstrate that schwannoma formation arises from a faulty peripheral nerve regenerative response involving cellular changes and interactions of several different cell types – neurons, Schwann cells and macrophages. Finally based on our discoveries I will share results of our novel therapeutic intervention utilizing mouse models of neurofibromatosis type 2 (NF2).

Alexander Schulz[1,2], Robert Buttner[1], Christian Hagel[3], Yan Cui[1], Susann Schirmer[1], Tobias Sperka[1], Anne-Marie Carlstedt[1], D. Wade Clapp[4] and Helen Morrison[1]

[1]Leibniz Institute on Aging, Fritz Lipmann Institute, 07745 Jena, Germany
[2]Department of Genetics and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT 06510, USA
[3]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany
[4]Department of Pediatrics, Herman B Wells Center for Pediatric Research Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46202, USA

SESSION 7: Signaling in Neurofibromatosis

Chairs: Karlyne Reilly, PhD, *National Cancer Institute;* Alison Lloyd, PhD, *University College London, UK*

Role of NF1 in Reward, Motor Learning and Opioid Actions in the Striatum

Session 7: Tuesday, June 13, 11:15am – 11:35am

Kirill Martemyanov, PhD, *Scripps Institute*

It is well recognized that, GPCRs can activate Ras-regulated kinase pathways to produce lasting changes in neuronal function. Mechanisms by which GPCRs transduce these signals and their relevance to brain disorders are not well understood. Here we identified a major Ras regulator, neurofibromin 1 (NF1), as a direct effector of GPCR signaling via Gβγ subunits in the striatum. We found that binding of Gβγ to NF1 inhibited its ability to inactivate Ras. Deletion of NF1 in striatal neurons prevented the opioid receptor induced activation of Ras and eliminated its coupling to Akt-mTOR signaling pathway. By acting in the striatal medium spiny neurons of the direct pathway, NF1 regulates opioid induced changes in neuronal excitability thereby sensitizing mice to rewarding effects of morphine. These results delineate a novel mechanism of GPCR signaling to Ras pathways and establish a critical role of NF1 in addiction.

Opposing Effects of Activating Mutations on Developmental Ras Signaling and Morphogenesis

Session 7: Tuesday, June 13, 11:35am – 11:55am

Rebecca Burdine, PhD, *Princeton University*

Germline mutations in RAS/MAPK pathway components cause developmental disorders termed RASopathies, including Noonan and Cardio-Facio-Cutaneous syndromes. These disorders are common, affecting 1/1000 births, and can include cardiac defects, orbital hypertelorism, and neurodevelopmental delays. While it is generally believed that RASopathies are caused by altered levels of RAS pathway activation, the actual signaling changes, and phenotypic consequences of those alterations, are not well understood.

Here, we utilize zebrafish and *Drosophila* systems to analyze RASopathy mutations during development. We developed a rapid assay in zebrafish to rank human mutations in MEK from RASopathy and cancer patients. We find our zebrafish ranking holds in *Drosophila,* suggesting the rank reflects intrinsic properties of the mutations and not species-specific effects. The assay can be utilized to rapidly assess potential mutations and variants of unknown function in RAS pathway components in the future.

Surprisingly, we find that activating variants of MEK, can both increase and decrease signaling levels of the pathway *in vivo* depending on the cellular location. We see this both in zebrafish and in *Drosophila* indicating that RASopathy phenotypes may result from combinations of activated and attenuated signaling during development. Indeed, we show gain-of-function and loss-of-function phenotypes in both systems resulting from the same mutation in MEK. This has important implications for development of pharmaceutical therapies for these disorders. While MEK inhibitors are rapidly being developed to trial in cancer patients, inhibition of MEK may actually worsen some phenotypes in RASopathy patients.

Granton A. Jindal[1,2,3,*], Yogesh Goyal[1,2,*], José L. Pelliccia[3], Kei Yamaya[2,3], Eyan Yeung[2,3], Alan S. Futran[1,2], Iason Kountouridis[2], Courtney A. Balgobin[3], Trudi Schüpbach[3], and Stanislav Y. Shvartsman[1,2,3], Rebecca D. Burdine[3]

[1]Department of Chemical and Biological Engineering, [2]The Lewis-Sigler Institute for Integrative Genomics and [3]Department of Molecular Biology
Princeton University, Princeton, NJ 08544

*Contributed equally

Platform: The Molecular and Immunologic Landscape of Cutaneous Neurofibroma

Session 7: Tuesday, June 13, 11:55am – 12:15pm

Robert Allaway, PhD, *Sage Bionetworks*

Neurofibromatosis type 1 (NF1) is a genetic disorder that results from germline mutations of the neurofibromin 1 gene (*NF1*). NF1 presents with a broad clinical spectrum; patients with NF1 exhibit a variety of symptoms, including predisposition to tumors such as cutaneous (or dermal) neurofibromas (cNF). Patients usually experience onset of cNF during late childhood or adulthood. While cNF are benign, they can cause discomfort and cosmetic issues. Furthermore, therapeutic options for cNF are generally restricted to surgical approaches.

The etiology of cNF is not well understood. It is thought that these tumors generally exhibit biallelic inactivation of *NF1* and that the nature of these inactivation events may play a phenotypic role in cNF development. It has also been established that endocrine signaling and the neurofibroma microenvironment contribute to cNF formation. For example, female NF1 patients frequently experience cNF formation and/or growth during puberty and pregnancy. In the cNF microenvironment, $NF1^{-/+}$ mast cells and $NF1^{-/-}$ Schwann cells are required for cNF formation.

To further elucidate the molecular and microenvironmental landscape of these tumors, the Children's Tumor Foundation, Sage Bionetworks, Mount Sinai, and HudsonAlpha collaborated to acquire, molecularly profile, and analyze a panel of cNF samples and patient-matched whole-blood samples donated to the CTF Biobank. Data were obtained using whole exome sequencing, RNA-seq, SNP arrays, and proteomics methods, and the data were released as a scientific resource (synapse.org/dermalNF).

We conducted downstream analysis of these data to investigate the pathologic characteristics of cNF. Using expression data from cNFs, we identified enrichment of several cancer and immune signatures, signatures associated with resting mast cell and M2 macrophage infiltration, and differentially expressed genes in subsets of cNF samples. The presence of these signatures suggests that there may be common signaling pathways that are exploited by both cNF and cancer, and supports previous work indicating that the microenvironment is an important contributor to cNF development. We also evaluated the germline and somatic mutation spectrum of cNF. At the pathway level, we observed that CREBBP and CDC27 pathway genes were commonly mutated. Finally, we performed integrative analysis and identified genes and pathways that were correlated with CREBBP and CDC27 mutation. In summary, analysis of these data highlighted molecular characteristics of cNF that may contribute to the pathology of these tumors.

Robert Allaway PhD[1], Sara JC Gosline PhD[1], Allison Galassie BS[2], Andrew Link PhD[2], Pamela Knight MS[3], Salvatore La Rosa PhD[3], Annette Bakker PhD[3], Justin Guinney PhD[1]

[1]Sage Bionetworks, Seattle, WA 98109, [2]Vanderbilt University, Nashville, TN 37232, [3]Children's Tumor Foundation, New York, NY 10005

Funding: Children's Tumor Foundation

Shutting Your TRAP to Kill Neurofibromas? The Oncogenic Role of the Mitochondrial Chaperone TRAP1 in NF1

Session 7: Tuesday, June 13, 11:35am – 11:55am

Andrea Rasola, PhD, *Department of Biomedical Sciences, University of Padova, Padova, Italy*

Metabolic rewiring plays a pivotal role in neoplastic progression. Hyperactivation of Ras/ERK signalling has a profound impact on cell bioenergetics, and in neurofibromatosis type 1 (NF1) is mandatory for tumor growth following loss of the Ras-GAP neurofibromin. Nonetheless, the metabolic features of NF1-related tumors are poorly investigated. We have found that cells lacking neurofibromin exhibit enhanced glycolysis and decreased respiration in a Ras/ERK-dependent way. In mitochondria of neurofibromin-deficient cells a fraction of active ERK1/2 associates with the complex II of the respiratory chain, succinate dehydrogenase (SDH), and with TRAP1, a chaperone that promotes the accumulation of the oncometabolite succinate by inhibiting SDH. TRAP1 silencing or mutagenesis at the serine residues targeted by ERK1/2 abrogates tumorigenicity, a phenotype that is reverted by addition of a cell-permeable succinate analog. Our findings reveal that Ras/ERK signaling controls the metabolic changes orchestrated by TRAP1, which in turn have a key role in the oncogenic growth of neurofibromin-deficient cells. Thus, TRAP1 inhibition is a promising anti-neoplastic strategy in NF1.

Tipping the Redox Balance in the Fight against NF2

Session 7: Tuesday, June 13, 12:35pm – 12:55pm

Chunling Yi, PhD, *Lombardi Comprehensive Cancer Center, Georgetown University Medical School*

NF2 tumors are typically indolent and refractory to standard chemotherapy that targets rapidly dividing cells. Using a genetically engineered liver tumor model, we recently revealed that the indolence of *NF2* mutant tumors are likely due to the activation of a Rac1-mediated redox switch, which induces p53/p16 cell cycle checkpoints, dampens the tumor growth rate. Here, we will present update on our latest efforts in investigating the roles of redox signaling during NF2 tumorigenesis and exploring the therapeutic potentials of targeting the redox network for treatment of *NF2* mutated tumors.

Yuhao Shi, Saumya R. Bollam, Shannon M. White, Chunling Yi
Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington DC

The Role of the Hippo-YAP Pathway in NF2

Session 7: Tuesday, June 13, 12:55pm – 1:15pm

Joseph Kissil, PhD, *Scripps Research Institute*

The Hippo-YAP pathway is a central regulator of cell contact inhibition, proliferation and death. Significantly, YAP is required for cell survival and proliferation in NF2 null Schwann cells and thus presents as a potential target for therapeutic intervention in neurofibromatosis type 2. We previously identified Angiomotin, as a regulator of YAP and Merlin function. There are conflicting reports regarding the role of Angiomotin (Amot) in regulating YAP. While some studies suggest a YAP-inhibitory function other studies indicate Amot is required for YAP activity. Here, we describe an Amot-dependent complex comprised of Amot, YAP and Merlin. The phosphorylation of Amot at Serine 176 shifts localization of this complex to the plasma membrane, where it associates with the tight-junction proteins Pals1/PATJ and E-cadherin. Conversely, hypophosphorylated Amot shifts localization of the complex to the nucleus, where it facilitates the association of YAP and TEAD, induces transcriptional activation of YAP target genes and promotes YAP-dependent cell proliferation. We propose that phosphorylation of Amot^{S176} is a critical post-translational modification that suppresses YAP's ability to promote cell proliferation and tumorigenesis by altering the subcellular localization of an essential YAP co-factor.

SESSION 8: Development of New Therapies for NF1 and NF2: A Model Strategy for Orphan Diseases

Session 8: Tuesday, June 13, 1:15pm – 1:35pm

D. Wade Clapp, MD, *Indiana University School of Medicine*

NF1 and NF2 are genetic diseases caused by tumor suppressor genes and characterized by their development of cancers in the central and peripheral nervous system. NF1 is caused by germ line mutations in the *NF1* tumor suppressor gene (TSG), which encodes a GTPase activating protein (GAP) called neurofibromin that forms a molecular complex with activated Ras-GTP and negatively regulates Ras signaling by accelerating GTP hydrolysis. Collectively, the tumors that develop in NF1 patients are a substantial cause of morbidity and premature mortality. In addition to its role as an initiating mutation in NF1-associated cancers, recent genome-wide sequencing studies uncovered frequent somatic *NF1* mutations in glioblastoma, acute myeloid leukemia, adenocarcinoma of the lung, and other sporadic cancers. NF2 is less common than NF1 (1:33,000 individuals worldwide vs 1:3000), though NF2 is prevalent as the initiating mutation of sporadic schwannomas and meningiomas that are important causes of CNS malignancies. The biochemical role of the NF2 tumor suppressor is incompletely understood, but is a source of activity by multiple laboratories.

Therapeutic advances in orphan diseases are routinely challenged by the lack of coordinated expertise in the many overlapping areas of science required in order to proceed from the bench to the bedside and back to the bench. However, the opportunities for advancements of therapies in NF1 and more recently NF2 point to encouraging drug targets and clinical advances. Coordination of many NF focused laboratories with supportive leadership from the NIH, DOD, and two vibrant Foundations (Children's Tumor Foundation and more recently NTAP), have allowed the genesis of a series of multi-investigator preclinical and clinical initiatives. These include the Department of Defense Clinical Consortium, a preclinical consortium funded by the CTF and NTAP, a preclinical consortium for NF2 called Synodos, and an NCI sponsored SPORE grant focused on neurofibromatosis type 1. Collectively, the discoveries from these groups have now resulted in the development of multiple encouraging phase 2 trials and even regulatory trials for NF1. Further, parallel strategies point to advancements in therapies for NF2 disease manifestations as well.

Poster Presentation (odd numbers)

SATURDAY, JUNE 10, 2017 (4:30 – 6:00 PM)

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Ammoun	Sylwia	5	Investigation and Targeting Cellular Prion Protein PrP^C in Neurofibromatosis Type II Related Tumours
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Cai	Wenli	21	MRI Texture Analysis for Identifying NF1 Radiogenomics: The Correlation Between NF1 Genotype and Imaging Phenotype
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Negative Regulation of the Neurofibromin 2 (*NF2*) Tumor Suppressor Gene by MicroRNA-92a Promotes Cell Proliferation, Migration, and Survival in Colorectal and Lung Cancer Cells

Krizelle Mae Alcantara, MS, *National Institute of Molecular Biology and Biotechnology, University of the Philippines Diliman*

Inactivation of the tumor suppressor protein Merlin leads to the development of benign nervous system tumors of neurofibromatosis type 2 (NF2). Cases of Merlin deficiency are also observed in human malignancies such as colorectal and lung cancers. Known mechanisms causing Merlin loss of function include deleterious mutations in the neurofibromin 2 gene (*NF2*) and aberrant post-translational modifications leading to Merlin degradation. However, other mechanisms that may contribute to *NF2* downregulation and/or Merlin inactivation remain largely unexplored. In this study, we examined the possible regulation of *NF2* by microRNAs (miRNAs), small non-coding RNAs that regulate target mRNA expression primarily through interaction with their 3'-untranslated region (3'UTR). *In silico* analyses predicted evolutionarily conserved miRNA response elements (MREs) for hsa-miR-92a within the *NF2*-3'UTR sequence. Dual luciferase assays performed on human colorectal carcinoma (HCT116) and lung adenocarcinoma (A549) cells demonstrated endogenous downregulation of reporter expression in cells transfected with wild-type *NF2*-3'UTR cloned into the miRNA target expression vector pmirGLO, downstream of luciferase. Downregulation was significantly enhanced in wild-type *NF2*-3'UTR co-transfected with miR-92a, but not in setups co-transfected with *NF2*-3'UTR containing mutated miR-92a MRE. Further, endogenous *NF2* mRNA and Merlin expression levels are significantly downregulated in cells transfected with miR-92a overexpression construct as evaluated by quantitative real-time PCR and Western blot. Transcript and protein expression was rescued by co-transfection of a target protector oligo specific for the miR-92a binding site within *NF2*-3'UTR. Cell-based assays were then performed to investigate the functional consequences of miR-92a regulation on the tumor suppressor role of *NF2.* Overexpression of miR-92a in HCT116 and A549 cells increased migration and proliferation rates, inhibited apoptosis, and altered F-actin organization compared to control cells. Overall, our study demonstrates that miR-92a negatively regulates *NF2* at both transcriptional and post-transcriptional levels, resulting to inhibition of Merlin's tumor suppressor functions. Our results provide functional proof of the unappreciated role of miRNAs in *NF2* regulation and tumor progression.

Full list of authors: Krizelle Mae Alcantara, MS, University of the Philippines Diliman; Reynaldo L. Garcia, PhD, University of the Philippines Diliman

Acknowledgment: This work was supported by funding from the Disease Molecular Biology and Epigenetics Laboratory (DMBEL), National Institute of Molecular Biology and Biotechnology, University of the Philippines Diliman.

Development and Implementation of a Robust Screening and Analysis Pipeline to Identify Drugs and Drug Targets for NF2-Associated Schwannoma and Meningioma

Robert Allaway, PhD, *Sage Bionetworks*

Neurofibromatosis type 2 (NF2) predisposes patients to benign nervous system tumors such as meningiomas and schwannomas. Of particular note are vestibular schwannomas as they can cause hearing loss, facial nerve paralysis, and other symptoms. These tumors can form as a consequence of a germline *NF2* mutation and a successive somatic loss-of-function of the second allele of the *NF2* gene. The protein encoded by *NF2,* merlin, is a structural protein that regulates signaling mechanisms such as PI3K/Akt/mTOR, RTKs, HDACs, and HIPPO. Merlin's tumor suppression therefore may be the result of interactions with multiple molecular partners.

Under the sponsorship of the Children's Tumor Foundation, the NF2 Synodos Consortium, a group comprised of collaborating laboratories in the U.S. and Europe, is focusing on the development, implementation, and continuous improvement of a drug testing pipeline for NF2-associated tumors. Our goals are 1) to optimize the pipeline for further drug screens, making all data available to catalyze further NF2-relevant research and 2) to implicate drug targets. While our intent is to establish a robust, genetically accurate system that can be used for future drug screening directly relevant to NF2-associated tumors, we also aim to use this platform in the near-term to identify compounds that can be tested in human trials and are likely to have clinical benefit.

The initial screening strategy implemented by the NF2 Synodos Consortium involved testing a panel of drugs relevant to NF2 biology in the cells of origin for schwannoma and meningioma with intact and mutant NF2. Of the 19 tested drugs, the HDAC inhibitor panobinostat, the HDAC/PI3K inhibitor CUDC-907, and the PI3K/mTOR inhibitor GSK2126458 were identified as lead compounds. These were then tested in NF2-deficient xenograft and genetically engineered mouse models of meningioma and schwannoma, respectively. To elucidate critical signaling pathways and potential targets in merlin deficient tumors, we examined the effect of NF2 deficiency on the transcriptome and kinome of schwannoma and meningioma cells. We also studied drug induced perturbations to the transcriptome and kinome of these cells to identify potential drug response/resistance mechanisms and combination targets.

In summary, the approach used here facilitates the discovery of compounds that target schwannoma and meningioma, and enables the identification of single-agent and combination drug targets. Here, we present the summary of the data generated by the consortium along with the brief results. The raw and analyzed data will be released as a resource through Sage Bionetworks' Synapse platform (http://www.synapse.org/ synodosnf2) for continued NF2 research conducted by external researchers. We also showcase how these data can be interactively and visually interrogated with a tool developed by Sage Bionetworks (the Synodos Data Explorer, SyDE).

The NF2 Synodos Consortium (full list of members at "About Us" http://www.synapse.org/synodosnf2)

Funding organization: Children's Tumor Foundation

Investigation and Targeting Cellular Prion Protein PrP^C in Neurofibromatosis Type II Related Tumours

Sylwia Ammoun, PhD, *Plymouth University Peninsula Schools of Medicine and Dentistry, UK*

NF2 patients, in addition to multiple schwannomas, develop also multiple meningiomas and spinal ependymomas. Therefore, to find the best treatment for NF2 disease common therapeutic targets for all three tumours must be defined and inhibited by either mono- or combination therapy.

We hypothesise that one such target is cellular prion protein (PrP^C). Our hypothesis is based on our current results which demonstrate that (a) PrP^C protein is strongly overexpressed in schwannoma compared to control Schwann cells and tissues and that this overexpression is negatively regulated by Merlin and depends on NF kappa B; (b) PrP^C overexpression translates to other Merlin-deficient tumours since its levels are highly increased both in Merlin-deficient human mesothelioma cell line TRA and Merlin-deficient human meningioma grade I tissues and primary cells; (c) PrP^C increases schwannoma cell proliferation, survival and cell-matrix adhesion acting via 37/67kDa non-integrin laminin receptor (LR/37/67kDa) and downstream ERK1/2, PI3K/AKT, and FAK signalling pathways; (d) PrP^C is released from schwannoma cells, both via exosomes and also as free peptides suggesting that it may act in autocrine and/or paracrine manner; (e) Schwannoma cells and tissues display strong intrinsic overexpression of multi-drug resistance (MDR1) protein P-gp which decreases upon cell treatment with prion inhibitor TCS. Additionally the forced overexpression of *PRNP* in Schwann cells increases P-gp expression.

We therefore suggest that decreasing PrP^C levels either by using humanized anti-PrP antibody PRN100 (clinical trials are planned by MRC Prion Unit for sporadic Creutzfeldt-Jakob disease) or proteasome inhibitor (targeting NF kappa B) Bortezomib (FDA approved for the treatment of multiple myeloma); or targeting PrP^C receptor, LR/37/67kDa, using new range of inhibitors such as NSC47924 or antibodies could be a good therapeutic strategy for schwannoma and other Merlin-deficient tumours.

Full List Authors: Lucy Provenzano, University Peninsula Schools of Medicine and Dentistry (PUPSMD), UK; Yan Ryan, PUPSMD, UK; David Hilton, MD, PhD, Department of Cellular and Anatomical Pathology, Derriford Hospital, UK; Jade Lyons Rimmer, PUPSMD, UK; Foram Dave, PUPSMD, UK; Emmanuel Atangana Maze, MSc, PUPSMD, UK; Adams Claire, PhD, PUPSMD, UK; Rosemarie Rigby-Jones, Department of Cellular and Anatomical Pathology, Derriford Hospital, UK; Sylwia Ammoun Plymouth, PhD, PUPSMD, UK; C Oliver Hanemann, Professor, PUPSMD, UK.

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The Role of Human Endogenous Retroviral Proteins in the Development of Merlin-deficient Tumours and as Potential Drug Targets

Sylwia Ammoun, PhD, *Plymouth University Peninsula Schools of Medicine and Dentistry, UK*

There is no effective treatment for Merlin-deficient tumours. Over the last years we have defined several therapeutic targets and successfully tested various drugs, using human schwannoma *in vitro* model. We curently test one ot these drugs, Sorafenib in phase 0 clinical trial on NF2 patients. However, more targets are required because of the risk of the activation of alternative/parallel pathways contributing to tumour progression. We have also previously identified the overexpression of multi-drug resistance protein P-gp (phosphoglycoprotein) in schwannoma which suggest a possible risk of intincic drug resistance in this tumour.

In this study we have identified a new potential therapeutic target, for schwannoma and possibly other Merlin-deficient tumours, Human Endogenous Retrovirus type K (HERVK). Endogenous retroviruses are viruses that have integrated themselves into germ-line chromosomes and thereby become part of the host genome sequence. HERVK is overexpressed in several cancers, is reported to be involved in drug resistance in ovarian cancer and childhood leukaemia and activates pathways known to be involved in schwannoma development. Our preliminary data demonstrate overexpression of the HERVK envelope (env), capsid/gag, Np9 and Rec proteins in schwannoma cells and release of capsid/gag protein. Env expression is Merlin-dependent and decreases upon $CRL4^{DCAF1}$ knock down in schwannoma cells. HERVK env and capsid act via ERK/FAK/AKT pathways leading to tumour growth which we successfully inhibited *in vitro* using anti-HERVK antibodies and anti-retroviral drug Ritonavir. The proliferation of normal Schwann cells was not affected by any of the treatment. Additionally, pre-treatment of schwannoma cells with Ritonavir increased potency of Sorafenib and Selumetinib treatment. We hypothesise that HERVK is involved in the development, progression of schwannoma and is a good potential therapeutic target.

Full List Authors: Emmanuel Atangana Maze, MSc, Plymouth University Peninsula Schools of Medicine and Dentistry (PUPSMD), UK; Shona Reeves, PUPSMD, UK; Bora Agit, PUPSMD, UK; David Hilton, MD, PhD, Department of Cellular and Anatomical Pathology, Derriford Hospital, UK; Emanuela Ercolano, PhD, PUPSMD, UK; C Oliver Hanemann, Professor, PUPSMD, UK; Robert Belshaw, PhD, PUPSMD, UK; Sylwia Ammoun, PhD, PUPSMD, UK.

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Washington University NF1 Human Induced Pluripotent Stem Cell (iPSC) Repository

Corina Anastasaki, *Department of Neurology, Washington University School of Medicine, St. Louis, MO*

Neurofibromatosis type 1 (NF1) is characterized by great clinical heterogeneity, with medical symptomatology ranging from benign neurofibromas and malignant cancers to bone abnormalities and cognitive defects. One obstacle to developing effective treatments for NF1-associated cognitive and behavioral problems, caused by a germline *NF1* mutation, is the limited availability of human brain biospecimens. To address this barrier, we have established a repository of human induced pluripotent stem cells (hiPSCs) as a renewable source of human cells capable of forming most differentiated cell types. Our hiPSC repository contains three types of lines. First, we have generated a library of 12 hiPSC lines directly derived from human keratinocytes or bladder epithelial cells. Second, we have engineered a series of 7 heterozygous and two homozygous isogenic mutant hiPSCs, each harboring specific NF1-patient mutations on an identical genetic background. Lastly, we are generating conditional knockout hiPSCs with loxP sites flanking *NF1* exons 41-42 for conditional Cre-mediated *NF1* gene inactivation. Taken together, this repository constitutes a valuable resource for the NF1 research community.

Full List Authors: Corina Anastasaki[1], Michelle Wegscheid[1], Yi-Hsien Chen[2], Ludwine M. Messiaen[3], David H. Gutmann[1]

[1]Department of Neurology, and [2]Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110, USA, and [3]Department of Genetics, University of Alabama, Birmingham, Birmingham, AL 35294, USA

The Role of *EVI2A* and *EVI2B* in NF1 Tumors and Leukemias

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

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease affecting the development and growth control of a variety of tissues. NF1 represents a major risk factor for development of malignancies, particularly malignant peripheral nerve sheath tumors (MPNST), optic gliomas, and leukemia. The *NF1* gene spans about 350 kb of genomic DNA and encodes a transcript estimated at 11 to 13 kb containing 61 exons. Three genes, *EVI2A, EVI2B,* and *OMGP* are embedded within intron 27b of the *NF1* gene. These genes are transcribed in the direction opposite that of the *NF1* gene. Little is known about the function of these genes. Both *EVI2A* and *EVI2B* encode putative transmembrane proteins. The mouse homologs (*Evi-2a* and *Evi-2b;* ecotropic viral integration sites) are associated with viral insertions involved in leukemia in mice and its relation to NF1 symptoms is unknown. It has been already identified that *Evi2b* as a direct target gene of C/EBPa, a transcription factor critical for myeloid differentiation. It is possible that these genes are related to the leukemia observed in NF1 patients, although there is no data confirming this association. Expression of these genes is altered by viral integration and this altered expression may predispose cells to myeloid disease. These genes might act as a modifier in the NF1 phenotypic variations. Therefore we investigated *EVI2A* and *EVI2B* gene in NF1 tumors and leukemia.

We analyzed 10 NF tumors, 20 leukemia and 3 NF1-leukemia by PCR based technics. DNA samples were sequenced to detect variations in each exon. The pathological status of tumor tissues was confirmed by routine pathological examination. Standard immunohistological procedure was performed for EVI2B protein and S100 in tumor samples to prove the existance of Schwann cells.

Our preliminary results shows that there are viral integrations in both exons of *EVI2B* in 5 of the leukemia patients. EVI proteins were detected in the membrane structure. We have received the first data related to EVI genes which seems to be important in NF1 tumorigenesis.

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Full List of Authors: Parisa Sharafi PhD, TOBB ETU University, Faculty of Medicine.
Banu Anlar MD, Ali Varan MD, Mualla Cetin MD, Hacettepe University, Faculty of Medicine.

SMARCB1 Mutant Schwann Cells Secrete Factors that Induce Increased Pain Sensitivity

Fatima Banine, *Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR*

Pain is a significant symptom for many patients with schwannomatosis. We found that inducible conditional disruption of the *SMARCB1* 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 *SMARCB1* in Schwann cells demonstrate behavioral phenotypes consistent with increased pain sensitivity. We find that dorsal root ganglion (DRG) neurons from mice with Schwann cell-targeted disruption of *SMARCB1* 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 wild type DRG cells grown in *SMARCB1*-null Schwann cell conditioned media demonstrated elevated TRPV1 expression compared to cells grown with wild type Schwann cell conditioned media. We find that this effect was not due to transcriptional upregulation of the *Trpv1* gene. We have now performed a proteomic analysis of the conditioned media from *SMARCB1*-mutant Schwann cells and find evidence of a novel signaling cascade that results in Trpv1 upregulation in neurons. Collectively, these data indicate that loss of *SMARCB1* in Schwann cells leads to the secretion of a factor or factors that induce the expression of TRPV1 in sensory neurons through a mechanism that does not involve increased *Trpv1* transcription.

Full List of Authors: Fatima Banine[1], Steven Matsumoto[1], Scott Foster[1], Brian Hammond[1], Cristina Fernandez-Valle[2], and Larry S. Sherman[1]

[1]Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, USA and [2]Department of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL, USA

Targeting NF1-Dysregulated Tumors Using a Synthetic Lethal Approach

Stephanie J. Bouley, BA, *Geisel School of Medicine at Dartmouth*

Neurofibromatosis type 1, a genetic disorder caused by loss of the gene neurofibromin 1 (NF1), predisposes patients to the development of aggressive, NF1-deficient neurological tumors, including malignant peripheral nerve sheath tumors (MPNSTs), highly resistant sarcomas with a 5-year survival of 16-38% (1-4). While there are clinical trials investigating new therapies for NF1-deficient MPNSTs, such as MEK inhibitor therapy, there still exists a critical need for novel therapeutics (5). Our group addressed this clinical unmet need by developing a yeast-based screening platform to identify compounds that are synthetic lethal with NF1 loss. Yeast lacking the *NF1* homolog *IRA2* (NF1 mutant) were screened with drug-like compounds representative of a >300,000 compound library. Compounds were considered a hit if they inhibited the growth of NF1 mutant cells at concentrations that had no effect on wild-type control strains. Our first screen, carried out in collaboration with Dr. Nancy Ratner at the University of Cincinnati Drug Discovery Center, also included testing compounds in NF1 wild-type and mutant MPNST cell lines, and resulted in the identification of our first lead compound UC1 (6). Using a high-copy suppressor screen, we identified CDK9 as UC1's target. In a second screen carried out in our yeast platform, we identified several other lead compounds, including Y100 and Y102. Y102 treatment resulted in the accumulation of reactive oxygen species and the autophagy marker p62, as well as an altered mitochondrial phenotype in NF1-dysregulated cells. Treatment with Y102 also resulted in accumulation of the mitophagy marker BNIP3L/Nix. Together our findings suggest that Y102 may modulate mitophagy, a selective form of autophagy. Currently, we are implementing genetic, proteomic, and biochemical strategies to identify cellular targets of Y102 as well as Y100. I hypothesize that NF1-deficient cancers can be selectively targeted with the proposed mitophagy modulator Y102 and that modulation of autophagy/mitophagy is an effective therapeutic strategy in cancers with NF1 loss. We expect that this work will result in the identification of new targets and therapeutic leads for aggressive neurological cancers driven by NF1 loss like MPNSTs.

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Full List Authors: Robert J. Allaway PhD, Geisel School of Medicine at Dartmouth; Matthew D. Wood MD, PhD, Geisel School of Medicine at Dartmouth; Helen W. Hou, Geisel School of Medicine at Dartmouth; William Seibel PhD, Cincinnati Children's Hospital, University of Cincinnati; Nancy Ratner PhD, Cincinnati Children's Hospital, University of Cincinnati; Konstantin H. Dragnev MD, Geisel School of Medicine at Dartmouth, Norris Cotton Cancer Center; Yolanda Sanchez PhD, Geisel School of Medicine at Dartmouth, Norris Cotton Cancer Center

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Adeno-Associated Viral Vectors Delivery of Apoptosis-Associated Speck-Like Protein (ASC) Inhibits Schwannoma Tumor Growth *In Vivo*

Gary Brenner, MD, PhD, *Massachusetts General Hospital/Harvard Medical School*

Schwannomas are tumors of the schwann-cell lineage that can cause pain, sensory/motor dysfunction, and even death through compression of peripheral nerves, the spinal cord, and the brain stem. Schwannoma treatment represents a major unmet clinical need. Here we report that expression of the pro-apoptotic intracellular signaling protein, ASC/TMS1, is specifically inhibited in schwannoma cells and not in Schwann cells. This suggests that ASC/TMS1 could act as a tumor suppressor in schwannoma tumor cells and thus represents a candidate transgene for gene therapy.

Our data show that ASC/TMS1 is down regulated in the HEI-193 human schwannoma cell line, cultured primary human schwannoma cells, and in human NF2 and sporatic vestibular schwannomas. Hypermethylation of ASC/TMS1 was identified as one of the mechanisms responsible for its downregulation. Ectopic over-expression of ASC in cultured schwannoma cell-lines induced apoptotic cell death through induction of caspases 3 and 9, as well as the BH3 interacting death domain agonist (BID).

Using a human xenograft schwannoma model in mice, we show that intra-tumoral injection of an adeno-associated virus (AAV) serotype 1 vector encoding ASC under control of the schwann-cell specific promoter, P0, lead to reduction of tumor growth and concomitant resolution of tumor-associated pain, without causing any vector-mediated toxicity. In conclusion, our data suggest that ASC may act as a suppressor of schwannoma development and supports AAV-mediated ASC gene delivery as a promising strategy for schwannoma treatment in humans.

Sherif G. Ahmed[1], Abdelanabi Ahmed[1], Mohamed Doha[1], Farnaz Hadaegh[1], Jessica Sagers[2], Rebecca Lewis[2], Stankovic Konstantina[2], Alona Muzikansky[3], Maguire CA[4], Anat Stemmer-Rachamimov[5], Giulia Fulci[6] and Gary J. Brenner[1].

[1] Department of Anesthesiology, Critical Care, and Pain Medicine, Massachusetts General Hospital, Boston, MA, United States, 02114; [2] Eaton Peabody Laboratories, Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts, USA; [3] Bisotatistics Center, Massachusetts General Hospital, Boston, MA, United States, 02114; [4] Neuroscience Center, Department of Neurology, Massachusetts General Hospital, Boston, MA, United States, 02114; [5] Molecular Pathology Division, Massachusetts General, Boston, MA 02114; [6] Cancer Center, Massachusetts General Hospital, Boston, MA, United States, 02114.

The Missing Gap in Neurofibroma Development

Jean-Philippe Brosseau, PhD, *Department of Dermatology and Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas*

Neurofibromatosis type I (NF1) is the most common human genetic disorder of the nervous system and manifests through inheritance or sporadic mutation of the *NF1* tumor suppressor gene, a negative regulator of oncogenic p21-RAS, which predisposes NF1 patients to develop multiple tumors. Neurofibroma is the most common tumor in NF1 and typically presents itself as a disorganized peripheral nerve structure composed of proliferative Schwann cells (NFSC) mixed with neurofibroma-associated fibroblasts (NAFs) and other cell types in an abundant collagen matrix. Despite the fact that the critical role of fibroblasts has been extensively acknowledged in numerous diseases, the absolute requirement of fibroblasts and collagen as well as their pathogenesis for neurofibroma development remain unknown.

We unbiasely profiled the extra cellular matrix content of human neurofibroma by mass spectrometry to define the specific types of collagen that are abundant in those tumors. We subsequently validated our findings in additional sets of human and mouse neurofibromas. To begin delineating the neurofibroma cells producing these specific collagen types, we conducted a systematic screen to identify neurofibroma specific fibroblast markers. We are currently using these markers to evaluate the respective contribution of Schwann cells and fibroblasts to collagen deposition using our recently developed fast turnover assays modeling neurofibroma *in vitro* and *in vivo.* Our results support a new collagen paracrine signaling that may be of therapeutic benefit for NF1-patient. CPL (2014) and JPB (2016) are Children's Tumor Foundation Young Investigator Awardees. Research is partially supported by a 2015 Exploration-Hypothesis Development Award from the Department of Defense Neurofibromatosis Research Program.

Full list authors: Toan Nguyen, BS, UTSW; Chung-Ping Liao, PhD, UTSW and Lu Q Le, MD, PhD, UTSW.

MRI Texture Analysis for Identifying NF1 Radiogenomics: The Correlation Between NF1 Genotype and Imaging Phenotype

Wenli Cai, PhD, *Massachusetts General Hospital and Harvard Medical School*

Background: Radiomics is an innovative image analysis technique used for comprehensive quantification of tumor phenotypes by applying a large number of quantitative imaging biomarkers that describe the characteristics of tumor intensity, image heterogeneity, and tumor shapes. The identification of the linkage of tumor imaging phenotypes to the underlying genomic composition of the tumor is termed as radiogenomics. The underlying hypothesis is that genomic and proteomic patterns can be expressed in terms of macroscopic image-based biomarkers (radiomics). However, little is known to date regarding whether this kind of radiogenomics linkage exist between NF1 gene expression and MRI imaging biomarkers, i.e. the correlation between NF1 genotype and MRI phenotype.

Purpose: The purpose of this study was to investigate NF1 radiogenomics, i.e., the genotype-phenotype correlations between NF1 gene expression and MRI texture features, by using radiogenomics image analysis techniques.

Materials and Methods: Twenty-nine (29) NF1 subjects who had known germline mutations determined previously by targeted next generation sequencing (NGS) underwent whole-body MRI scanning using a fat suppressed fluid sensitive sequence (STIR). Each tumor was segmented and a set of fifty-nine (59) statistical and shape texture features was calculated using our in-house volumetric image analysis platform, "3DQI". A radiomics heatmap was generated after applying unsupervised hierarchical clustering of 59 image textures across all detected lesions. The clustered texture patterns were compared with gene mutation location and type, tumor type (DN: discrete form vs PN: plexiform), tumor size and locations to identify the genotype-phenotype correlations. A 5-fold cross-validation method was used to construct and validate the predictive model for distinguishing PN from DN using texture parameters in conjunction with random forest.

Results: A total of 218 neurofibromas (97 DN and 121 PN) were identified in 19 of the 29 subjects. Seven (7) major texture patterns were identified in the MRI radiomics heatmap. Statistical analysis revealed that gene mutation locations and types, and tumor types (DN vs. PN) were significantly associated with identified texture patterns ($P<0.01$), whereas tumor size and locations showed no correspondence with texture features ($P=0.79$ and 0.42, respectively). In addition, the predictive accuracy for distinguishing PN from DN was 76.7%, 84.5%, and 93.5% for tumors of >5cc, >20 cc and >60cc, respectively, by using the top five most important texture features.

Conclusion: This preliminary study has identified that specific gene mutations and tumor types in NF1 have strong genotype-phenotype correlations with MRI image texture patterns that warrant further investigation and validation.

Full list of authors: Yunpeng Liu, Justin Jordan, Miriam Bredella, Scott Plotkin, Gordon Harris
Affiliations: Massachusetts General Hospital and Harvard Medical School

Developing Animal Models with Patient Specific NF1 Mutations

Anil Kumar Challa, PhD, *Department of Genetics, University of Alabama at Birmingham*

Preclinical animal models to study NF1 disease *in vivo* have been previously limited to the use of null alleles, or conditional knockout alleles using cre-loxP technologies in the mouse. The UAB Neurofibromatosis Program is developing animals with patient-specific mutations to expand the repertoire of available models for studying disease processes and developing therapeutic interventions. Using conventional gene targeting in mouse embryonic stem (ES) cells, we have established and characterized mice harboring an NF1 nonsense mutation (corresponding to human variant c. 2041 C>T; p. Arg681*, *NF1*^{Arg681*}), as well as a missense mutation (c.2542G>C; p.Gly848Arg) associated with the development of multiple plexiform neurofibromas along spinal nerve roots ("spinal" NF). To accelerate the generation of animal models, we have recently employed the CRISPR-Cas9 system to create a workflow that can effectively engineer desired patient mutations in rodents and other species. Our initial efforts are focused on modifying the mouse ortholog with a 16 bp deletion in Exon 20 (c.2393_2408del16), an insertion (TT) in Exon 22 (c.2919_2920insTT), and a missense mutation in Exon 38 (c.5425C>T, p.Arg1809Cys). Additionally, mice and rats harboring the c.3827G>A, p.R1276Q mutation predicted to be located in a key domain important for *NF1*-GRD–Ras interactions, and associated with "spinal" NF are being developed. We are also making efforts to model a relatively frequent (34 reported cases) missense mutation at position c.1466A>G (p.Tyr489Cys) that appears to be pathogenic due to the activation of a cryptic splicing site in exon 13 and causing a subsequent 62bp deletion. This requires a larger modification to "humanize" the region by replacing the mouse exon 13 with the corresponding human exon. We present the design, methods and results from our ongoing efforts. Overall, our results indicate CRISPR/sgRNAs validated in cultured blastocysts can be used, along with synthetic single stranded oligonucleotides as homology-driven repair templates, to generate G_0 animals with targeted mutations, and the mutations are successfully transmitted through the germline.

Author List: Ashley N. Turner, MS, Laura Lambert, PhD, Shelly Graham, BS, Daniel Kennedy, BS, Leila S Quick, Bruce R. Korf, MD, PhD, and Robert A. Kesterson, PhD. Department of Genetics, University of Alabama at Birmingham.

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Merlin Plays an Important Role in Centrosome Disjunction

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

Neurofibromatosis type 2 (NF2) is a genetic disorder characterized by the development of multiple nervous system tumors, such as vestibular schwannomas. NF2 is caused by mutations in the *NF2* gene, which encodes the merlin protein that regulates multiple signaling pathways in several cellular compartments. In addition, somatic *NF2* mutations have been detected in multiple cancer types, including breast cancer. Presently, the mechanism by which *NF2* inactivation leads to tumorigenesis is not completely understood. We have shown that *NF2* is strongly expressed in the developing brain and in regions containing migrating cells, including the neural tube closure. Using *Nestin-CreER,* we demonstrated that *NF2* inactivation during early gestation impaired neuroprogenitor cell proliferation and caused neural tube defects. In contrast, mice with *NF2* inactivation during mid-to-late gestation developed schwannomas at a high frequency. Similarly, we showed that *NF2* inactivation in luminal epithelial cells during mid-to-late pregnancy using *Wap1-Cre* and during early pregnancy using *Blg-Cre* markedly decreased cell proliferation, leading to impaired lobuloalveolar morphogenesis. Interestingly, 100% of these mice with *NF2* knockout in mammary epithelial cells developed mammary tumors following multiple gestation cycles. The decreased cell proliferation during development and tumor formation at later stages due to *NF2* loss in neural and mammary epithelial cells implies that merlin either inhibits or supports cell proliferation depending on the biological context. To further examine the role of merlin during tumorigenesis, we found that mitotic neuroprogenitor and mammary epithelial cells lacking *NF2* displayed abnormal spindle formation and chromosome segregation due to defects in centrosome duplication and separation. We have generated merlin-deficient MCF10A mammary epithelial cells using shRNA or the CRISPR/Cas 9 technology. Both *NF2*-depleted and *NF2*-null MCF10A cells also exhibited abnormal centrosome separation, resulting in abnormal centrosome clustering or multiple centrosome formation. Double immunostaining detected merlin in the centrosomes. As β-catenin is a Nek2 substrate involved in centrosome separation, we found that depletion of merlin affected β-catenin phosphorylation at the centrosome. Together, these results suggest that merlin plays an important role during the centrosome separation process. Experiments are in progress to examine possible therapeutic implications of our findings.

Full List Authors: Long-Sheng Chang,[1,2,3] Jie Huang,[1,2] Elena Akhmametyeva,[1,2] Sarah Burns[1,2]
[1]The Research Institute at Nationwide Children's Hospital and [2]Department of Pediatrics, The Ohio State University

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Synthetic Lethality to BET Inhibitors in Malignant Peripheral Nerve Sheath Tumors

Jonathan M. Cooper, PhD, *UT Southwestern Medical Center*

BET bromodomain inhibitors have emerged as a promising therapy for numerous cancer types in pre-clinical studies, but molecular indicators of tumor response are lacking. Through modeling tumor evolution by studying genetic lesions underlying the development of neurofibromatosis type 1 (NF1)-associated malignant peripheral nerve sheath tumors (MPNST), we identified a BRD4 dependency in MPNST that serves as a controlled model system to delineate mechanisms of sensitivity or resistance to BET bromodomain inhibitors in this disease. In this study, we show that loss of P53 is associated with increased BRD4 levels and partial sensitivity to BET inhibition in MPNST, a lethal sarcoma with no effective medical treatment. Strikingly, genetic depletion of BRD4 profoundly sensitizes P53-inactivated MPNST cells to diverse BET inhibitors in culture and in vivo, while P53-wildtype normal cells are spared. Collectively, genetic and pharmacological inhibition of BRD4 reveals that BET inhibitor resistance in P53-inactivated MPNST requires wild-type BRD4 to support growth in a bromodomainindependent manner. Our findings provide a framework for understanding BET inhibitor sensitivity and resistance within distinct molecular subsets of MPNST. In addition, discovery that a synthetic lethality exists between P53 inactivation and reduced BRD4 levels nominates a P53-inactive/BRD4-low patient subpopulation as that which might best respond to a BET inhibitor therapeutic strategy for MPNST.

Author List: Amish J. Patel[1,2]*, Jonathan M. Cooper[1]*, Kun Chen[1,5], Zhiguo Chen[1,5], Chung-Ping Liao[1,5], Yong Wang[1], Lu Q. Le[1,3,4]
[1]Department of Dermatology, [2]Cancer Biology Graduate Program, [3]Harold C. Simmons Cancer Center, [4]UTSW Comprehensive Neurofibromatosis Clinic, University of Texas Southwestern Medical Center. [5]These authors contributed equally. *Co-first author.

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NF1 -/- SCs Resist Neuron-Activity Dependent Quiescence Signaling

Robert A. Coover, PhD, *Cincinnati Children's Hospital, Experimental Hematology and Cancer Biology*

Schwann cells (SCs) in the adult peripheral nerve are quiescent. Neuronal activity-dependent release of ATP is implicated in suppression of immature SC proliferation in culture; however, *in vivo* relevance has not been established. Here, we demonstrate that nerve activity suppresses both myelinating and non-myelinating SC proliferation *in vivo.* We blocked nerve conduction with bupivacaine hydroxide (BupOH) or tetrodotoxin (TTX) in wild type mice. At 5 days post treatment, proliferation was significantly increased. All proliferating cells were Sox10+, indicating that they are SCs, and a significant portion were myelinating SC determined by MBP association (~30%). We also administered intramuscular injections of apyrase to examine the effects of ATP removal in intact nerves. Proliferation significantly increased in SCs treated with apyrase vs heat inactivated apyrase. Notably, these SCs were also associated with myelin and co-labeled with SC markers of myelination. Thus, in the absence of activity-dependent quiescence signaling, mature SCs proliferate. We also demonstrate, *in vitro* and *in vivo,* that quiescence signaling is reduced in a common inherited disorder, neurofibromatosis type 1 (NF1). SCs with an inactivating mutation in *NF1* were resistant to growth suppression by ATP. Additionally, we determined that the P2y2 receptor is responsible for the growth suppressive effects of ATP in primary SCs and iHSCs. We characterized the stimulation of SCs by ATP and found that purinergic stimulation was reduced in mouse primary *NF1*-/- SCs as compared to wildtype cells and was rescued by increased levels of ATP. Proliferation resulting from SC specific conditional knock out of *NF1* in a GEM model of neurofibromatosis type 1 was suppressed by exogenously supplied ATP *in vivo.* Long term treatment of our NF1 GEM with ATP resulted in fewer and smaller plexiform neurofibroma, and reduced cell proliferation. Thus, nerve SC proliferation is normally under active suppression and purinergic stimulation may provide viable therapy for the treatment of glial derived neoplasms.

Full List Authors: Coover, Robert A.; Guo, Li; Chaney, Katherine; Aschbacher-Smith, Lindsey; Jankowski, Michael P.; Ratner, Nancy.

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Hematopoietic Cells in the *NF1* +/- Tumor Microenvironment Accelerate MPNST Development Without Altering Chemotherapy Response

Rebecca Dodd, PhD, *University of Iowa*

NF1 haploinsufficiency is a hallmark of neurofibromatosis type 1 and is important for neurofibroma development. 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 appear to have worse outcomes than patients with sporadic MPNSTs, but the underlying mechanism is not understood. To define the impact of the tumor microenvironment on MPNST biology and outcomes, we have developed unique mouse models that reflect the genetic status of *NF1* in the stroma of different MPNST patient populations.

We use Adenovirus-Cre injections to generate MPNSTs in $NF1^{Flox/Flox}$; $Cdkn2a^{Flox/Flox}$ and $NF1^{Flox/-}$; $Cdkn2a^{Flox/Flox}$ paired littermate mice to model tumors from NF1-wild-type and NF1-associated patients, respectively. We find that *NF1* haploinsufficiency (*NF1* +/- stroma) accelerates tumor onset, which is accompanied by an influx of immune cells comprised of CD11b+ cells, monocytes, and mast cells. Furthermore, mast cells are also enriched in human NF1-associated MPNSTs. Bone marrow transplants confirm that *NF1* +/- hematopoietic cells accelerate MPNST formation. Using these models in a preclinical trial, we demonstrate that *NF1* status in the tumor microenvironment does not alter MPNST response to multi-agent chemotherapy. Current studies are combining CRISPR/Cas9 technology with Cre-driven lineage-specific depletion to further define the population of myeloid cells influencing MPNST biology. We are investigating the biological mechanisms and therapeutic implications of these myeloid populations. Taken together, these studies are clarifying the role of the *NF1* haploinsufficient tumor microenvironment in MPNSTs.

Additional Authors: Chang-Lung Lee (Duke University), Tess Overton (Duke University), Wesley Huang (Duke University), William Eward (Duke University), Lixia Luo (Duke University), Yan Ma (Duke University), Davis Ingram (MD Anderson Cancer Center), Keila Torres (MD Anderson Cancer Center), Diana Cordona (Duke University), Alexander Lazar (MD Anderson Cancer Center), and David Kirsch (Duke University)

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Systematic Evaluation of SRC and MEK Inhibitors as Potential Therapeutics for NF2-associated Schwannomas: Results of *In Vitro* and *In Vivo* Screens

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

Loss of merlin function in Schwann cells causes deregulated signal transduction from multiple receptor tyrosine kinases leading to activation of downstream kinases including MEK (MAPK/ERK kinase) and SRC. Over the last six years, we have established a rapid drug screening platform to accelerate development of drug therapies for NF2-associated schwannomas. Our three step testing funnel consists of: 1) cell based drug screens using mouse and human merlin-deficient Schwann cells, followed by 2) *in vivo* validation using a mouse allograft model, and finally 3) confirmation of drug efficacy in primary cultures of human vestibular schwannoma (VS) cells with known *NF2* gene mutations. Using this paradigm, we evaluated four SRC kinase inhibitors and five MEK inhibitors approved by the FDA or in clinical trials for oncology individually and for some in combination. We found that multiple SRC inhibitors caused a strong G1 cell cycle arrest at nanomolar concentrations but had modest activity *in vivo.* Two MEK inhibitors reduced cell viability with a high maximal effect (>80% loss of viability) and low nanomolar IG_{50} concentrations, and triggered caspase dependent apoptosis. *In vivo,* one MEK inhibitor significantly reduced schwannoma growth in the two week drug study. The combination of SRC and MEK inhibitors together was not superior to the effect of MEK inhibitor alone in the allograft model. Vestibular schwannoma cells grown in the presence of varying concentrations of SRC and MEK inhibitors responded with a loss of viability ranging between 10-40% of the vehicle control during the 48 hour assay. These results support further investigation of SRC and MEK inhibitors as therapeutic agents for patients with NF2 or sporadic schwannomas with *NF2* gene mutations.

Alejandra Petrilli, PhD, Marisa A. Fuse MS, Maria Clara Franco PhD, Stephani Klingeman Plati MS, Konstantin Brjnos
Neuroscience Division, Burnett School of Biomedical Science, College of Medicine, University of Central Florida
Christine Dinh, MD, Xue-Zhang Liu, MD, PhD, Fred F. Telishci, MD. Department of Otolaryngology, University of Miami Miller School of Medicine

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Dual Inhibition of c-Met and Src Induces Apoptosis of Neurofibromatosis Type 2-Associated Schwannomas

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

Neurofibromatosis type 2 (NF2) is a benign tumor disorder caused by a loss of function of the merlin tumor suppressor. Mainstream treatment options for NF2 have been limited to surgery and radiotherapy, however, off-label use of FDA-approved cancer drugs is becoming more common. C-Met and Src kinase are upregulated in human vestibular schwannomas and promote increased proliferation of Schwann cells, suggesting that they would be promising targets for NF2 therapeutics. Here we show that the dual inhibition of c-Met and Src kinase with cabozantinib and saracatinib reduced growth of merlin-deficient mouse Schwann cells (MD-MSC) *in vivo* in an orthotopic sciatic nerve mouse model by inducing caspase-dependent apoptosis. When given in combination, cabozantinib and saracatinib were more effective in reducing graft size over 14 days than either drug alone. Whereas inhibition of c-Met and Src kinases individually reduced viability of cultured MD-MSC by triggering a G1 cell cycle arrest, the drug combination induced caspase-dependent apoptosis downstream of Fas receptor activation. Moreover, the combination of cabozantinib and saracatinib reduced growth of primary human vestibular schwannoma cells in culture. This pre-clinical study demonstrates that simultaneous inhibition of c-Met and Src signaling in MD-MSC triggers apoptosis and reveals vulnerable pathways that can be exploited to develop effective NF2 therapies.

Full list of authors: Stephani Klingeman Plati, M.S., University of Central Florida; Christine T. Dinh, M.D., University of Miami Miller School of Medicine; Julia Soulakova, Ph.D., University of Central Florida; Alicja J. Copik, Ph.D., University of Central Florida; Christophe J. Echeverri, Ph.D., Cenix BioScience GmbH; Xue Zhong Liu, M.D., Ph.D., F.A.C.S., University of Miami Miller School of Medicine; Fred F. Telischi, M.E.E., M.D., F.A.C.S., University of Miami Miller School of Medicine; Long-Sheng Chang, Ph.D., Nationwide Children's Hospital; Maria Clara Franco, Ph.D., University of Central Florida; Cristina Fernandez-Valle, Ph.D., University of Central Florida.

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Use of Tumor-Secreted Exosomes to Define New Biomarkers and Targets to Prevent Malignant Peripheral Nerve-Sheath Tumor Transformation

Teresa González Muñoz, MS, *Microenvironment and Metastasis Group, Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain*

The development of dermal and plexiform neurofibromas and their transformation into malignant peripheral nerve sheath tumors (MPNSTs), a highly aggressive sarcoma with poor prognosis, are serious complications of neurofibromatosis type 1 (NF1). At the moment, there is not a reliable method to early detect this NF1 malignant transformation. Although it is known that the NF1 microenvironment contributes to the initiation and progression of these tumors, relevant tumor-stromal interactions remain incompletely understood. We have recently demonstrated that tumor-derived exosomes can support primary tumor growth and metastatic progression in other cancer types. Here, we report for the first time evidences that circulating exosomes may be useful to monitor neurofibroma transformation and/or MPNST. We isolated and characterized exosomes from human MPNST cell lines and plasma from 19 NF1 patients. We found that the number and protein content of exosomes secreted by human MPNST cell lines and plasma circulating exosomes from NF1 patients were significantly increased when compared to controls. Mass spectrometry analysis of exosomes derived from MPNST cell lines showed endoglin, a transforming growth factor beta (TGF-beta) co-receptor with an important function in angiogenesis, as one of the top candidates to be secreted by MPNST tumor cells. Furthermore, specific targeting of endoglin decreased significantly MPNST growth and metastasis into lymph nodes *in vivo.* Overall, our data suggest that analysis of circulating exosomes in NF1 patients may be useful for early detection of subjects with malignant transformation of neurofibroma and support the use of endoglin as a new potential target to block NF1 progression.

Author List: Angela Di Giannatale MD[2], Claudia Savini PhD[1], Susana García-Silva PhD[1], Alberto Benito-Martin PhD[3], Cristina Merino BSc[1], David Pisapia MD[4], Wu Jianqiang MD[5], Nancy Ratner PhD[6], Kaleb Yohay MD[7], and Héctor Peinado PhD[1,3].

[1]Microenvironment and Metastasis Group, Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO), Madrid, Spain; [2]Department of Oncohematology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [3]Children's Cancer and Blood Foundation Laboratories, Department of Pediatrics, Drukier Institute for Children's Health, Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA; [4]Department of Neurosurgery, Weill Cornell Medicine, New York, NY, USA; [5]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA; [6]Department of Pediatrics, Cancer and Biology and Neural Tumours Program, Cincinnati Children's Hospital, Cincinnati, OH, USA; [7]Department of Neurology, New York University Langone, New York, NY, USA.

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Consequences of RTK Amplification and p53 Loss in MPNSTs

Jamie Grit, BS, *Van Andel Research Institute*

Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive, chemoresistant sarcomas that develop in patients with neurofibromatosis type 1. Loss of *TRP53* and amplification of the receptor tyrosine kinase (RTK) *MET* is observed 25-50% of cases. To investigate the role of MET and p53 in MPNST progression, we developed three MPNST mouse models: an *NF1* null and MET overexpression MPNST model (NF1-MET), an *NF1* and *TRP53* heterozygous knockout model (NF1-p53), and a *NF1* null model (NF1). Using orthotopic tumorgrafts from these models, we tested the efficacy of MET (capmatinib) and MEK (trametinib) inhibition on MPNST growth. Trametinib moderately inhibited tumor growth in all of the models; however, combined inhibition of MEK and MET was highly effective in both the NF1-MET and NF1 models. NF1-p53 tumors were the least responsive to combined inhibition and upregulated AKT signaling in response to single agent therapy. These data suggest that alternate RTK signaling pathways downstream of MET may compensate for MEK inhibition, and that p53 loss promotes kinome reprogramming in response to targeted inhibition in MPNSTs. To determine the effects of MEK, MET, PI3K, and mTOR inhibition on kinome signaling, we generated MPNST cell lines from each of our MPNST models. Western blot analysis revealed that NF1-MET MPNST cells are highly sensitive to MET inhibition, NF1-p53 MPNST cells maintain ERK and AKT activity in the presence of MET inhibition, and NF1-p53 MPNST cells uniquely upregulate MEK signaling in response to PI3K/mTOR inhibition. These results suggest p53 plays a unique role in kinome reprograming and resistance to kinase inhibitors. Currently, we are determining the effects of combined kinase inhibition on proliferation of MPNST cells and utilizing reverse phase protein arrays to evaluate the effect of MET and MEK inhibition on the phospho-proteome in our MPNST models. By understanding these complex signaling networks we will identify combinations of targeted therapies that can be used to effectively treat MPNSTs.

Author List: Matt Pridgeon, MD, Van Andel Research Institute, Spectrum Health; Elizabeth Tovar, PhD, Van Andel Research Institute; Jackie Peacock, PhD, Van Andel Research Institute; Julia Wulfkuhle, MD, George Mason University; Emanuel Petricoin, MD, George Mason University, Carrie Graveel, PhD, Van Andel Research Institute; Matt Steensma, MD, Van Andel Research Institute, Spectrum Health

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Plasma and Cerebrospinal Fluid Pharmacokinetics of Selumetinib in Non-Human Primates (NHP)

Andrea Gross, MD, *Pediatric Oncology Branch, National Institutes of Health*

Background: Selumetinib (AZD6244 hyd sulfate) is an orally bioavailable agent targeting mitogen-activated protein kinase (MEK) 1/2. Selumetinib has shown activity in children with NF1 plexiform neurofibromas and refractory low-grade gliomas. The central nervous system (CNS) penetration of selumetinib in humans is unknown.

Methods: We analyzed selumetinib in the cerebrospinal fluid (CSF) of NHP as a surrogate for CNS tissue penetration. Four adult male rhesus macaques were each given a single dose of selumetinib capsules 2.5 mg/kg (human equivalent dose 50 mg/m^2) by mouth on an empty stomach. Selumetinib was quantified in serial paired plasma and CSF samples from 0 to 48 hours after administration by Covance Bioanalytical Services (lower limit of quantification 0.5 ng/mL). Free selumetinib was estimated at 2.3% of total based on reported plasma protein binding of 97.7% in NHP. Pharmacokinetic (PK) parameters including area under the curve of selumetinib concentration versus time (AUC) were calculated using non-compartmental methods and CSF penetration was calculated from the ratio of $AUC_{(CSF)}$: $AUC_{(plasma)}$.

Results: Selumetinib was detected in plasma in all 4 NHPs, but in the CSF of only one NHP (ZH60). The mean ($\pm$ SD) $AUC_{0-\infty}$ and $t_{1/2}$ in plasma were 3350 $\pm$ 489 ng•h/mL and 10.8 $\pm$ 2.5 hours, respectively. In ZH60, the CSF AUC_{0-48} hr was 10.6 ng•h/mL. The CSF penetration of total and free selumetinib in this animal was 0.4%, and 14%, respectively. The mean plasma AUC and $t_{1/2}$ were comparable to that of children (2842 ng•h/mL and 6.9 hours, respectively) at a similar dose in a phase I trial. However, the average peak concentration in children occurred at approximately 1 hour with a C_{max} of 841 ng/mL, while the C_{max} in NHP plasma ranged from 1 – 4 hours and averaged 198.8 ng/mL.

Conclusions: The CSF penetration of oral selumetinib is limited; in one NHP it was 0.4% and 14% when corrected for plasma protein binding of selumetinib. The fact that plasma $AUC_{0-\infty}$ and half-lives in NHP are similar to previously reported human plasma PK indicates that this model may accurately reflect human CSF penetration as well.

Full List Authors: Andrea M. Gross, Cindy McCully, Katherine E. Warren MD, Brigitte C. Widemann MD; Pediatric Oncology Branch, National Cancer Institute.

Polo-like Kinase 1 as a Therapeutic Target for Malignant Peripheral Nerve Sheath Tumors

Jianman Guo, PhD, *Perelman School of Medicine, University of Pennsylvania*

Neurofibromatosis type 1 (NF1) patients have a high risk for malignant peripheral nerve sheath tumors (MPNST), which are often inoperable and do not respond well to current chemotherapies or radiation. To identify potential therapeutic targets in MPNSTs, we screened the MPNST cell line ST88-14 against ~2000 drugs of known mechanisms of action, including ~600 cancer relevant drugs. The screen identified four inhibitors of Polo-like kinase 1 among the most potent hits. Since Ras transformed cells are especially sensitive to PLK1 inhibitors and our earlier work identified PLK1 as part of the aurora kinase pathway in MPNST, we explored PLK1 and its relationship to aurora kinase in MPNST. PLK1 inhibitors were potent inhibitors of MPNST regardless of p53 and NF1 status, and also inhibited NF2 null cell lines. Furthermore, one PLK1 inhibitor, BI6727, in both single dose and combined with an Aurora kinase inhibitor, MLN8237 stabilized tumor volume and significantly increased survival of mice with MPNST xenografts. Therefore, PLK1 may be a therapeutic target for MPNSTs.

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Author list: Jianman Guo, PhD[1], Katherine E. Chaney, PhD[2], Gabriela Witek, PhD[1], Ami V. Patel, PhD[2], Hong Xie, PhD[1], Danny Lin, PhD[1], David C. Schultz, PhD[1], Jeffrey Field, PhD[1] and Nancy Ratner, PhD[2].
[1]University of Pennsylvania, Philadelphia, PA; [2]University of Cincinnati, Cincinnati, OH, USA

Decoding NF1 Microdeletions, Microinsertions and Nonsense Mutations

Meng-Chang Hsiao, PhD, *Department of Genetics, University of Alabama at Birmingham*

Microdeletions, microinsertions (<21 bp) and nonsense mutations contribute to ~48% of all mutations leading to NF1 (Messiaen and Wimmer, 2008). However, the extent to which specific mechanisms are involved in causing these mutations has not been well explored yet. Here, we analyzed the frequency and characteristics of 2,116 microdeletions/microinsertions and 1,731 nonsense mutations (out of a total number of 8,100 unrelated *NF1* mutation-positive probands) from the UAB Medical Genomics Laboratory and inferred their likely mutational mechanisms. Regarding microdeletions and microinsertions, 16 hotspots were identified (each with a frequency > 0.1%), which are highly associated with non-B DNA structures and short tandem repeats. On the other hand, the majority of the *NF1* nonsense mutations are associated with CpG dinucleotides (1,046/1,731, 60%). Particularly, 18 nonsense mutation hotspots were identified (each with a frequency > 0.45%), which all occurred at CpG dinucleotides. Only one CpG dinucleotide that theoretically could result in a nonsense mutation (c.8371C>T, p.R2791*, located in the last exon (exon 58)) was never observed yet in our cohort, nor in any other database. Furthermore, it is believed that a termination codon must reside further than 50-55 nucleotides upstream of the splice donor of the penultimate exon, in order for nonsense-mediated mRNA be able to decay the transcripts (Popp and Maquat, 2016). However, the *NF1* mutational spectrum does not show *any* truncating mutations beyond c.8135, which is 179 nucleotides before the exon 57 end. This study provides perspective in molecular diagnostics as well as in molecular mechanisms for NF1 etiology and suggests that truncating mutations downstream of c.8135 may not result in an NF1 phenotype.

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Full List Authors: Meng-Chang Hsiao PhD, Yunjia Chen PhD, Tom Callens BS, Ludwine Messiaen PhD (Department of Genetics, University of Alabama at Birmingham, Birmingham, Alabama)

Investigating the Role of Adenylate Cyclase on cAMP-dependent Kinase (PKA) Activation and Adenosine Receptor Expression in *NF1*-null MPNST Cells

JoAnna E. Hughes, MS, *Hood College, Frederick, MD*

The second messenger, cyclic-AMP (cAMP), is produced by adenylate cyclase (ADCY), and previous reports indicate that cAMP expression is altered in response to *NF1* mutations via mechanisms that are as yet unclear. There are nine transmembrane isoforms of ADCY and one soluble isoform expressed in a tissue-specific manner. The effects of these isoforms vary between types including the activation or inhibition of cAMP-dependent protein kinase (PKA). The expression profile of ADCY isoforms differs between normal Schwann cells and malignant peripheral nerve sheath tumors (MPNSTs) which may account for the change in cAMP expression after loss of *NF1.* Futhermore, ADCY expression and function is modulated by the G-protein coupled adenosine receptors, encoded by the *ADORA* genes, which may have either stimulating or inhibiting effects on the production of cAMP. We aimed to overexpress ADCY isoforms in *NF1*-null MPNST cells as a means to dissect the mechanism by which cAMP expression is altered after *NF1* loss.

We confirmed variations in ADCY transmembrane isoforms between non-tumor and MPNST cell lines, and ADCY3, 6, 7, and 9 were chosen as a focus in this study due to their varied expression between the cell lines. In untreated non-tumor cells, there was a significant decrease in adenosine receptor 1 (*ADORA1*). *ADORA1* stimulates the MAPK and phospholipase C (PLC) pathways, which inhibits ADCY. Whereas in MPNST cells, *ADORA2B,* which also stimulates the MAPK pathway, showed the highest expression. While overexpression of ADCY isoforms did not change PKA activity in a statistically significant manner, overexpression of ADCY9 led to significantly increased expression of *ADORA1* in MPNSTs. There were also significant changes in *ADORA* between cell types depending upon which ADCY isoform was overexpressed. Two *ADORA* isoforms had decreased expression in MPNST cells when accompanied by ADCY 3 or 7 overexpression. Overexpression of ADCY3 was associated with a decrease in *ADORA1* expression, whereas ADCY7 overexpression was associated with decreases in *ADORA1* and *ADORA2A* which increases cAMP levels by activating ADCY.

These data indicate that changes in ADCY and *ADORA* expression in MPNSTs may account for changes in cAMP expression after loss of *NF1.* Further studies are needed to assess the impact of adenosine receptors and ADCY/cAMP expression on Schwann cell tumor proliferation and NF1 protein function.

Additional Authors: Georgette N. Jones, PhD, Hood College, Frederick, MD

Perturbation of the Hyaluronan-Rich Nerve Sheath Tumor Microenvironment to Improve Drug Efficacy and Delivery

Bryant J. Keller, BS, *Department of Genetics, Cell Biology, and Genetics, Masonic Cancer Center, University of Minnesota – Twin Cities*

Neurofibromatosis type 1 (NF1) is a genetic disease caused by a mutated copy of the gene encoding the Neurofibromin protein (*NF1*). Biallelic inactivation 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 NF1 patients. These MPNSTs are the deadliest of the soft tissue sarcomas, showing a 40% 5-year survival rate. The tumors prove very difficult to treat due to their location and what we believe to be a constrictive microenvironment which collapses the tumor vasculature and raises internal pressure. Although targeted drugs have been implemented, nonspecific chemotherapy and surgical resection remain the only standard of care. We hypothesize that the lack of efficacy is due not to the potency of the drugs, but to drug delivery in the tumors.

Pancreatic ductal adenocarcinoma exhibits elevated levels of hyaluronic acid (HA) in the extra cellular matrix (ECM) which constricts the microenvironment; collapsing vasculature and increasing interstitial fluid pressure. This greatly decreases drug penetration. A PEGylated recombinant human hyaluronidase (PEGPH20) has been implemented with traditional chemotherapeutics to break down this HA barrier and open the environment to improve drug efficacy and delivery in both mice and humans.

We show that human neurofibromas and MPNSTs, and Genetically Engineered Mouse Peripheral Nerve Sheath Tumors (GEMPNSTs), contain high HA levels and collapsed vasculature. Microarray data from Neurofibroma and MPNST Schwann cells reveals overexpression of the Hyaluronan Synthase 2 (*HAS2*) gene. In our GEMPNST models, treatment with PEGPH20 not only degrades the surrounding HA but facilitates increased penetration of the small molecule drug doxorubicin.

Longevity and pharmacodynamics studies are being implemented with doxorubicin and two targeted therapies (RAD001, an mTOR inhibitor and PD-0325901, a MEK inhibitor). Both targeted drugs individually have induced highly significant increases in longevity in the GEMPNSTs. We hypothesize the observed monotherapy effects reflect a system highly amenable to combination therapy. Therefore, we are testing RAD001 and PD-0325901 in combination for efficacy. We are also assessing the ability of these drug combinations to further synergize with PEGPH20 which, if given prior to treatment, can lead to increased drug delivery in tumors. Doxorubicin, which has a modest affect in our GEMPNST model, is also being tested in combination with PEGPH20 to analyze treatment efficacy and drug penetration. Altogether, we believe that degradation of the tumor-associated HA in nerve sheath tumors will lead to increased drug perfusion/localization and provide therapeutic benefits.

Other Authors: Adrienne Watson PhD[2], Kyle Williams PhD[1], Rory Williams BS[1], Leah Anderson BS[1], Marjorie Carlson BS[1], Justin Knight BS[1], Colleen Forster BS[3], Kwangmin Choi BS[4], Nancy Ratner PhD[4], Co-Senior: Paolo Provenzano PhD[1], Co-Senior: David Largaespada PhD[1] Affiliations: [1]University of Minnesota, [2]Recombinetics Inc., [3]BioNet, [4]Cincinnati Children's Hospital

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NF1 Mutation Structure-Function Analyses Using a Full-Length Mouse cDNA

Bob Kesterson, PhD, *University of Alabama at Birmingham*

To determine the functional consequences of NF1 patient mutations and variants of unknown significance, we have established a heterologous cell culture expression system using a full-length mouse *NF1* cDNA and human cell lines. We demonstrate that a full-length mouse *NF1* cDNA (longest known coding *NF1* mRNA of 2841 amino acids) placed downstream of the CMV promoter will produce a >250 kDa neurofibromin protein that is capable of modulating Ras signaling when expressed in wild type or in *NF1*-deficient cells. For the latter, knockout HEK293 and human iPS cell lines have been established wherein *NF1*-deficiency was induced via CRISPR/Cas9-induced genome editing. When the mNf1 cDNA is transiently transfected into HEK293 cells (or expressed in stable cell lines), Western blotting shows overexpression of neurofibromin in both *NF1* replete ($NF1^{+/+}$) and deficient ($NF1^{-/-}$) cells. Moreover, the re-expression of mouse neurofibromin from the cDNA is able to suppress the elevated pERK seen in the $NF1^{-/-}$ HEK293 cells, thereby demonstrating that the mouse neurofibromin protein is functional in the human cell line. Ongoing studies using site-directed mutagenesis to establish mutant cDNAs representing NF1 patient variants and presumptive mutant alleles will be shown, wherein mutant cDNAs are assessed for their ability to produce mature neurofibromin and restore significant *NF1* activity (suppress elevated p-ERK, alter cAMP levels, and other kinase pathways) when compared to the WT cDNA. Initial characterization of different cDNAs representing various types of predicted mutations including out-of-frame deletions (c.2393_2408del16), nonsense point mutants (c.2041C>T, p.R681*), and missense mutations (c.5425C>T, p.Arg1809Cys and c.3827G>A, p.R1276Q) associated with different phenotypes clinically will be presented. We demonstrate that a relatively frequent cryptic splicing mutation (c.1466A>G, p.Tyr489Cys reported 34 cases that leads to a 62bp deletion in exon 13) will however produce functional neurofibromin if a full length mRNA is made (i.e. mouse p.Tyr489Cys *NF1* cDNA is functional). The ability to express mouse *NF1* cDNAs in human cell lines has also led to the validation of antibodies that recognize mature and truncated versions of neurofibromin proteins with different specificity to either C-terminus (SC-67, rabbit polyclonal) or N-terminus (H-12, mouse monoclonal) regions.

Kairong Li, PhD, Mei-Jan Chen, MD, Anil Challa, PhD, Jing Li, Ashley Turner, Deeann Wallis, PhD, and Bruce R. Korf, MD, PhD. Department of Genetics, University of Alabama at Birmingham.

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Pathway Dependence and Drug Synergism in NF1-Associated Malignant Peripheral Nerve Sheath Tumors Using the Zebrafish Model

Dong Hyuk Ki, PhD, *Dana-Farber Cancer Institute and Boston Children's Hospital*

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are very aggressive and often metastatic soft tissue sarcomas, which are frequently found in patients who have neurofibromatosis type 1 (NF1). Currently, surgical excision is the only curative therapy for MPNST, although many patients have unresectable or metastatic tumors at diagnosis and the recurrence rate after surgery is high. Chemotherapy regimens are only partially effective and associated with significant toxicity that can severely reduce quality of life.

Objective: To develop a faithful *in vivo* MPNST transplantation assay to determine drug efficacy and host toxicity to identify promising drugs and drug combinations. Our long range goal is to define active combinations of three or more non-cross resistant drugs that exhibit synergistic and sustained tumor cell cytotoxicity at dosages tolerable to the developing recipient zebrafish.

Methods: Primary MPNSTs were harvested from *sox10:mCherry;nf1a+/-;nf1b-/-;p53m/m* zebrafish and the cells were mechanically dispersed. Approximately 100-120 MPNST cells were implanted into embryos at 2 day post-fertilization (dpf), either into the posterior aspect of the yolk cell or into the pericardium. Twenty-four hours after implantation, the fluorescent tumor cross-sectional area was imaged and MEK, Hsp90, mTOR, topoisomerase inhibitors at various concentrations, or DMSO vehicle were added to the fish water. The transplanted embryos were incubated for 4 days in the vehicle or each individual drug. Quantitative assessment of the cross-sectional area of remaining fluorescent tumor cells was performed at 7 dpf and fish were raised in the absence of drug and imaged every 7 days to assess the durability of the response.

Results: The pericardial transplantation assay proved superior, because the tumor cells formed an easily quantifiable mass in a region that lacks non-specific fluorescence and the tumor cells grew vigorously in vehicle treated fish over the 4-day incubation period. Drugs in clinical use for MPNST such as PD-0325901, ganetespib, and AZD2014 showed drug responses at their maximum tolerated doses in the pericardial implantation assay, and topotecan elicited the best cytotoxic response against transplanted MPNST cells.

Conclusion: We developed a robust *in vivo* pericardial transplantation model to test drug efficacy using our zebrafish model of MPNST. We identified topotecan as a promising anti-tumor drug for NF1-associated MPNST, and we are now assessing FDA approved drugs to identify those that synergize with topotecan to induce sustained MPNST cell death at tolerable dosages for the host.

Full List Authors: A. Thomas Look MD, Dana-Farber Cancer Institute and Boston Children's Hospital.

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Investigating RAS and RTK Signaling in MPNST Progression and Drug Resistance

Candace N. King, *Van Andel Institute Graduate School, Grand Rapids, MI*

Neurofibromatosis type 1 (NF1) is an inherited tumor predisposition syndrome affecting 1 in 3000 live births. NF1 is caused by an autosomal dominant mutation resulting in the loss of function of NF1, which encodes for the tumor suppressor neurofibromin. Loss of heterozygosity of NF1 in Schwann cells leads to the development of the benign plexiform neurofibroma (PNF) and 10% of these develop into malignant peripheral nerve sheath tumors (MPNST). Several studies have implicated the receptor tyrosine kinases (RTK) MET and EGFR in NF1-related MPNST disease progression; however the necessity for RTK signaling in the context of NF1 loss and Ras activation is unclear. Genomic amplification of *MET* (25%), *EGFR* (25%), and *PDGFRA* (29%) frequently occurs in MPNSTs. Our studies of NF1 in the context of *MET* amplification revealed that NF1-MET MPNST xenografts are more sensitive to MET inhibition compared to MEK inhibition. Unfortunately, clinical studies using the EGFR inhibitor erlotinib were unsuccessful in MPNSTs and suggest that compensatory signaling pathways may drive MPNST resistance to single kinase inhibition. Since MET signaling has been found to compensate for EGFR inhibition in lung cancer and BRAF inhibition in melanomas, we predict that drug resistance may be driven by similar kinome adaptations in MPNSTs. We hypothesize that coordinated Ras and RTK activation supports robust kinome signaling and MPNST drug resistance, which may be abrogated by combination kinase inhibition. We are using human MPNST cells to determine the genomic context that influences RTK and MEK signaling in NF1-related MPNSTs and identify therapeutic drug combinations that prevent drug resistance. Using Western blot analysis we have evaluated the MET, EGFR, ERK, and AKT signaling activity in human MPNST cells lines to determine the potential dependence each line has on RTK signaling. We also performed a CytoSNP array to determine the genomic status of NF1 along with major RTKs and possible undiscovered therapeutic targets. Currently, we are investigating the effects of the tyrosine kinase inhibitors capmatinib (MET), erlotinib (EGFR), and trametinib (MEK) on cell viability and RTK signaling. Overall these studies will add to the knowledge base of mechanisms that drive MPNST RTK signaling, drug response, and mechanisms of resistance.

Carrie Graveel, PhD. Laboratory of Musculoskeletal Oncology. Van Andel Institute. Grand Rapids, MI
Matt Steensma, MD. Laboratory of Musculoskeletal Oncology. Van Andel Institute. Grand Rapids, MI

LATS1 Phosphorylates FERM Domain of ERM Proteins to Modulate its Activity

Shinji Kuninaka, MD, PhD, *Division of Gene Regulation, Institute for Advanced Medical Research, Keio University School of Medicine*

Lats1 is a main effector kinase of the Hippo pathway which is activated by the FERM domain protein Merlin. To elucidate a physiological role of LATS1, we performed phosphoproteomic screen using LATS1 kinase and found ERM (ezrin-radixin-moesin) protein as a new substrate. LATS1 directly and preferentially phosphorylated FERM domain of RADIXIN (RDX) *in vitro.* LATS1's phosphorylation sites were clustered at F3 lobe of the FERM domain which included previously known activation phosphorylation site (Thr235). When serine/threonine residues at the F3 lobe were substituted with aspartic acid (D), it lost ability to interact with a tail domain, suggesting LATS1 activates ERM proteins. Furthermore, our genetic analysis showed the lethal mutant of *Drosophila moesin* (*Moesin*^{G0323}) was rescued by ectopic expression of *moesin* 4D, in which serine/threonine residues of F3 lobe was substituted with aspartic acid. These results suggest LATS1 as a new regulator of ERM proteins via FERM domain phosphorylation.

Tatsuyuki Chiyoda,[1] Takayuki Kuraishi,[3] Nobuyuki Tanno,[2] Yuji Otsuki,[2] Kiyomi Kimura,[2] Daisuke Aoki,[1] and Hideyuki Saya[2]

[1]Department of Obstetrics and Gynecology, [2]Division of Gene Regulation, Institute for Advanced Medical Research, [3]Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo 160-8582, Japan

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TRAPping the Metabolic Adaptations of NF1-Associated Tumors

Ionica Masgras, PhD, *University of Padova, Padova, Italy*

The numerous emerging inter-connections between oncogenic signaling cascades and changes in cell metabolism have identified the metabolic rewiring of tumors as a novel and potentially successful therapeutic target. We have recently demonstrated that dysregulation of Ras/ERK transduction axis impacts on tumor metabolism through phosphorylation and thus activation of the mitochondrial chaperone TRAP1, which mediates inhibition of mitochondrial respiration and leads to succinate-dependent stabilization of the pro-tumorigenic transcription factor HIF1alpha (1). These findings have raised the attention on the potential contribution of metabolic changes in neurofibromatosis type 1 (NF1)-associated cancers, where hyper-activation of Ras/ERK signaling is crucial.

Here we show that inhibition of TRAP1 expression in *NF1*-deficient DNSCs (mouse embryonic day 13.5 dorsal root ganglia (DRG)/Nerve root sphere cells), the cells of origin of neurofibromas in a NF1 mouse model, strongly impairs tumor development; furthermore, TRAP1 silencing is associated with significant changes in the metabolic phenotype of tumor cells as expression of several glycolytic markers is markedly reduced. We have also exploited the possibility that increased TRAP1 expression level could be associated with neurofibroma progression toward MPNST (malignant peripheral nerve sheath tumors) when additional loss of tumor suppressor genes, i.e. p53, occurs. We find that increased TRAP1 expression exacerbates the shift toward the glycolytic metabolism occurring after p53 loss, suggesting a plausible synergism in promoting the tumorigenic potential of *NF1*-deficient cells.

Our work sheds light on TRAP1-mediated metabolic adaptations in neurofibromas, which could provide selective advantages to NF1 tumor cells. Understanding these metabolic changes and their possible implication in malignant progression of neurofibromas has the potential to pave the way for the design of novel anti-neoplastic drugs for NF1-associated tumors against which there are no current effective treatments.

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Author List: Ionica Masgras PhD, University of Padova; Zhiguo Chen PhD, MD, University of Texas Southwestern Medical Center; Marco Pizzi MD, University of Padova; Lu Q. Le, PhD, MD, University of Texas Southwestern Medical Center; Andrea Rasola PhD, University of Padova.

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Dissecting the Role of the CRMP2-Neurofibromin Complex on Pain Behaviors

Aubin Moutal, PhD, *University of Arizona*

Neurofibromatosis type 1 (NF1) is a relatively common genetic disease primarily linked to nervous system tumors that are associated with significant chronic pain. Enhanced pain sensitivity in NF1 patients may involve the sensitization of small-diameter nociceptive sensory neurons that are known to mediate nociceptive neurotransmission. Membrane relocalization of the presynaptic N-type voltage-gated Ca^{2+} channel (CaV2.2) facilitates calcitonin gene-related peptide (CGRP) release, a neurotransmitter involved in pain. CRMP2, a protein interacting with Neurofibromin, is an important mediator of CaV2.2 trafficking. Here, we examine *how* the CRMP2-neurofibromin complex controls the activity of CaV2.2. A peptide-tiling array identified a peptide within CRMP2 that bound the C-terminus of neurofibromin: CRMP2 Neurofibromin regulating peptide 1 (t-CNRP1; where tat is the charged transduction domain from HIV-1). We tested if uncoupling the neurofibromin-CRMP2 signaling cascade could (1) affect Ca^{2+} channel activity and trafficking in sensory neurons; (2) regulate evoked release of CGRP in sensory neurons from wildtype and $NF1^{+/-}$ mice; (3) induce functional phenotypic changes in sensory neurons; and (4) affect nociceptive function in a rodent model of neuropathic pain. t-CNRP1 inhibited K^{+}-evoked Ca^{2+} influx in sensory neurons, decreased CaV2.2 membrane localization, which was related to inhibition of the CaV2.2/CRMP2/neurofibromin protein complex as evidenced by GST-pull down and proximity ligation assay (PLA). t-CNRP1 decreased the evoked release of CGRP from $NF1^{+/-}$ mice sensory neurons. Functional fingerprinting of sensory neuron population using constellation pharmacology showed phenotypic changes in sensory neurons treated with t-CNRP1. Finally, intrathecal administration of t-CNRP1 reversed nociceptive behavior in rodent models of inflammatory pain and post-surgical pain. These results identify t-CNRP1 as a novel tool that uncouples the CRMP2/neurofibromin and CRMP2/CaV2.2 interactions, curbs CaV2.2 activity, CGRP release and reverses nociceptive behavior, thus highlighting the potential therapeutic significance of targeting the neurofibromin-CRMP2-CaV2.2 axis for treatment of pain.

Xiao-Fang Yang[1], Aubin Moutal[1], Yue Wang[1], Shreya Sai Bellampalli[1], Angie Dorame[1], Lindsey A. Chew[1], Erik T. Dustrude[1,4], Brian S. Schmutzler[4,5], May Khanna[1], Cynthia M. Hingtgen[4,5,6], Todd Vanderah[1,2] and Rajesh Khanna[1,2,3,]*

Departments of [1]Pharmacology, [2]Anesthesiology, and [3]Neuroscience, College of Medicine, University of Arizona, Tucson, AZ 85724, USA; [4]Paul and Carole Stark Neurosciences Research Institute, Departments of [5]Pharmacology and Toxicology, and [6]Neurology, Indiana University School of Medicine

*Corresponding Author: Dr. Rajesh Khanna, Department of Pharmacology, College of Medicine, University of Arizona, USA Office phone: (520) 626-4281; Fax: (520) 626-2204; Email: rkhanna@email.arizona.edu

A Strategy to Identify an Effective Therapy for NF2-Associated Vestibular Schwannomas Revised

Janet Oblinger, PhD, *Nationwide Children's Hospital & The Ohio State University*

Current treatment options for neurofibromatosis type 2 (NF2)-associated vestibular schwannomas (VS) and meningiomas are limited to surgery and radiation; however, serious complications can occur with these treatments, and radiation can induce secondary malignancies. Also, incomplete tumor resection is not uncommon and is a main cause of tumor recurrence. Development of an effective medical therapy for NF2-associated tumors is urgently needed. Previously we reported that while the histone deacetylase inhibitor AR-42 caused tumor regression in *NF2*-deficient meningiomas, it merely inhibited tumor growth in schwannomas. Similar findings were observed in a clinical trial of AR-42 in NF2 patients. Meningiomas treated with AR-42 exhibited tumor regression; in contrast, AR-42 only slowed VS growth. In one patient with multiple tumors, his meningiomas remained small while the growth of his VS rebounded after cessation of AR-42 treatment. These results suggest that a more effective treatment of VS may require a drug combination. To investigate possible underlying causes for the differential response of meningiomas and VS to AR-42, we performed mutational analysis of tumors from two NF2 patients. In addition to *NF2* mutations, VS from both patients harbored mutations in the *NUP98* gene. Also, a duplication of exon 2-3 in the *MYC* gene was detected in one of the patients. Intriguingly, we detected the same genetic changes in the left and right VS and meningioma from the same patient. Immunostaining revealed strong nuclear MYC staining in 20 VS analyzed, but meningiomas showed little nuclear MYC expression. Depletion of MYC by shRNAs suppressed VS but not meningioma cell growth, suggesting that drugs targeting MYC expression may be effective in VS. Consistently, we showed that the bromodomain inhibitor JQ1, which transcriptionally downregulates MYC, inhibited VS cell proliferation. Combining JQ1 with AR-42 resulted in enhanced growth suppression. We are presently investigating additional drug combinations with the ultimate goal of identifying an effective therapy for VS. Further, we have established telomerase-immortalized cell lines from NF2 patient tumors for further investigation.

Full List Authors: Sarah Burns[1,2], Janet Oblinger[1,2], Elena Akhmametyeva[1,2], D. Bradley Welling[3,4], Long-Sheng Chang[1,2,3]

[1]Center for Childhood Cancer and Blood Diseases, Nationwide Children's Hospital, Departments of [2]Pediatrics and [3]Otolaryngology, The Ohio State University, and [4]Department of Otolaryngology, Harvard Medical School/Massachusetts General Hospital

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Natural Silvestrol-Related Rocaglates as Potential Treatments for Vestibular Schwannomas and Meningiomas

Janet Oblinger, PhD, *Nationwide Children's Hospital & The Ohio State University*

Vestibular schwannomas (VS) and meningiomas are intracranial tumors that are often caused by inactivation of the *NF2*/merlin tumor suppressor gene. These tumors cause serious morbidities, including deafness, vertigo, facial paralysis, hydrocephalus, cranial nerve palsies, seizures, and brainstem compression. Currently, surgical excision and radiation are the treatment options for these tumors, since an FDA-approved targeted therapy is not yet available. We and others have previously shown that VS and meningiomas often exhibit high levels of activated AKT, which can promote protein biosynthesis. As uncontrolled growth of tumor cells often requires a high degree of protein translation, we found that both of these tumors frequently over-express eIF4A, eIF4E and eIF4G, components of the eukaryotic initiation factor 4F (eIF4F) complex that critically regulates protein translation initiation. Intriguingly, the eIF4A inhibitor silvestrol, which is a member of the rocaglate family isolated from *Aglaia* plants in tropical rainforests, was consistently found to be a potent inhibitor of VS and meningiomas. However, due to its complex structure and large molecular weight, silvestrol has suboptimal pharmacokinetic and pharmacodynamic properties. To this end, we have investigated the growth-inhibitory activity of 10 silvestrol-related compounds isolated from *Aglaia perviridis.* These compounds have the same scaffold as silvestrol, but lack the bulky, sugar-like dioxanyl ring that is thought to hinder silvestrol's bioavailability. We found that three of these silvestrol related rocaglates, didesmethylrocaglamide, methyl 4′-demethoxy-3′,4′-methylenedioxyrocaglate, and rocaglaol strongly inhibited the growth of schwannoma and meningioma cells with IC_{50} values similar to or better than that of silvestrol (less than 100 nM). Importantly, the IC_{50} values for these rocaglates in normal meningeal cells were greater than 300 nM, suggesting an improved therapeutic window. Like silvestrol, didesmethylrocaglamide and rocaglaol decreased the levels of phospho-AKT, PCNA, cyclin D1, and Aurora A. Studies are in progress to verify the *in vivo* efficacy of these compounds in our tumor models.

Full List Authors: Janet Oblinger[1,2], Sarah Burns[1,2], A. Douglas Kinghorn[4], Long-Sheng Chang[1,2,3]

[1]Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Children's Hospital, Departments of [2]Pediatrics & [3]Otolaryngology, The Ohio State University, [4]Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy

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

Understanding the Structure-Function Relationship of NF1-G Proteins Interaction

Dipak N. Patil, PhD, *The Scripps Research Institute*

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, a 320 kda protein which contains several domains. NF1-GRD domain that serves as a negative regulator of Ras by its GAP domain is thought to be responsible for most of the biological activities. However, the molecular targets of NF1 in many processes are not clearly understood. Hence, unfurling of the physiological functions of NF1 through gaining better knowledge of the signaling pathways associated with NF1 could lead to identification of new therapeutic targets. Our study is focused on understanding the role of NF1 in GPCR signaling through Gβγ subunit.

In our study, we found that activation of GPCR can dynamically regulate association between Gβγ and NF1. We found direct interaction of Gβγ to NF1 Sec14/PH module. Using Ras GTP hydrolysis assay, we observed that the binding of Gβγ to NF1 inhibits its ability to inactivate Ras. Based on this, we tried to understand the structure-function relationship of the Gβγ-Sec14/PH interaction and pathogenic mutations in Sec14-PH domain found in NF1 patients. We also tested the role of lipid specifically PIP2 in stabilizing the Gβγ-Sec14/PH complex. In this direction, to evaluate the role of residues involved in the interaction to understand the role of pathogenic mutations along with basis of inhibition of GAP activity, the structure of the NF1-Gβγ complex and it's the physiological consequences are currently under investigation.

Full List Authors: Dipak N. Patil, PhD; Keqiang Xie, PhD; Kirill A. Martemyanov, PhD;

The Scripps Research Institute, Jupiter, FL 33458, USA.

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Frequency of Germline Pathogenic Variation in *NF1* and Eight Other RASopathy Genes in the Exome Aggregation Consortium (ExAC) Database: A Pilot Study.

Alexander Pemov, PhD, *Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD*

ExAC is the largest public database of human variation, comprising high-quality variant calls across 60,706 human exomes. Importantly, the curators of the database "have made every effort to exclude individuals with severe pediatric diseases from the ExAC data set," which makes the database a valuable resource for studying variation in Mendelian disease genes in unaffected populations. The RASopathies are a group of inherited disorders caused by mutations in predominantly RAS-MAP kinase pathway genes. We downloaded all coding, non-synonymous germline variants in nine genes: *NF1, HRAS, KRAS, NRAS, BRAF, RAF1, SPRED1, MAP2K1* and *MAP2K2* from the database (excluding 7,601 The Cancer Genome Atlas (TCGA) samples). There were 1,188 mutations in the nine genes (range: 36 (*NRAS*) to 498 (*NF1*), of which almost half were found only once in the dataset and 76% had frequency below 0.01%. We then analyzed the variants using InterVar (ACMG/AMP 2015 guideline) method and phenotype-genotype databases to predict the pathogenicity of the variants. As expected, the majority of the variants were classified as "benign," "likely benign," and "variants with unknown significance." We also compared the ExAC variants with the variation annotated in the Human Gene Mutation Database (HGMD). In the nine genes, we found 60 variants present in both HGMD and non-TCGA ExAC. Forty-one variants were found in *NF1* alone, of which nine were loss-of-function or canonical splice-site mutations. After excluding variants for which the evidence of pathogenicity was less convincing (labeled "DM?" in HGMD), there were 28 disease-causing mutations. Assuming that there was one *NF1* mutation per individual, this observation implies that in ExAC the frequency of *NF1* variants associated with neurofibromatosis type 1, is 28/$(60{,}706_{Total}\text{-}7{,}601_{TCGA})$=0.00053 (~1/1,900), which is within the range of published NF1 prevalence in the general population. Review of the primary literature of the pathogenicity of the 28 published *NF1* variants is underway. There were eight "pathogenic" or "likely pathogenic" mutations in four genes (*H-, K-, NRAS* and *BRAF*), all of them were rare, with frequencies ranging $8.2X10^{-6}$ – $2.5X10^{-5}$. There were no "pathogenic" or "likely pathogenic" variants in *BRAF, RAF1, SPRED1, MAP2K1* or *MAP2K2.*

Conclusions: In this pilot study of a large exome database, we found that prevalence of pathogenic variation in *NF1, BRAF, H-, K-* and *NRAS* is close to published estimates. Future work focuses on phenotype spectrum and penetrance of NF1 and other RASopathies in large, exome-sequenced populations linked to an electronic medical record.

Full list authors: Jung Kim, PhD and Douglas R. Stewart, MD, both Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD

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The BCR-ABL Inhibitors Ponatinib and Dasatinib Have Cytostatic Effects on Merlin-Deficient Human Schwann Cells and Cultured Primary Vestibular Schwannoma Cells

Alejandra Petrilli, PhD, *University of Central Florida*

Mutations in the *NF2* gene cause neurofibromatosis type 2 (NF2), a non-malignant tumor-forming disease of the nervous system. Currently, there are no FDA approved drug therapies for NF2. Work by us and others has shown that NF2 schwannomas and schwannoma cells have higher levels of activated SRC and receptor tyrosine kinases such as platelet derived growth factor receptor, and ephrin receptors than normal human Schwann cells (HSC) and nerve. Ponatinib, a third generation BCR-ABL/SRC inhibitor, is a multi-tyrosine kinase inhibitor that targets key proliferation pathways overactive in schwannomas, and received FDA approval in 2012 for leukemia. We found that ponatinib decreased the viability of multiple merlin-deficient HSC lines in a dose dependent manner. When merlin-deficient and merlin-expressing HSC were cultured in the absence of serum and mitogens, merlin-deficient HSC were significantly more sensitive to ponatinib than the merlin-expressing HSC.[1] Ponatinib was more potent than dasatinib, another FDA approved second generation BCR-ABL inhibitor in reducing cell viability. Similarly, flow cytometry studies revealed that ponatinib was more potent than dasatinib in triggering a G1 cell cycle arrest of the merlin-deficient HSC. Moreover, in agreement with the merlin-deficient HSC results, ponatinib also decreased viability of cultured human vestibular schwannoma cells. Results of ponatinib and dasatinib efficacy in an orthotopic allograft NF2 schwannoma mouse model will be presented. These studies are expected to identify an FDA approved BCR-ABL drug as a potential treatment for NF2 schwannomas.

Full List Authors: Marga Bott MS, University of Central Florida, Marisa Fuse MS, University of Central Florida, Stephani Klingeman Plati MS, University of Central Florida, Alicja Copik PhD, University of Central Florida, Christine Dinh MD, University of Miami Miller School of Medicine, Olena Bracho BS, University of Miami Miller School of Medicine, Xue-Zhong Liu MD, PhD, FACS, University of Miami Miller School of Medicine, Fred Telischi MEE, MD, FACS, University of Miami Miller School of Medicine, and Cristina Fernández-Valle PhD, University of Central Florida.

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A Functional and Connectomic Approach to Understanding Neurocognitive Deficits in Mouse Models of NF1

J. Elliott Robinson, Postdoctoral Scholar, *California Institute of Technology*

Up to 80% of patients with neurofibromatosis type 1 (NF1) exhibit neurocognitive deficits, including impaired executive functioning, learning disabilities, and attention deficit hyperactivity disorder (ADHD). Transgenic mouse models reveal that ADHD-like symptoms may be due to developmental perturbations in dopaminergic projection neurons, which regulate attention, reward processing, and motivated behavior. We are employing novel viral vectors; advanced technologies for circuit mapping, such as *in vivo* and *ex vivo* optogenetics, CLARITY, neurite tracing, and mGRASP (mammalian GFP reconstitution across synaptic partners); and 2-photon calcium imaging to investigate how abnormal circuit structure and function influences behavioral phenotypes in two different mouse models of NF1.

Authors: J. Elliott Robinson, Elisha Mackey, and Viviana Gradinaru

Affiliation: California Institute of Technology, 1200 E California Blvd, Pasadena, CA 91125

Tissue-engineered Skin Model Derived from Neurofibromatosis Type 1 Patients to Study Cutaneous Tumor Genesis

Vincent Roy, MSc, *Departement of Surgery, Faculty of Medecine, Laval University; Regenerative Medecine, Centre de Recherche du CHU de Québec-Laval University, LOEX/HEJ, CHU de Québec-Laval University*

Background: Neurofibromatosis type 1 (NF1) is an autosomal dominant multisystemic disorder caused by aberrations in the neurofibromin gene. Typically, patients develop multiple cutaneous tumours that grown from axon of peripheral nerve, called neurofibromas and schwannomas. These benign tumours are generally composed of Schwann cells (SC) and fibroblasts, but others cells type can also be found. Highly variable clinical manifestations between NF1 patients are observed. Actually, there is no specific treatment for this stigmatizing disease.

Objective: The purpose of this study is to develop a tissue-engineered human skin model derived from NF1 patients to characterized and understand the formation of neurofibromas and schwannomas.

Methods: We used a spheroid suspension culture method to generated neurofibroma-like and schwannoma-like tumours. Schwannomas are composed of immortalized SC isolated from an NF1 tumor and neurofibromas are composed of an equal number of the same SC lineage and fibroblasts isolated from skin biopsies. The auto-assembly model was used to generate tissue-engineered skin (TES) *in vitro* with fibroblasts and keratinocytes isolated from NF1 patients (n=3). Spheroids were added within the skin between the dermis and the epidermis.

Results: We first determined the best conditions for the formation of spheroids. Surface area of spheroids was significantly increased already at day 3 and continued until day 10 after seeding. Spheroids growth was significantly faster than control cells. Immunofluorescence revealed that spheroids/tumors-like, seeded with NF1-TES, are in a proliferative state. Furthermore, non-apparent activation of apoptosis within spheroid is detected. Histological analysis revealed similar patterns normally found in schwannomas and neurofibromas.

Conclusion: Our NF1 skin model could become a unique tool to better characterize the pathogenic mechanism associated with skin tumor genesis in NF1. Tumorigenic properties of different *NF1* mutations could also be assessed. Ultimately, it could provide better tools to develop new therapies for patients through development of precision/personalized medicine strategies.

Vincent Roy[1,4], Lydia Touzel-Deschênes[1,4], Édouard Marques[1,4], Todd Galbraith[1,4], Peter Kannu[2], Hélène T. Khuong[3,4], Nicolas Dupré[1,4] and François Gros-Louis[1,4]

[1]Departement of Surgery, Faculty of Medecine, Laval University;
[2]Departement of Paediatrics, Faculty of Medecine, University of Toronto;
[3]Departement of Neurological Sciences, Faculty of Medecine, Laval University;
[4]Regenerative Medecine, Centre de Recherche du CHU de Québec-Laval University, LOEX/HEJ, CHU de Québec-Laval University.

Mammary Adenocarcinoma is Highly Penetrant in NF1-deficient Rats with Evidence of Genotype-Phenotype Correlation

Matt Steensma, MD, *Van Andel Research Institute; Helen DeVos Children's Hospital; Michigan State University College of Human Medicine; Grand Rapids, MI*

Introduction: Breast cancer is an established phenotype of Neurofibromatosis Type 1 (NF1). Epidemiological studies confirm that women with NF1 maintain a significantly increased risk of developing breast cancer under the age of 50. Recent work also suggests that NF1-related breast cancer survival is diminished regardless of age at presentation, gender or tumor receptor status. Moreover, up to 25% of sporadic breast cancers harbor *NF1* mutations and comprehensive genomic analyses have revealed that *NF1* may be an important driver in sporadic breast cancer. These data are compelling for the role of neurofibromin-mediated tumor suppression in breast cancer development and disease progression. Our work addresses a current gap-in-knowledge as to how germline *NF1* deficiency influences cancer development in the breast.

Methods: We present the first germline NF1-deficient rats bearing mutations in the GAP-related domain (GRD) of the *NF1* gene ortholog. Germline *NF1*-haploinsufficiency was induced using CRISPR-Cas9 gene editing in the Sprague-Dawley rat using a targeted 50bp deletion in the GRD, as well as random indels. Since non-homologous end joining (NHEJ) repair that follows CRISPR-Cas9 generated double strand breaks often results in variability at the target site and short flanking regions, we were able to generate two classes of *NF1* mutants: (i) distinct "in frame" missense germline mutations (-57del; -63del; -54del) in 3 rats, and (ii) distinct "frameshift" mutations that result in altered protein coding and premature stop codons (+1, -8, +4, -10; -11,-20; -11,-4) in 3 rats. Male and female G_0 animals were crossed to wildtype Sprague-Dawley rats to confirm germline transmission and phenotypes.

Results: Germline *NF1* deficient female rats exhibit penetrant mammary adenocarcinoma beginning at 4-8 weeks of age in the G0, G1, and G2 generations. Histologic examination revealed the early presence of hyperproliferation in mammary tissue (~3 weeks of age), along with classic findings of ductal carcinoma in situ adjacent to sites of adenocarcinoma. Spontaneous lymph node metastasis was consistently observed. Both missense and nonsense mutant rats exhibited a range of multiple primary tumors along the ventral mammary stripes: G_0=2-7 sites; G_1=0-10 sites; G_2=0-10 sites. Based on pedigree analysis, the breast cancer phenotype was significantly more penetrant but not exclusive to the nonsense mutant lines. Immunohistochemistry analysis of breast tumors verified ER+/PR+/Her2+ expression in the vast majority of tumors. Neurofibromin expression was abrogated in the breast neoplasia, but was variably present in normal tissues. These results strongly support the role of *NF1* as a breast cancer driver and highlight potential differences in the potency of various GRD mutations.

Patrick Dischinger, VAI; Curt Essenburg, VAI; Elizabeth Tovar, PhD; Jamie Grit; Candace King; Bruce Korf, MD, PhD; Ashley Turner, MS; Anil Challa, PhD; Robert Kesterson, PhD; Carrie Graveel, PhD

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Cancer Stem Cell as the Target for MPNST Tumorigenesis and Relapse

Daochun Sun, PhD, *Brain Center, Memorial Sloan Kettering Cancer Center, New York, NY*

Malignant peripheral nerve sheath tumor (MPNST) is a type of soft tissue sarcoma that arises from the neural crest lineage and commonly associated with nerve trunks. Surgical removal is the major treatment conjugated with chemo/radiation therapy when applicable. However, the complete tumor clearance is limited by the resectability, especially when associated with large peripheral nerve. The local recurrence of MPNST is 40~45% with high morbidity.

We previously harnessed a specific transgene, using the components of rat endogenous *Nestin* promoter and enhancer, to find a subset of glioblastoma (GBM) cells that are relatively quiescent and show cancer stem cell properties. This transgene has herpes simplex virus thymidine kinase gene (*TK*) and green fluorescent protein (*Nes-TK-GFP*), and labels a small quiescent cell population in MPNSTs from both *cisNf1*$^{+/-}$*;Trp53*$^{+/-}$ (*cisNP*) spontaneous model and skin progenitor (SKP) based allograft model. Ganciclovir chow treatment kills the dividing cells expressing the transgene and significantly decreases the tumor growth in both models. We also found that the transgene labeled GFP positive cells enrich in the sciatic nerve region compared to the distal tumor mass in MPNST allografts. Tumor primary culture from the sciatic nerve vicinity forms more and significantly larger spheres *in vitro* than the distal tumor mass. The BrdU and EdU sequential labeling assay after chemotherapy regimens in MPNST allografts shows that the GFP positive cells can continuously repopulate the tumor in a hierarchical organization. These results indicate that the transgene labeled cells may serve as CSCs to propagate tumor growth and relapse after the chemotherapy, which provides a novel therapeutic strategy to MPNST.

Author List: Zilai Wang PhD, Elsa Vera PhD, Sameer Farouk Sait MD and Luis Parada PhD, Memorial Sloan Kettering Cancer Center
Funding: Department of Defense Congressionally Directed Medical Research Programs

Meta-analysis of Neurofibromatosis 1 (NF1) and Internalizing Outcomes

Leah R. D. Thomas, Student, *California Polytechnic State University, San Luis Obispo*

Background: Neurofibromatosis type 1 (NF1), an autosomal genetic disorder affecting about 1 in 3,000 persons, is associated with a variety of medical, developmental, cognitive, and behavioral outcomes. Individuals with NF1 are at risk for various mental health difficulties, with many studies reporting increased rates of anxiety and depressive disorders. To date, however, the strength of the association between NF1 and internalizing outcomes (e.g., anxiety and depression) remains unclear. Thus, the objective of this meta-analysis is to summarize risk for internalizing outcomes in individuals with NF1 and to explore how sample-level and methodological factors are associated with the strength of association between NF1 and internalizing outcomes.

Methods: This meta-analysis is part of a larger, ongoing meta-analysis examining a range of neuropsychological, cognitive, academic, and behavioral outcomes in NF1 samples. PubMed, Science Direct and Web of Science were used to find all relevant studies meeting the inclusion criteria of case-control studies with NF1 patients with anxiety, depression, and emotional difficulties. All included studies have patients with NF1 and healthy or community sampled controls. Random effects meta-analysis will estimate the mean of all relevant true effects. Subgroup analysis, meta-regression analyses, and quality assessment analyses will examine the influence of sample and methodological variables on the observed effect size.

Results: The literature search generated 4,578 unique studies, of which 336 studies were reviewed based on their full text. We are currently performing data extraction and expect that approximately 20-30 studies will be included in this meta-analysis in the end.

Conclusion: This meta-analysis will further our understanding of the prevalence and severity of internalizing disorders affecting NF1 patients. This will help professionals to support the emotional needs of individuals with NF1.

Full List Authors: Michael Vargas B.S., California Polytechnic State University, San Luis Obispo; Jacklyn Engle, Student, California Polytechnic State University, San Luis Obispo; Alan Aung, Student, California Polytechnic State University, San Luis Obispo; Maria T. Acosta MD, Children's National Health System & George Washington University; Jessica A. Kaczorowski Ph.D., California Polytechnic State University, San Luis Obispo, Taylor F. Smith Ph.D., California Polytechnic State University, San Luis Obispo;

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Leveraging the Macrophage Bioenergetic Profile in Neurofibromatosis Type 1

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

Emerging evidence suggests that macrophages play a critical role in several manifestations of NF1 including cardiovascular disease, malignant neurofibroma transformation, osteopenia, and glioma formation. Unfortunately, pharmacotherapies directed at suppressing macrophage recruitment, inhibiting macrophage function, or limiting the effects of macrophage byproducts has yielded mixed results with some pre-clinical models demonstrating disease progression. These findings reflect a poor understanding of macrophage function in NF1-related disease and represent a critical barrier to developing strategies directed at neurofibromin-deficient macrophages. Therefore, we sought to define neurofibromin's role in macrophage metabolism and polarization. Previous research has shown increased macrophage mobilization in NF deficient mice in response to LPS as well as higher levels of pro-inflammatory monocytes in the circulation, which corresponds with M1 macrophage polarization. Based on our previous observations, we hypothesize that neurofibromin-deficient macrophages exhibit a bioenergetic profile that enhances autonomous cell survival and provides energy substrates for their client cells within the microenvironment. BMDM isolated from wild type (WT) and neurofibromin-deficient mice (NF1 flox/flox Lysm+) were incubated with interferon-gamma (IFN-gamma) and LPS to induce an M1 phenotype and characterized with the Seahorse XF analyzer, measurement of reactive oxygen species (ROS) and nitric oxide (NO) production, glucose uptake analysis and an mRNA microarray of metabolic related genes. We also began to investigate the effect of BMDMs on the local environment by incubating endothelial cells (EOCs) with BMDM conditioned media and observing tube formation, migration and proliferation of EOCs. Unstimulated (M0) NF1-/- macrophages exhibit an altered bioenergetic profile when compared with WT macrophages as evidenced by increased glycolytic capacity and basal oxygen consumption. Co-incubation with Ras inhibitors suggests glycolysis and mitochondrial respiration in NF1-/- macrophages is likely mediated via activation of the PI-3K-Akt signaling pathway. NF1-/- M0 cells also took up more glucose and had altered levels of glycolytic enzyme mRNA. The difference in bioenergetic potential observed in NF1-/- M0 cells was amplified when incubated with IFN-gamma and LPS to induce an M1 macrophage. Conditioned media from NF deficient M1 macrophages had altered levels of end point metabolites. When applied to EOCs this media elicited changes in tube formation and proliferation when compared to media obtained from WT M1 cells. Our results show NF deficient macrophages have an altered metabolic phenotype and that the end products released into the environment have a distinct effect on BMDM client cells. These results indicate an importance of BMDM metabolism and warrants further investigation in pursuit of novel therapeutic targets.

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Full list authors: Farlyn Hudson BS, Paine College, Augusta Ga, Brian Stansfield MD Medical College of Georgia, Augusta, GA.

Understanding the Mechanisms of *RSPO2* Overexpression in Malignant Peripheral Nerve Sheath Tumors

Germán L. Vélez Reyes, BS, *Department of Pediatrics, Masonic Cancer Center, University of Minnesota, Twin Cities*

Neurofibromatosis type 1 syndrome (NF1) is a cancer predisposition disorder caused by loss-of-function mutations in the neurofibromin 1 gene (*NF1*), a Ras-GAP that negatively regulates the Ras signaling. Plexiform neurofibromas and malignant peripheral nerve sheath tumors (MPNST) are common tumor manifestations in NF1. In 2013, our lab conducted a *Sleeping Beauty* (SB) forward genetic screen and gene expression analyses that indicated a role for R-spondin2 (*RSPO2*) and β-catenin (*CTNNB1*) signaling as drivers of MPNST development, progression and maintenance. R-spondins are secreted Wnt agonists that synergize with the many Wnt ligands to modulate Wnt signaling.

Despite the associations between *RSPO2* and multiple malignancies, little is known about the genetic regulation of *RSPO2* expression and how RSPO2 signals to promote tumor initiation and maintenance of MPNSTs. Intriguingly, *RSPO2* overexpression is associated with detection of an *EIF3E-RSPO2* fusion transcript in a subset of MPNST cell lines and tumors in the absence of genomic inversions, deletions, and/or insertions to explain this fusion transcript. Thus, we consider it to be a so-called "transcriptionally induced chimera" or TIC, which occurs via RNAPII read-through from *EIF3E* to the downstream *RSPO2* gene. We hypothesize that *RSPO2* overexpression occurs via the loss of insulator activity bringing its promoter under the influence of distant enhancers and/or promoters, leading to the simultaneous generation of TICs and loss of proper epigenetic control of gene expression.

In order to understand the role of CTCF in the regulation of *RSPO2* expression, we found that the knockdown of *CTCF* using an siRNA leads to an increase in the expression of *RSPO2* in human immortalized Schwann cells and MPNST cell lines. We have also identified 6 CTCF DNA binding sites in the *EIF3E-RSPO2* intergenic region using ENCODE and Chromatin Immunoprecipitation (ChIP) with PCR. We also utilized Chromosome Conformation Capture technology (3C) to determine if the proximity of the two gene loci plays a role in the formation of the fusion transcript and *RSPO2* overexpression. Using 3C-PCR in both human immortalized Schwann cell lines and the *RSPO2*-expressing MPNST cell line S462, we found that: (1) S462 native chromatin conformation differs from that of immortalized human Schwann cells and (2) that the *EIF3E* promoter region comes in close contact with the *RSPO2* promoter in S462 cells, whereas this event does not occur in immortalized Schwann cells.

In our studies, we have found a possible mechanism of oncogene activation in MPNSTs with possible implications for other human cancers. In the future, we would like to further understand the role of CTCF binding in the genesis of MPNSTs and its global effects on gene expression in human Schwann cells.

Other Authors: David Largaespada PhD, Department of Genetics, Cell Biology, and Development, Department of Pediatrics, University of Minnesota, Twin Cities. Nancy Ratner, PhD, Department of Pediatrics, University of Cincinnati.

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Transcriptomic Changes in NF1 Deficient Cells

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

In efforts to define pathways responsible for the initial development of neurofibromas, we have been evaluating transcriptomic changes in a non-malignant *NF1* null cell line. We created *NF1* null HEK293 cells using CRISPR/Cas9 technology and performed transcriptomic analysis in comparison to HEK293 wild type (*NF1* +/+) cells. We have found that there are 352 genes that are up-regulated and 445 genes down-regulated by 2 fold or more ($q < 0.05$) in the null cells. Some of the more interesting genes that are up-regulated include: *CACNG2* (33 fold), *ETV4* (19 fold), *LAMB3* (15 fold), and *FOSL1* (7 fold). Some of the more interesting genes that are down-regulated include: *SLTRK5* (43 fold), *GABRB2* (26 fold), *RORB* (22 fold), and *ESRRG* (16 fold). Ingenuity Pathway analysis has revealed that the top conical pathways include: GABA Receptor Signaling ($p = 9.73E-06$), Regulation of the Epithelial-Mesenchymal Transition Pathways ($p = 1.55E-04$), and Axon Guidance Signaling ($p = 1.8E-04$). In addition, the top three Diseases and Biological functions associated with these expression changes are "Dematological Diseases and Conditions" ($p < 5.43E-06$), "Organismal injury and abnormalities" ($p < 2.64E-05$), and "Cancer" ($p < 2.64E-05$). The RAS-MAPK pathway was not significantly affected, possibly due to its activity being modified post-translationally as opposed to transcriptionally. We are in the process of validating various gene expression changes by q-RT-PCR, which we hope will lead to definition of new pathways and functions of neurofibromin.

Mei-Jan Chen MD, Anil Challa PhD, Bruce R. Korf, MD, PhD, and Bob Kesterson, PhD. Department of Genetics, University of Alabama at Birmingham.

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The Role of the Runt-related Transcription Factor Family in Neurofibromagenesis

Christina Wei, PhD, *Division of Experimental Hematology and Cancer Biology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital*

Neurofibromatosis type 1 (NF1) patients are predisposed to neurofibroma formation but the driver(s) that contribute to neurofibromagenesis 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 cellular context. We previously showed that genetic loss of Runx1 transiently delayed neurofibroma formation. To test if compensation among Runx factors occurs, we dually targeted genetic deletion of Runx1 and Runx3 in Schwann cells (SCs) and Schwann cell precursors (SCPs). We significantly delayed neurofibroma formation and increased survival in the *NF1$^{fl/fl}$;DhhCre* neurofibroma mouse model. We also identified a potential Runx down-stream target, the transcription factor 4 (TCF4). Western blots showed that 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. In preliminary studies, pharmacological inhibition of the 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.* These results implicate 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 R01 NS097233 to JW).

Full list of authors:
Christina Wei, PhD[1], Eva Dombi MD[2], Hongzhu Liu, MS[1], Ashley Hall, BS[1], Kwangmin Choi PhD[1], P. Paul Liu PhD[3], Gang Huang PhD[1] and Jianqiang Wu, M.D MSc[1]

[1]Division of Experimental Hematology and Cancer Biology, Cancer and Blood Diseases Institute, Cincinnati Children's Hospital, Cincinnati, OH 45229, USA
[2]Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD 20892, USA
[3]National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA

Functions of Neurofibromin in Cortical Neural Circuit Development

Lei Xing, PhD, *UNC Neuroscience Center*

Neurofibromatosis type 1 (NF1) is an autosomal dominant inherited disease caused by loss of function mutations in the gene *NF1,* which results in ERK/MAPK hyperactivation. Besides cutaneous manifestations, NF1 patients frequently exhibit brain growth alterations, neurodevelopmental delay, learning deficits, motor deficits, and high prevalence of autism and seizures. However, molecular and cellular mechanisms underlying the pathogenesis of these central nervous system associated-neurological phenotypes in NF1 are largely unknown.

In this study, we investigated the effect of NF1 loss-of-function in the developing cortex by inactivating NF1 in radial glial cells using an *Emx:Cre* line. We found that homozygous NF1 conditional knock out (*NF1$^{loxp/loxp}$;Emx:Cre*) mice exhibit phenotypes similarly observed in human patients, suggesting *NF1$^{loxp/loxp}$;Emx:Cre* mice are valid models to investigate the function of NF1 in cortical development and circuit formation. We found a significant increase in cortical thickness reminiscent of macrocephaly in *NF1$^{loxp/loxp}$;Emx:Cre* mice at postnatal day 14. We found that the increase in cortical thickness is associated with overproduction of oligodendrocytes, rather than cortical principle neurons. On the contrary, the thickness of upper layers (Layer 2-4) was reduced presumably due to the premature transition from neurogenesis to gliogenesis. While the cortical laminar structure was largely normal in mutant mice, we found the extension of long-range projecting corticospinal axon in the spinal cord was drastically reduced. Similar effect on corticospinal axon extension was recapitulated when ERK/MAPK was directly activated by a constitutive active form of Mek1.

We then tested the layer V neuron-autonomous effect of NF1 inactivation using a *Retinol binding protein 4:Cre* (*Rbp4:Cre*) line in which Cre activity is restricted to layer V corticospinal neurons in the cortex. We found that the spinal cord innervation of corticospinal axons was significantly reduced in lumbar segments of the spinal cord in *NF1$^{loxp/loxp}$;Rbp4:Cre* mice. This results suggest that NF1-mediated regulation of ERK/MAPK signaling is critically required for the proper establishment of cortical layer V connection with subcortical targeting regions. Given the critical function of cortical layer V projection neurons in motor learning and voluntary and skilled movement control, our results suggest that the aberrant development of cortical long-range projections may contribute to motor deficits in NF1.

Full List Authors: Lei Xing, PhD, UNC Neuroscience Center; William D. Snider, MD, UNC Neuroscience Center

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Psychophysiological Correlates of Pain in NF1: The Role of Heart Rate Variability and Psychological Flexibility

Taryn M. Allen, PhD, *National Cancer Institute*

Background: Individuals with neurofibromatosis type 1 (NF1) and plexiform neurofibromas (PNs) may experience chronic pain. An emerging body of literature has examined the role of the autonomic nervous system (ANS) in persistent pain conditions. Heart rate variability (HRV) is an index of ANS functioning, and reflects the time elapsed between consecutive heart beats. Patients with chronic pain tend to exhibit lower HRV, which has been associated with difficulty adapting to physical and emotional stressors (e.g., weaker psychological flexibility) and poorer emotion regulation. No known research has examined the role of autonomic functioning and psychological processes in individuals with NF1 and pain.

Methods: Adolescents and young adults (AYA; $n=37$; $M=24.2$ years old, 43.2% male) with NF1, at least one PN, and chronic pain completed baseline questionnaires to assess their pain and psychological functioning as part of a larger randomized intervention trial. Participants also underwent an electrocardiogram (ECG) in the supine position for 5 minutes. A subset of participants ($n=16$) underwent a second baseline appointment approximately 8 weeks after their initial visit, and completed the same questionnaires and a second ECG. Spectral analyses of ECGs yielded a measure of parasympathetically-mediated HRV. Exploratory analyses of cross-sectional data examined correlations between HRV, pain, and psychological functioning. Using longitudinal data, two linear regression models examined predictors of HRV and pain intensity.

Results: Baseline correlations revealed that lower HRV is related to greater inflexibility ($r=-.50$, $p<.01$) and more pain interference ($r=-.34$, $p=.05$). However, HRV, psychological inflexibility and pain interference were all unrelated to pain intensity. Regression models indicate that baseline psychological inflexibility significantly predicts HRV at 8-week follow-up, while controlling for gender, pain intensity, and disease severity ($\beta=-.62$, $t=-2.80$, $p<.05$). Further, baseline HRV significantly predicted pain intensity at follow-up while controlling for disease severity and age ($\beta=-.42$, $t=-2.94$, $p=.01$).

Conclusion: This is the first study that links pain to physiological (HRV) and psychological processes in individuals with NF1. Indeed, the data suggest that psychological flexibility influences autonomic functioning *and* that autonomic functioning influences pain intensity in AYA with NF1 and PNs. This suggests a complex relationship between mind-body processes in the experience of pain in this population. Behavioral interventions that target psychological and ANS processes in the context of pain (e.g., Acceptance and Commitment Therapy; mindfulness-based interventions) may be particularly valuable for this population.

Full List Authors: Kari L. Struemph, PhD[1], Mary Anne Toledo-Tamula, MA[2], Andrea Baldwin, CRNP[1], Brigitte Widemann, MD[1], Pamela Wolters, PhD[1], Staci Martin, PhD[1]
[1]National Cancer Institute; [2]Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, Inc.

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Characteristics of Patients that Pursue Genetic Testing of *NF1* and *SPRED1* and Outcomes of Testing at a Large Neurofibromatosis Center in New York City: A Retrospective Analysis

Kara Anstett, MS, CGC, *Comprehensive Neurofibromatosis Center, NYU Langone Medical Center*

Genetic testing can be a valuable adjunct to clinical care for individuals with neurofibromatosis (NF) and schwannomatosis (SWN), by providing confirmation of diagnosis for those that do not meet clinical criteria, increasing precision in providing a risk assessment for family members, and assisting with family planning decision-making for adult patients. While some clinics in the United States that care for patients and families with NF and 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.

The addition of a genetic counselor to the NF Center in September of 2015 has resulted in increased availability of genetic testing and related services for all appropriate patients within our Center. As a result, over 45 patients have undergone *NF1* and combined *NF1*/*SPRED1* genetic testing to assess for neurofibromatosis type 1 (NF1) and Legius syndrome in our clinic in the 21 months since the addition of a genetic counselor to the multidisciplinary team.

Using a retrospective, quantitative approach, we analyzed our cohort of patients for which *NF1* and *NF1*/*SPRED1* genetic testing was completed, including demographic information such as age, gender, and ethnic background, indication for testing, and the presence or absence of family history of NF1. The known diagnosis and clinical features of patients prior to genetic testing were divided into five groups: isolated café-au-lait macules (CALs), CALs and skin fold freckling only, clinical diagnosis of NF1 due to features in addition to CALs and freckling, presumed mosaicism due to distribution of clinical findings, and unspecified NF/other. All patients for which clinical features were consistent with NF1 or Legius syndrome had genetic testing that included *NF1* and *SPRED1* analysis, while patients with additional features consistent with NF1 only had single gene analysis. The outcomes of genetic testing on lymphocytes and biopsy specimen were then determined for each clinical features group.

Our analysis highlights the characteristics of patients that choose to pursue genetic testing in our patient population, and the outcomes of genetic testing for patients with each pre-test clinical diagnosis, including unexpected results. We also present select cases of interest, including atypical clinical presentations, mosaicism, and individuals with multiple gene variants.

Full List Authors Kaleb Yohay, MD, NYU Langone Medical Center.

NF1 is Not Enough?

Dusica Babaovic-Vuksanovic, MD, *Mayo Clinic*

Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder characterized by development of pigmentary skin changes, neurogenic tumors and other manifestations involving multiple systems. Diagnosis of NF1 has been based on clinical findings as proposed by NIH consensus criteria. Development of molecular testing for NF1 allowed for confirmatory testing, targeted testing in families with identified genetic defect as well as for diagnosis of patients who do not have clear clinical presentation. Extra-familial and intra-familial clinical variability in NF1 is remarkable, with a wide range of disease severity and variety of complications in affected individuals. Given that NF1 is one of more common hereditary conditions, presence of a second genetic disorder, in addition to NF1 is not unexpected. The Mayo Clinic Neurofibromatosis Clinic sees between 200-250 NF1 patients per year. During comprehensive clinical evaluations we have diagnosed several patients who, in addition to NF1, had another hereditary disorder that contributed to phenotypic complexity and challenges for long term management. Examples include patients who had NF 1 and Prader-Willi Syndrome, Down syndrome, myofibromatosis, non-syndromic craniosynostosis and other less common conditions. While variability in NF1 expression is very high, clinicians participating in NF clinics should keep high level of suspicion for presence of another disorder in patients with NF1 whose phenotype exceeds the average expected for this condition.

Diagnosing NF1 Using Multi-Gene Cancer Panels: An Emerging Trend and the Implications for NF Clinicians

Amanda Bergner, MS, CGC, *Ambry Genetics*

Background: Historically, individuals referred to an NF clinician for consideration of neurofibromatosis 1 (NF1) have presented with clinical features consistent with NF1and/or a family history of NF1. As next-generation sequencing (NGS) is becoming more widely available, a distinct pattern of referral for NF1 clinical evaluations is emerging. The gene for NF1 (*NF1*) is now included on many multi-gene cancer panels, and individuals are being referred for an NF1 clinical evaluation *after* receiving a genetic diagnosis of NF1.

Methods: All sequential oncology cases submitted to one lab for germline genetic testing panels containing *NF1* between July 2015 and December 2016 were retrospectively reviewed. Cases with an *NF1* gene mutation or variant of uncertain significance (VUS) were identified, and available clinical data and genetic test results were reviewed. Follow-up clinical correlation evaluations at a major NF Center were conducted for several cases.

Results: 100 cases were found to have an *NF1* mutation, 77% of which presented for testing due to a personal and/or family history of breast cancer. 42% had no reported clinical features or family history of NF1 and 16 (38.1%) of these cases received mosaic test results. 8/100 cases were also found to have a mutation in a separate known cancer gene. 1217 cases were found to have an *NF1* VUS. 4 probands were evaluated for clinical correlation at a major NF Center, which confirmed the diagnosis of NF1 for 2. The other 2 cases were not found to have clinical features of NF1; one had a mosaic test results and the other had a VUS test result.

Discussion: This new type of referral presents many challenges to the NF clinician, including managing the unexpected nature of the diagnosis later in life, determining the relationship (or lack thereof) between a variety of cancers and NF1, and discussing the heritable nature of NF1 after child-bearing has already occurred. In addition, this data highlights the need for NF clinicians to understand more about the interpretation of *NF1* genetic testing results, including 1) distinguishing between mutations and variants of uncertain significance, 2) interpreting mosaic test results, 3) correlating clinical symptoms with test results, and 4) recommending follow-up genetic testing, as appropriate. Given the source of these new referrals, ongoing education of our oncology colleagues about the primary features of NF1 and when to refer patients to an NF specialist will become important.

Full List Authors: Kaleb Yohay, MD, NYU Langone Medical Center; Zoe Powis, MS, CGC, Ambry Genetics; Kara Anstett, MS, CGC, NYU Langone Medical Center

To Reflex or Not to Reflex: Genetic Testing Patterns for Neurofibromatosis 1 (NF1) and Legius Syndrome

Amanda Bergner, MS, CGC, *Ambry Genetics*

Background: Germline molecular testing for NF1 and Legius syndrome using next generation sequencing (NGS) and deletion/duplication analysis (del/dup) is being offered by an increasing number of diagnostic laboratories. Clinicians now have the option to pursue testing for one or both of these genes, concurrently or sequentially, using one or both testing methodologies. It is unclear what testing patterns for *NF1* and *SPRED1* are most prevalent, and what the comparative detection rates are for various approaches.

Methods: All sequential cases submitted to one lab for germline genetic testing of *NF1* and/or *SPRED1* by any testing methodology in any sequence between January 2014 and December 2016 were retrospectively reviewed. Cases in which a gene mutation was identified were selected and reviewed.

Results: 597 probands underwent testing: 53.2% (318) were tested only for *NF1,* 5.4% (32) were tested only for *SPRED1,* and 41.4% (247) were tested for both. Of the probands tested for both, 15.4% (38) underwent concurrent NGS and del/dup of both genes, 30.4% (75) underwent NGS for *NF1* and *SPRED1* with reflex to del/dup of both, and 54.2% (134) underwent NGS and del/dup for *NF1* with reflex to NGS and del/dup for *SPRED1.* The overall detection rate was 33.3% (199/597), with mutations in *NF1* accounting for 97.0% (193/199). NGS detected 92.5% (184/199) of all mutations, with the remainder identified by del/dup. Specific detection rates for all probands undergoing *NF1* NGS was 32.3% (178/551), *NF1* del/dup was 2.9% (15/522), *SPRED1* NGS was 2.5% (6/238), and *SPRED1* del/dup was 0% (0/218).

Discussion: As the options for molecular interrogation of *NF1* and *SPRED1* increase, clinicians must ensure that they are ordering relevant testing that is most likely to detect a mutation, if present. Although most mutations in this cohort were detected using NGS, an important minority of NF1 cases were identified using del/dup, underlining the need for both methodologies to achieve maximum detection rates. Del/dup for *SPRED1* appears less relevant. Interestingly, there was no significant difference in detection rates for first-round testing if a reflex from *NF1* NGS and del/dup to *SPRED1* NGS was ordered or if a reflex from *NF1* and *SPRED1* NGS to *NF1* del/dup was ordered. For individuals who have clinical features consistent with both NF1 and Legius, there may be no benefit to selecting one sequence over the other. This is particularly relevant when insurance coverage, not clinician preference, dictates the type of testing that can be ordered.

Full List Authors: Patrick Reineke, Ambry Genetics; Zoe Powis, MS, CGC, Ambry Genetics

Qualitative Analysis of Patient Cohort with NF1-Related Glomus Tumors

Nicole Bertsch, MS, CGC, *Mayo Clinic*

Background: Glomus tumors are benign tumors of the glomus body and considered an uncommon manifestation of neurofibromatosis type 1 (NF1). They are mainly found under the nail in fingertips and toes of patients with NF1 and are associated with a characteristic triad of symptoms: severe pain, localized tenderness, and sensitivity to cold. Magnetic resonance imaging (MRI) can aid in the diagnosis of glomus tumors, and these lesions are often successfully removed via surgical excision, although they can recur. The NF1 clinic at our institution saw two patients within the span of a month presenting with symptoms of glomus tumors. We subsequently sought to analyze the characteristics of patients in our clinic with NF1-related glomus tumors.

Methods: We conducted a retrospective chart review of 7 patients out of a cohort of approximately 400 patients who met criteria for a clinical diagnosis of NF1 over the past 5 years. These 7 patients were evaluated by providers in our NF1 clinic and identified to have typical symptoms of glomus tumors. A qualitative approach was used to analyze characteristics of these patients regarding the symptoms, diagnosis and treatment of their glomus tumors.

Results: The following major themes were identified: (1) Patients experienced symptoms for years prior to the diagnosis and treatment of their glomus tumors, (2) patients' symptoms significantly impacted their quality of life, (3) patients reported the use of unsuccessful therapies prior to undergoing surgical excision and successful removal of the glomus tumor, and (4) MRI was used in the diagnostic process but was misinterpreted and/or not diagnostic in some patients.

Conclusions: In our clinic, glomus tumors present as a "diagnostic dilemma." There appears to be a lack of awareness surrounding the symptoms, treatment, and impact of glomus tumors on patients with NF1. Education about this rare but potentially debilitating manifestation is essential for a timely diagnosis and improvement in patients' quality of life. Given the difficulty in diagnosis, glomus tumors may be more common in patients with NF1 than current data suggests.

Author List: Dusica Babovic-Vuksanovic, MD, Mayo Clinic

Spinal and Para-Spinal Plexiform Neurofibromas in NF1 Patients; A Novel Scoring System for Clinical-Radiological Correlation

Felix Bokstein, MD, *Tel-Aviv Sourasky Medical Center and the Tel-Aviv University*

Background and purpose: Neurofibromas are the hallmark of neurofibromatosis type I, and a major cause for morbidity and mortality. The aim of this study was to characterize radiological presentation of NF1 patients with significant spinal disease, and to correlate it to clinical presentation and outcome.

Methods: We conducted a historical cohort study of adult NF1 patients with spinal involvement. Longitudinal clinical evaluation included pain and neurological deficit. Differences in the mean distribution of the tumors between groups of patients at presentation was evaluated using Student's independent samples T-test. Tumor sub-groups that demonstrated a significant difference in prevalence ($p<0.05$) or a trend ($p<0.1$) between the clinical groups were further analyzed. The effect of tumor sub-type on the outcome measures (pain, neurological deficit, neurological deterioration), was evaluated using logistic regression. A radiological risk score was computed based on the odds ratios of the significant categories for each patient, and correlated with the outcome.

Results: 34 of 257 adult NF1 patients qualified for inclusion in this study. Three independent factors were found to be associated with increased risk for neurological deficit: 1. Bilateral tumors at the same level in the cervical region that approximated each other ("kissing neurofibromas"); 2. Para-spinal tumors at the lumbar region; 3. Intra-dural lesions. Based on these factors we calculated a combined risk-score for neurological deficit for each patient. We found a clear correlation between patient status and the calculated risk score. Patients with neurological deficits were found to have a higher risk score (9 ± 8.3), than patients not suffering from neurological deficits (2.5 ± 2.9, $p<0.05$). Patients that progressed during the follow-up period had a significantly higher score at presentation than stable patients (9.9 ± 8.73 vs. 3.9 ± 5.3 respectively, $p<0.05$).

Conclusion: In this series, neurological deficit is correlated with tumor burden and subtype. We found no direct correlation with pain. Our novel radiological classification scoring system may be used to predict increased risk for neurological morbidity.

Mauda-Havakuk Michal MD, PhD[1,2]*, Shofty Ben MD[1,3]*, Ben-Shachar Shay MD[1], Ben-Sira Liat MD[1,2], Constantini Shlomi MD[1,3], and Bokstein Felix MD[1,4].

[1]The Gilbert Israeli NF Center, [2]Pediatric Radiology Unit, [3]Pediatric Neurosurgery, [4]Neuro-Oncology service; at Dana Children's Hospital, Tel-Aviv Medical Center, and the Tel-Aviv University, Tel-Aviv, Israel.

Social Dysfunction Correlates with Learning Disabilities in Children with Neurofibromatosis Type 1

Ying Cai, BA, *UCLA*

Background: Neurofibromatosis type 1 (NF1) is one of the most common neurological genetic disorders caused by a single gene. Learning disabilities are found in 50-80% of individuals with NF1. NF1 is often associated with poor language and visual-spatial skills as well as worse performance on academic achievement tests. Social and attention problems, depression, somatic complaints, and aggressive behavior are also present at higher rates in NF1. The aim of this study was to evaluate the relationship between learning disabilities, social, emotional and behavior deficits in children with NF1 using standardized neuropsychological measures.

Methods: This study included children 7-16 years of age with an NF1 diagnosis ($N=44$, age mean$=11.9$ years) and a demographically matched, typically developing control population ($N=31$, age mean$=12.1$ years). Parents of participants completed the Social Responsiveness Scale (SRS) and Behavior Rating Inventory of Executive Function (BRIEF) questionnaires to assess reciprocal social behavior and executive function respectively. Estimates of general intellectual function, visuospatial learning/memory and working memory were also obtained for all participants.

Results: The NF1 population demonstrated a significant impairment ($p<0.001$) in social behavior compared to controls on the SRS with respect to cognition, expressive communication, motivation and autistic mannerisms. BRIEF assessment showed a significant impairment ($p<0.001$) in executive functioning in children with NF1. Cognitive testing showed that children with NF1 had lower IQs as well as deficits in visuospatial processing/attention and working memory. We found a strong correlation between social/emotional function and IQ among NF1 patients ($p=0.024$, $R^2=0.196$), but not in controls ($p=0.660$, $R^2=0.011$). Four out of five SRS subscale scores were strong predictors of IQ scores in children with NF1 but not in controls.

Conclusions: Compared to a control peer group, children with NF1 demonstrate significant deficits in social behavior, executive function and IQ which contribute to the morbidity of this genetic disorder. Children with NF1 and lower IQ may be at risk for worse social/emotional functioning. Early identification of these deficits is critical to developing interventions to maximize the academic, social, behavioral and emotional outcome in children with NF1.

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Cutaneous Neurofibromas In NF1: A Quantitative Natural History Study

Ashley Cannon, PhD, MS, CGC, *Department of Genetics, University of Alabama at Birmingham*

A major feature of NF1 is the development of localized cutaneous neurofibromas (cNFs). cNFs manifest in >99% of adults with NF1 and are responsible for major negative effects on quality of life. Previous reports have correlated increased burden of cNFs with age and pregnancy, but longitudinal data are not available to establish a quantitative natural history of these lesions. The purpose of this study is to conduct a prospective natural history study of 22 adults with NF1 using reliable outcome measures to quantify cNF number and size. Over the 8-year study timeframe, mean cNF volume increased by 2.33x in the back, 1.56x in the abdominal, and 1.47x in the leg/arm regions over 96 months. The approximate increase in number of cNF was 3.2 in the back, 1.8 in the abdominal, and 0.4 in the leg/arm regions. This study demonstrates that the number and volume of cNFs significantly increase over an 8-year timespan; however, the rate of increase is variable by body region. This is the first reported longitudinal natural history study of cNFs. These findings may provide insight into cNF development and benefit researchers considering clinical trials targeting cNFs.

Full list of authors: Mei-Jan Chen, MD[1], Kevin P. Boyd, MD[2]
Amy Theos, MD[2], Peng Li, PhD[3], David Redden, PhD[3], Bruce Korf, MD, PhD[1]

[1]Department of Genetics, University of Alabama at Birmingham
[2]Department of Dermatology, University of Alabama at Birmingham
[3]Department of Biostatistics, University of Alabama at Birmingham

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Autism Spectrum Disorder Symptomatology in Children with Neurofibromatosis Type 1: Effect of Measurement Approach

Christina L. Casnar, MS, *University of Wisconsin - Milwaukee*

Social problems are a common concern of parents of children with neurofibromatosis type 1 (NF1). There has been a recent surge of research examining the prevalence of autism spectrum disorders (ASD) and ASD symptomatology in children with NF1 with mixed findings. The primary aim of this study was to examine ASD symptomatology in children with NF1 using a comprehensive assessment of ASD symptoms. Participants included 33 children with NF1 between the ages of 9 and 13, along with their parent. Standardized parent-report questionnaires were used to assess social responsiveness (Social Responsiveness Scale, Second Edition: SRS-2) and ASD symptomatology (Social Communication Questionnaire: SCQ). A gold-standard observational diagnostic assessment measure for ASD was used to examine the nature and severity of ASD symptomatology (Autism Diagnostic Observation Scale, Second Edition: ADOS-2). Results indicate that 30% of parents observed mild to moderate symptomatology on the SRS-2. While the group mean for the SRS-2 score fell in the average range, independent one-sample t-test indicated significantly higher scores than the normative mean on the SRS-2 Total Score, $t(31) = 3.17$, $p = .003$. No children exceeded cutoff on the SCQ. Children with NF1 showed very few difficulties on the ADOS-2; for most children, most algorithm items received a score of 0. Some very mild difficulties were noted on four algorithm items for some children with NF1, including slight difficulties with reporting events (30%), conversation skills (24%), use of gestures (27%) and quality of social responses (24%). Two children were classified ASD based on the ADOS-2 algorithm; neither was clinically diagnosed with ASD due to lack of significant restricted and repetitive behaviors or interests. Results indicate that children with NF1 are demonstrating elevated ASD symptomatology per parent report on a non-diagnostic measure of autism-related symptomatology, however those difficulties are generally not severe or pervasive enough to be evident on either a diagnostic screening measure or on structured clinician observation. The findings highlight the importance of completing a comprehensive assessment of the core ASD symptoms related to socio-communicative difficulties and repetitive behavior when assessing social difficulties of children with NF1.

Full List Authors: Christina L. Casnar, MS, University of Wisconsin – Milwaukee; Brianna D. Yund, MS, University of Wisconsin – Milwaukee; Kristin Lee, University of Wisconsin – Milwaukee; & Bonita P. Klein-Tasman, PhD, University of Wisconsin - Milwaukee

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AIDE for Patients with NF1: Systematic Developmental Screening and Behavioral Assessment

Lucie Côté, RN, *Université de Montréal CHU Sainte-Justine*

Neurofibromatosis type 1 (NF1) is associated with a wide variety of neurodevelopmental disorders in children. Motor skills, language, affective and behavioral regulation, learning, adaptive behavior, attention, and sleep can all be altered at various degrees in NF1. While the prevalence varies throughout the literature, most authors estimate that the majority of children with NF1 have at least one deficit regarding cognitive or motor abilities.

In order to detect early developmental delay within our NF1 population, we integrated the AIDE (Approche Interactive au Développement de l'Enfant) web based screening system. AIDE is an adaptation of the CHADIS (Child Health & Development Interactive System) platform. All children with NF1 between 6 months and 5 years of age were systematically enrolled. Depending on age at the time of screening, parents were asked to complete standardized questionnaires including: ASQ-3, ASQ-SE, Mc Arthur-Bates, M-CHAT, SCQ, BEARS, PSI-SF and CBCL. Testing was conducted around 6, 12, 18, 24, 36, 48 and 60 months.

Between November 2015 and February 2017, 44 non-selected patients and their families were enrolled. Of those 32 (18 females and 14 males) completed at least one questionnaire. Median at age at time of completion of the first tests was 27.5 months (range: 6.1-53.2 months). Sixteen patients (48,5%) were found to have at least one affected domain. The most common difficulties included: gross motor skills (11/16, 68,8%), fine motor skills (8/16, 50%) and socio-affective development (8/16, 50%). Most (10/16, 62.5%) had at least two affected domains. All patients with specific difficulties were referred to physical therapists, occupational therapists, and speech therapists within two months. In all children, therapists confirmed developmental delay suggesting a high positive predictive value.

Developmental screening through a web-based system such as AIDE can be implemented to a NF1 clinic. It offers systematic and reliable developmental evaluations. Within our population, almost half of our children with NF1 had developmental delay, which was rapidly detected and accelerated referral to therapists.

Karine-Sophie Vandal RN, CHU Sainte-Justine, Geneviève Lupien PT, CHU Sainte-Justine, Marie-Pierre Lamothe OT, CHU Sainte-Justine, Rabia Sabah Meziane ST, CHU Sainte-Justine, Vincent Domon-Archambault PSYD, CHU Sainte-Justine, Albert Larbrisseau MD, CHU Sainte-Justine, Sébastien Perreault MD, CHU Sainte-Justine
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How Executive Function Profiles Differentially Impact Social Problems in Children with Congenital and Neurodevelopmental Disorders

Stephany M. Cox, PhD, *Children's National Health System*

Children with neurofibromatosis type 1 (NF1) and congenital heart disease (CHD) exhibit executive function (EF) and social deficits that overlap with features of Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD). Executive dysfunction is associated with social deficits in children with neurodevelopmental and chronic medical disorders. Recent literature suggests unique EF profiles across disorders, which may have a unique impact on social functioning. The aims of this investigation are to identify EF phenotypes in the included groups, examine the impact of EF on social outcomes, and evaluate how behavioral and cognitive regulation aspects of EF impact social functioning in children with congenital (NF1, CHD) and neurodevelopmental disorders (ADHD, ASD).

Parent reports of EF, attention, and social functioning were collected (BRIEF and CBCL) on 158 children with NF1 (n=32), CHD (n=53), ADHD (n=37), and ASD (n=36) ages 6-17 (M=9.49, SD=2.99; 51.6% male). A simple linear regression was calculated to predict social functioning (CBCL Social Problems) based on the Behavioral Regulation Index (BRI) and Metacognitive Index (MCI) from the BRIEF. BRI is important for modulating emotions and behaviors, while MCI relates to cognitive self-management of tasks and monitoring performance.

Chi-square analyses revealed significant differences in the prevalence of clinically elevated scores among all groups ($p=.004$). Scales with the highest frequency of clinical elevations also differed by group: ADHD (Working Memory: 73%), ASD (Shift: 63.9%), CHD (Working Memory: 35.8%), and NF1 (Planning/Organization: 48.4%). Executive function deficits were predictive of social problems in children with ADHD ($p=.004$); however, there was not a specific EF profile that predicted impairments. In contrast, cognitive regulation was predictive of social problems in children with ASD ($p=.007$). In both medical populations, behavioral regulation predicted social problems ($p<.001$); additionally, cognitive regulation was indicative of social problems in children with CHD ($p=.038$). After analyzing the subscales that compose BRI, inhibition ($p<.001$) was the driving factor of social problems for children with NF1.

Although all groups exhibited EF deficits associated with social problems, the underlying mechanism driving their social difficulties differ, emphasizing the importance of tailoring interventions specific to disorder type.

Full Author List: Jacquie Sanz, PhD[1,2]; Tess Kennedy, BA[1]; Anthony Gioia, BS[1]; Kristina Hardy, PhD[1,2]; Yael Granader, PhD[1,2]; Karin S Walsh, PsyD[1,2]
[1]Children's National Health System; [2]George Washington University School of Medicine

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High Signal Intensity Lesions in the Spinal Cord of Children and Adolescents with Neurofibromatosis Type 1

Gabriel Dabscheck, MBBS Hons FRACP, *Neurology Department, Royal Children's Hospital, Murdoch Children's Research Institute, Melbourne Australia*

To date, studies in NF1 patients have not reported NF1 associated bright spots in MRI spines. This is a case series of five NF1 patients with interval monitoring of T2 bright intramedullary spinal lesions to help determine clinically if the T2 bright spots in MRI spines in children with NF1 are the corollary of bright spots seen in MRI brains of children with NF1. Five patients with clinically confirmed NF1 had interval MRI spines with an average of 24.8 interval months apart (12 months to 52 months). Four patients had a single T2 bright spinal cord lesion whereas one patient had five separate lesions. Lesions were seen diffusely scattered throughout the spinal cord. Three patients were given contrast, none of them showed contrast enhancement. No patients had neurological symptoms (weakness, spasticity, sensory disturbance or bladder and bowel involvement) attributable to their T2 bright spinal cord lesions. Four patients had stable appearance over the interval of scanning and one patient had a reduction in signal intensity over the interval.

The advent of MRI identified T2 bright spots in the brain in children and adolescents with NF-1 in the basal ganglia, thalami, dentate nuclei, cerebellar peduncles, optic radiations and brainstem. We describe a series of children with T2 bright spots in the spinal cord incidentally found in five NF1 patients. The patients remained asymptomatic over the period of observation. Our series suggest that T2 bright spots seen in the spinal cord of children and adolescents with NF1 represent myelin vacuolisation similar to the better described benign T2 hyperintensities in the brain of the same patient population. As long as patients do not show symptoms, regular neuroimaging follow-up of these hyperintense foci does not seem to be necessary.

Additional authors: Andrea Ruegger-Lustenberger, MD, Neurology Department, Royal Children's Hospital Melbourne, Jordan R. Hansford BSCH, MSc, MBBS, FRACP Children's Cancer Centre, Royal Children's Hospital Melbourne, Lee Coleman MB BS FRACP, Department of Radiology, Murdoch Children's Research Institute, Melbourne Australia

Effect of Age and Neurofibromatosis Type 1 Status on White Matter Integrity in the Optic Radiations in Children

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

Introduction: Reliable measures of vision are essential in children with neurofibromatosis type 1 (NF1) who are predisposed to optic pathway gliomas, cognitive differences and attention deficit that can challenge standard ophthalmologic assessments. Diffusion tensor imaging (DTI) of the optic radiations has been proposed as a biomarker of vision in children with optic pathway gliomas.[1] However, DTI measures vary with age and white matter maturation, and normal values for DTI measures such as fractional anisotropy (FA), radial diffusivity (RD) and mean diffusivity (MD) have not been defined in young children.

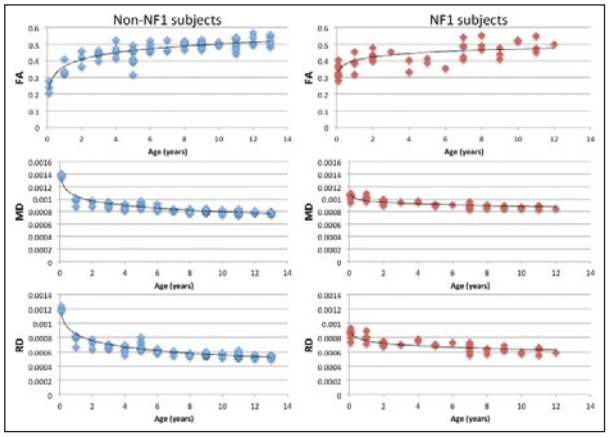

Figure 1: Scatterplots of DTI measures vs. age in children with NF1 (red) and without NF1 (blue)

Methods: We analyzed clinical DTI in 40 healthy children with NF1 and 55 healthy controls between 0-14 years of age. Subjects were excluded for prematurity or neurologic/ophthalmologic conditions that may affect white matter integrity or vision. 3T Diffusion MR (Siemens Trio, Skyra or Verio; Erlangen, Germany) was acquired with 30 gradient directions, 128 x 128 matrix, b=1000s/mm^2, 2mm isotropic voxel, and b=1000 s/mm^2. Automated probabilistic tractography was performed between the lateral geniculate nucleus and the occipital pole.[2] Regression models of age (linear, exponential, logarithmic, quadratic) on DTI measures were considered. Potential covariates included NF1 status and gender, and interaction terms between age and NF1 status were investigated ($p<0.2$ threshold of significance).

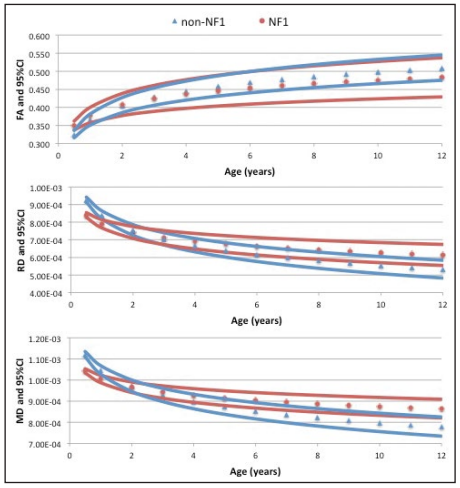

Figure 2: 95% Confidence intervals for DTI measures for subjects with and without NF1 between 0.5-12 years of age

Results: DTI measures of white matter integrity (FA, RD and MD) vs. age in subjects with and without NF1 are shown in Figure 1. In both NF1 and non NF1 subjects, age was significantly correlated with DTI measures, and ln(age) was determined to be the best-fit model. While NF1 status was not a significant covariate in the model, NF1*ln(age) met the predetermined threshold for significance ($p=0.073$). Therefore, NF1 and non-NF1 subjects were modeled separately (Figure 2). Between 0-14 years, a doubling of age predicted a 0.860x10^{-4}mm^2/s decrease in RD for children without NF1, but only 0.493x10^{-4}mm^2/s decrease for children with NF1.

Conclusions: This study demonstrates potentially altered developmental trajectory in the white matter optic radiations of children with NF1 and defines normal DTI values among children, essential to the development of DTI as a biomarker of vision.

References: [1]de Blank et al, *NeuroOncol* 2013. [2]de Blank et al, ISMRM 2016, abstract 3058

Acknowledgements: Neurofibromatosis Therapeutic Acceleration Program and the St. Baldrick's Foundation.

Full Author List: Jeffrey I Berman[a], Marisa Prelack[a], Amy Waldman[a], Michael J Fisher[a], [a]Children's Hospital of Philadelphia

Exploring the Utility of ^{18}F-fluorothymidine PET in the Diagnosis of Malignant Peripheral Nerve Sheath Tumors in Patients with Neurofibromatosis Type 1

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

Background: Malignant peripheral nerve sheath tumors (MPNST) are aggressive soft tissue sarcomas with poor prognosis. In patients with neurofibromatosis type 1 (NF1) the lifetime risk of developing MPNST is 12-15%, and malignant transformation often occurs within preexisting plexiform neurofibromas (PN). ^{18}F-fluorodeoxyglucose (FDG) PET sensitively identifies areas concerning for malignant transformation, however the intensity of the metabolic activity may overlap between histologically benign, atypical pre-malignant, and malignant lesions. A more specific indicator of malignant degeneration is needed. ^{18}F-fluorothymidine (FLT) PET imaging highlights the activity of thymidine salvage during DNA synthesis, and serves as a surrogate of cell proliferation, therefore may be better suited to detect malignant transformation than FDG.

Objectives: The goal of this study is to evaluate the utility of FLT-PET in the diagnosis of MPNST, compared to FDG-PET.

Methods: NF1 patients with lesions suspicious for malignant transformation, or confirmed MPNST were enrolled on NCI protocol NCT02211768, and underwent clinical evaluation, volumetric MRI, FDG and FLT-PET/CT imaging, and biopsy/resection of one or more concerning lesions. FDG and FLT-PET/CT were obtained within 2 days of each other, except for one patient scanned 33 days apart. The maximum standardized uptake values (SUV_{max}) of FDG and FLT recorded one hour after injection were compared for all nerve sheath tumors with above background FDG uptake.

Results: The study enrolled 10 patients (age range 12-51 years), 3 with MPNST diagnosis (1 known metastatic MPNST with disease progression on clinical trial, 1 newly identified, and 1 diagnosed 6 months later). From 12 tissue samples 1 MPNST and 11 atypical neurofibromas (ANF) were confirmed. 91 lesions (median 7, range 1-30 per patient) were identified with above background avidity on FDG-PET, median SUV_{max} 3.15 (range 1.1-13.2); 34 lesions had SUV_{max} >3.5. Above background FLT avidity was detected in 43 lesions, median SUV_{max} 1.8 (range 0.9-4.48), and background level was collected for others lesions. The highest FLT uptake was observed in the newly diagnosed high grade MPNST. All FLT avid lesions localized to areas of FGD uptake, and higher FLT uptake correlated with higher FDG uptake.

Lesions (N=91)	No biopsy (N=72)	ANF (N=11)	MPNST (N=8*)
FDG SUV_{max}: median (range)	3.06 (1.38-11.04)	6.12 (3.24-13.2)	2.50 (1.1-10.5)
FLT SUV_{max}: median (range)	1.27 (0.71-3.14)	1.82 (1.1-4.09)	1.29 (0.7-4.48)

*Including 4 metastatic lung lesions and 2 sites of local recurrence in a patient with progressive disease on treatment with a tyrosine kinase inhibitor.

Conclusion: PET avid lesions were identified in each study subject, including MPNST, biopsy confirmed ANF and clinically benign tumors (no biopsy). FLT uptake was observed in fewer lesions and at lower levels than FDG. FLT uptake co-localized with FDG avidity. Correlations with lesion size, lesion growth rate, and histopathological markers, including Ki67 are pending. The moderate FLT uptake in high grade or progressive metastatic MPNST suggest that these tumors may have evolved to bypass the thymidine salvage pathway. The value of FLT-PET in predicting imminent or early malignant transformation needs to be further studied.

Full list of authors: Christine Higham[1], Liza Lindenberg[2], Esther Mena[2], Seth Steinberg[3], Anne Goodwin[1], Andrea Baldwin[1], Sucharita Bhaumik[4], Joanne Derdak[1], Peter Choyke[2], Brigitte Widemann[1]

Affiliations: [1]Pediatric Oncology Branch, [2]Molecular Imaging Program, [3]Biostatistics and Data Management Section of National Cancer Institute, Bethesda, MD, [4]Wintrop University Hospital, Department of Pediatric Hematology Oncology, Mineola, NY.

Retrospective MRI Review in Neurofibromatosis Type 1 Patients Diagnosed with Malignant Peripheral Nerve Sheath Tumor

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

Background: In patients with neurofibromatosis type 1 (NF1) the lifetime risk of developing malignant peripheral nerve sheath tumor (MPNST) is 12-15%. Known risk factors for MPNST include microdeletion of *NF1,* family history of MPNST, prior radiation treatment, and large plexiform neurofibroma (PN) burden. Atypical neurofibromas (ANF) have been described as precursor lesions to MPNST, with *CDKN2A/B* deletion the initiating step towards malignant transformation. While ANF is a histological diagnosis, there may be imaging signs that indicate early transformation.

Objectives: To characterize imaging signs of early malignant transformation we performed a retrospective review of MRIs pre-dating the diagnosis of MPNST in patients with NF1.

Methods: Study subjects were identified at two NF centers, University Hospital Hamburg-Eppendorf in Germany, and the National Cancer Institute in Bethesda, Maryland. We confirmed the location of MPNST on the MRI performed at the time of diagnosis, and looked for distinguishing imaging features at the site of MPNST on prior MRIs.

Results: We identified 22 NF1 patients with histologically confirmed MPNST, and at least one MRI of the same body area that was performed before the MPNST diagnosis. On prior MRIs, a preexisting PN, or a distinct nodular lesion were present at the location of MPNST in all patients. We found examples of structural remodeling of the pre-existing PN and gradual development of a predominant nodule that eventually transformed to MPNST. Further image analysis is ongoing.

Conclusion: In a subset of MPNST patients, gradual development of distinct nodular lesions can be observed pre-dating the MPNST diagnosis. The risk of malignant transformation in NF1 patients presenting with distinct nodular lesions needs to be prospectively studied.

Full list of authors: Johannes Salamon[1], Anne Goodwin[2], Andrea Baldwin[2], Brigitte Widemann[2], Victor Mautner[1]

Affiliations: [1]Department of Neurology, University Hospital Hamburg-Eppendorf, Hamburg, Germany, [2]Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD.

Educational Achievements in Danish Adults with Neurofibromatosis Type 1 – A Nationwide Study

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

Background: Cognitive function may play a key role in obtaining an education and thus for integration into adult society. It has been suggested that neurofibromatosis type 1 (NF1) may imply impaired cognitive function in some children especially in the domains of learning and attention, resulting in lowered academic performance. However, knowledge about the consequences of these impairments on the children's abilities to succeed in the education system in late adolescence and young adulthood is unexplored.

Objectives: To investigate educational attainment in patients with NF1 using a population-based NF1-free cohort as a comparison group.

Methods: In a nationwide, population-based cohort study, 550 persons with NF1 born between 1965 and 1984 were identified and compared to a matched population-based NF1-free comparison cohort (n=4295), frequency matched on sex and date of birth. The Danish Hospital Register, Central Population Register and Statistics Denmark were used to identify study subjects and educational attainment. Multinomial logistic models were applied to investigate the odds ratios (ORs) and 95% confidence intervals (CIs) for obtaining a short (<=9 years, mandatory school) or medium (10-12 years, upper secondary or vocational) education compared to a long education (>12 years, higher education) at age 30. Analyses were adjusted for birth year, gender, psychiatric disease, and mothers' education. The variance of the estimates was adjusted for within-cluster correlation (clusters being families).

Results: Among persons with NF1 some 41% obtained short education and 21% long education while corresponding proportions were 20% and 34% among the comparison group. The odds of obtaining short education versus long education were more than 3-fold higher among persons with NF1 compared to the NF1-free persons (adjusted OR, 3.24; 95% CI 2.60 to 4.50) and 1.39 times higher for obtaining medium education (adjusted OR, 1.39; 95% CI 1.07 to 1.80). NF1 increased the probability of short education significantly irrespective of mother's education and if the mother has a long education NF1 decreased the probability of long education.

Conclusion: The findings of this first population-based study provide further evidence that cognitive implications of NF1 result in lower educational achievements.

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Full List Authors: Jeanette Falck Winther DMSc, Danish Cancer Society Research Center; Pernille Envold Bidstrup PhD, Danish Cancer Society Research Center; Elisabeth Wreford Andersen PhD, Danish Cancer Society Research Center; Line Kenborg, PhD, Danish Cancer Society Research Center; Hanne Hove DMSc, Rigshospitalet; John R Østergaard DMSc, Aarhus University Hospital; Jens Richard M Jepsen PhD, Lundbeck Foundation Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS) & Center for Neuropsychiatric Schizophrenia Research (CNSR); Sven Asger Sørensen DMSc, University of Copenhagen; John J Mulvihill MD, University of Oklahoma; Christoffer Johansen DMSc, Danish Cancer Society Research Center & Finsen Center, Rigshospitalet; Susanne Oksbjerg Dalton PhD, Danish Cancer Society Research Center

Neurofibromatosis Type 1(NF1) Associated Congenital Pseudarthrosis of the Tibia & Fibula Misdiagnosed as Non-Accidental Injury (NAI)

Judith Eelloo, RGN, RSCN, MPhil, *Complex NF1 Service, St Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, UK*

Background: Congenital tibial pseudarthrosis (CTP) presents with anterolateral bowing of the lower leg in infancy which often progresses to fracture and non-union (pseudarthrosis). CTP occurs in 2-3% of children with NF1. The distal end of the fibula and other long bones can also be affected.

Objective: We describe three children in whom NF1 related congenital tibial or fibular pseudarthrosis was initially misdiagnosed as NAI.

Presenting problem: The table summarises patient characteristics, presenting problem, safeguarding assessment & action taken by social services. Café-au-lait lesions were present in all 3 patients. The diagnosis of NF1 was confirmed by genetic testing in all patients.

Case	1	2	3
Age/Gender	20 Months;F	7Months;F	3Months;F
Presentation	Hard lump above the right lateral malleolus	Anterolateral bowing of the right lower leg from birth	Bilateral bowing of the lower legs from birth Pain when left leg moved
Radiographic Findings	Fracture of the distal right fibula with pseudarthrosis	Anterolateral bowing of the right distal tibia with fracture and pseudarthrosis	Fracture of the left distal tibia with pseudarthrosis Anterolateral bowing of right tibia & fibula
Safeguarding referral	Yes	Yes	Yes
Safeguarding investigations	No	Yes	Yes
Removed from parents	No	Yes	Yes
Family history of NF1	Yes	No	Yes

LEGEND: F - female; Safeguarding referral - referral to social services & Paediatric assessment; Safeguarding investigations – full radiological skeletal survey, CT head scan & fundoscopy; Removed from parents – temporarily removed from parents into foster care or placed with a relative.

Discussion: Carefully taken family history, thorough clinical examination and awareness of classical radiological features of CTP would have avoided unnecessary safeguarding assessment/investigations in these patients, and their temporary removal from parents (Cases 2&3).

Conclusion: Clinical and radiological features of congenital tibial & fibular pseudarthrosis, associated with NF1, may be mistaken for NAI.

Judith Eelloo, Farhan Ali, Rui Santos, Grace Vassallo, Siobhan West, Elizabeth Howard, Susan Huson, Elizabeth Rowles, Zulf Mughal.

Case Report: Patient with Neurofibromatosis Type 1 Newly Diagnosed with Co-Occurring Multiple Sclerosis after Presenting with Shortness of Breath

Celia Engelson, MS, FNP-C, *Comprehensive Neurofibromatosis Center, NYU Langone Medical Center*

Coincidence of multiple sclerosis (MS) and neurofibromatosis type 1 (NF1) is very rare but occurs at rates higher than expected with unclear but possible biological basis for the association. MS is a chronic inflammatory disease characterized by demyelination of the axons of the central nervous system. It is most commonly seen in the northern parts of North America and Europe, where the prevalence is approximately 0.1-0.2% of the population (5-6:100,000 per year). NF1 is a common autosomal dominant neurocutaneous disorder (1:3000 per year) characterized by café au lait macules, skin fold freckling, and neurofibromas. Unlike MS, NF1 is not associated with demyelination.

Despite differences in presentation, there is a higher than expected association between MS and NF1. It has been hypothesized that this association is related to a mutation in the oligodendrocyte myelin glycoprotein gene, which plays a role in myelination and is embedded within the intron of the NF1 gene. Another theory suggests a causative relationship between NF1 and MS due to the possible autoimmune impact of Schwann cell proliferation present in NF1.

We present a case study in which we describe a patient with a known clinical diagnosis of NF1, scoliosis, meningocele, and pectus excavatum who presented with periodic episodes of shortness of breath. Brain and spine imaging identified numerous demyelinating lesions suggestive of MS. She was referred to an MS specialist and the diagnosis of MS was confirmed. She has since started treatment with Copaxone.

To better understand the worldwide incidence of co-occurring MS and NF1, we conducted a review of the literature.

While the relationship between MS and NF1 remains unclear, studies suggest a small increased risk of MS in those with NF1. At our NF Center, we know of at least two patients with co-occurring diagnoses of MS and NF1.

Full List Authors: Kaleb Yohay, MD, Director, Comprehensive Neurofibromatosis Center, NYU Langone Medical Center

Home Practice and the Differential Improvement of Quality of Life among Patients with Neurofibromatosis Randomized to a Mind-Body Intervention

Christopher J. Funes, MS, *Massachusetts General Hospital*

Objectives: The purpose of this study was to summarize home practice patterns in patients with neurofibromatosis (NF) randomized to an 8-week group mind-body intervention (The Relaxation Response Resiliency Program for NF; 3RP-NF) delivered via live videoconferencing and to examine the association between home practice and quality of life (QoL) improvements from baseline to post-intervention and 6-month follow-up.

Methods: Data are derived from a single-blind RCT of the 3RP-NF versus an attention placebo control. Participants randomized to the 3RP-NF group were included in the current analyses (N=31). Daily practice logs were used to assess home practice of relaxation response (RR) and appreciation. Descriptive statistics were used to summarize amount of and adherence to home practice throughout the intervention, and zero-order correlations were used to assess associations with improvements of QoL.

Results: Participants reported engaging in RR home practice on an average of 28.55 days (SD=10.79) and appreciation home practice on an average of 24.39 days (SD=13.48). Adherence was generally high with an average rate of 83% and 76% for RR and appreciation home practice, respectively. Significant associations were observed between frequency of RR home practice and improvements of physical QoL at follow-up ($r=.355$, $p=.050$). Adherence to RR home practice was associated with improvements of psychological QoL at post-treatment ($r=.410$, $p=.022$).

Conclusions: Skills and exercises taught in the 3RP-NF are acceptable and feasible for NF patients. Significant correlations were observed for the frequency of home practice, rather than duration of practice, suggesting that developing a habit of practicing skills regularly may be more important for QoL improvements than the total amount of time spent on home practice.

Full List Authors: Emily Zale MS, Massachusetts General Hospital, Syracuse University; Christina Luberto PhD, Massachusetts General Hospital, Harvard Medical School; Ana-Maria Vranceanu PhD, Massachusetts General Hospital, Harvard Medical School

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Prevention of Plexiform Neurofibroma Rapid Regrowth Following Surgical Resection

Molly Hemenway, DNP, MS, RN, CPNP-AC, *University of Colorado, Children's Hospital Colorado*

Pharmacological treatment of plexiform neurofibromas (PN) in the setting of Neurofibromatosis Type I (NF1) alone produced poor treatment response until recent publications highlighting the use of MEK inhibitors as a hopeful next targeted therapy. Surgical resection alone for PN is often complicated with rapid regrowth of the PN to as large or greater than the size at surgical removal within just a few months. The indications for surgical removal of PN at Children's Hospital Colorado (CHCO) include pain (uncontrolled with pharmacological treatment), loss of function, and cosmetic need. Through collaboration with the Plastic Surgery team at CHCO, our multidisciplinary team has developed a treatment pathway for patients receiving surgical resection or debulking of their PN to prevent rapid regrowth following surgery. First, patients are identified with plexiform neurofibromas that meet the criteria for surgical resection. Second, patients are referred to our dedicated NF1 plastic surgeon. Following consultation with the surgeon, if the patient elects to have surgery, our research nurse and Neuro-Oncology nurse practitioner (NP) meet with the patient and family regarding the Tissue Bank Study. Tissue obtained in the course of debulking, for clinically indicated reasons that is not needed for diagnostic purposes, will be collected at the time of surgery, snap frozen in liquid nitrogen, and then kept in the-70°C freezer in the pathology department at Children's Hospital Colorado/The University of Colorado and/or used to establish primary cultures. Our Tissue Bank provides the opportunity to study the molecular biology of tumors in the setting of NF1, with the hope that in the future, biological knowledge will help design more effective treatment and prevention of disease. Next, the Neuro-Oncology NP discusses treatment with an mTOR inhibitor for three months following the surgical resection to prevent rapid regrowth. Following PN resection, the Plastic Surgery and Neuro-Oncology teams meet with the patient at two weeks post-operatively to determine adequate healing to start the mTOR inhibitor. If the patient's incision is healing well, the mTOR inhibitor is started and the Neuro-Oncology team monitors for side effects, effectiveness, and labs for the next three months. If no regrowth has occurred at three months of therapy, the inhibitor medication is stopped. The patient has continued monitoring with the Neuro-Oncology team for at least three months more to assess for PN regrowth. To date, eleven patients have been treated according to this treatment pathway with only two patients experiencing regrowth in the six months following surgery. Our treatment pathway with an mTOR inhibitor three months following surgical resection has shown to be well tolerated with only two of eleven recent cases with any regrowth of the previously resected plexiform neurofibromas.

Nick Foreman, MD, University of Colorado, Children's Hospital Colorado
Brooke French, MD, University of Colorado, Children's Hospital Colorado

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Case Study: Girl with NF1 has BRAF Mutant Pilocytic Astrocytoma

Molly Hemenway, DNP, MS, RN, CPNP-AC, *University of Colorado, Children's Hospital Colorado*

Children's Hospital Colorado (CHCO) is a tertiary care center treating 200 patients with Neurofibromatosis Type I (NF1) each year from a seven state region. Recently, a 9 year old girl, alias "Gabby", had surgical resection of a rapidly growing tumor which was diagnosed as pilocytic astrocytoma, WHO Grade I, with BRAF mutation. The mutation in BRAF resulted in an anticipated single amino acid substitution (V600E). Gabby presented to CHCO at 5 years old. She had completed treatment for optic pathway/hypothalamic glioma with carboplatin and vincristine at four years old at an outside hospital. No biopsy was performed at diagnosis. At seven years old on routine MRI, Gabby was noted to have progressive disease. She was treated with vinblastine and when six months later she had further progression, bevacizumab was added to the vinblastine backbone. Her MRI after six months of therapy with vinblastine and bevacizumab showed slight progression and she was developing peripheral neuropathies (due to vinblastine), her treatment was changed to bevacizumab and everolimus. Gabby tolerated this therapy well for six months with stable MRI scans. At that time she stopped bevacizumab due to proteinuria and continued with everolimus. She continued to have stable scans. After three months of monotherapy with everolimus Gabby's creatinine level was rising and everolimus was stopped. The MRI scan after two months without therapy unfortunately showed progressive disease. Due to concurrent vision loss in addition to her MRI showing progression of the superior part of the tumor at the hypothalamic/chiasmal interface, bevacizumab was restarted to treat her tumor and vision loss. Unfortunately, following one dose of bevacizumab she had a significant bleed into the tumor and a stroke. Therapy was halted and her tumor was monitored as she completed rehabilitation. Six months later, her tumor was growing rapidly on MRI and she had a surgical resection. Pathology returned showing a pilocytic astrocytoma with BRAF mutation. Her case prompts the question of whether more children with NF1 and brain tumors should have a surgical biopsy as more targets for therapies may be present other than those known to NF1.

Nick Foreman, MD, University of Colorado, Children's Hospital Colorado
Todd Hankinson, MD, University of Colorado, Children's Hospital Colorado
Jean Mulcahy-Levy, MD, University of Colorado, Children's Hospital Colorado

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Neoadjuvant Ifosfamide and Epirubicin in the Treatment of Malignant Peripheral Nerve Sheath Tumors (MPNSTs)

Angela C. Hirbe, *Division of Medical Oncology, Department of Medicine, Washington University School of Medicine; Siteman Cancer Center*

Background and Objectives: Malignant peripheral nerve sheath tumors (MPNSTs) are aggressive soft tissue sarcomas with poor overall survival. Response to chemotherapy has been debated for these tumors.

Methods: We performed a retrospective analysis of the patients at our institution with a biopsy-proven diagnosis of MPNST that underwent neoadjuvant chemotherapy prior to surgery.

Results: We retrospectively identified five patients who received neoadjuvant chemotherapy with epirubicin and ifosfamide that demonstrated a 30% reduction in tumor growth and a 60% response rate by RECIST criteria. Additionally, a metabolic response was observed in all three patients who received serial PET scans during neoadjuvant treatment. The clinical benefit rate, which includes stable disease, was 100%.

Conclusions: Our data suggest that MPNSTs do respond to epirubicin and ifosfamide based chemotherapy and prospective studies are warranted to further define the clinical benefit.

Angela C Hirbe[1,2]*, Pippa Cosper[3], Brian A Van Tine[1,2]
[1]Division of Medical Oncology, Department of Medicine, Washington University School of Medicine, 660 South Euclid Ave, St Louis MO, 63110; [2]Siteman Cancer Center, Saint Louis M0, 63110; [3]Department of Radiation Oncology, Box 660 South Euclid Ave, St Louis MO, 63110

Medical Resource Utilization of Outpatient Care for Children with Neurofibromatosis Type 1

Rabia Idrees, BS, *The George Washington University School of Medicine*

Background: Manifestations of neurofibromatosis type 1 (NF1) can affect the central nervous system (CNS), peripheral nervous system (due to plexiform neurofibromas), musculoskeletal system, and cognitive/behavioral function. While most research focuses on the incidence, symptoms, treatment and outcomes of specific NF1 manifestations, the magnitude of medical resource utilization for each manifestation is unknown. The current study sought to identify which manifestations of NF1 utilize the most healthcare resources and to validate the accuracy of using ICD-9 diagnostic codes to identify patients with NF1.

Methods: The electronic health record at The Children's Hospital of Philadelphia was queried to identify patients with the ICD-9 code for NF1 (237.71) who were evaluated between January 2011 and December 2015. Patients were excluded if the diagnosis of NF1 could not be confirmed. The frequency of MRI scans and specialty provider visits over the five-year study period were compared across disease manifestations. The positive predictive value (PPV) of identifying patients using the ICD-9 code was calculated.

Results: Nine-hundred-eleven unique patients with NF1 met inclusion criteria (age 0.7-69.5 years, median = 12.9; 51% female). Cognitive/behavioral (42%), CNS abnormalities (37%) and plexiform neurofibromas (32%) were the most common manifestations followed by musculoskeletal (21%) and other (19%). A total of 13,643 outpatient provider visits occurred with Oncology (23%) and Ophthalmology (18%) being the most frequently utilized specialty. Patients underwent a total of 4,527 MRIs with brain MRI being the most common (48%). Sixty-three percent of MRIs required sedation/anesthesia. The ICD-9 code for NF1 accurately identified patients with a confirmed diagnosis of NF1 (PPV = 94.4%) if the code was present once in the subject's chart. The PPV increased to 98.2% if at least two visits included the ICD-9 code for NF1.

Conclusions: CNS manifestations of NF1 demonstrated the greatest utilization of resources (combined MRI acquisitions and specialty visits). The ICD-9 code 237.71 accurately identified patients with NF1. Understanding the costs associated with the frequency and type of resources utilized (i.e., diagnostic imaging and specialty visits) may encourage academic/industry development of novel therapeutics and creation of algorithms to optimize clinical care.

Full List of Authors: Rabia Idrees, BS[1]; Michael J. Fisher, MD[2]; Rachel Hachen, MD[2]; Brian C. Callaghan, MD[3]; Robert A. Avery, DO, MSCE[2]; [1]The George Washington University School of Medicine; [2]The Children's Hospital of Philadelphia; [3]The University of Michigan School of Medicine.

Prevalence of Cerebral Vasculopathy in Children with Neurofibromatosis Type 1

Mona Jacobson, RN, MSN, CPNP-PC, *Children's Hospital Colorado, University of Colorado School of Medicine*

Introduction: Children with neurofibromatosis type 1 (NF1) are at risk for vascular abnormalities throughout the entire arterial system. Although previous studies have found a prevalence of cerebral vascular abnormalities of 2-7% in children with NF1, cerebral vasculopathy is likely underestimated because patients are often asymptomatic. Some studies also indicate that a diagnosis of optic glioma pathway (OPG) tumor increases risk for vasculopathy. We hypothesized that patients with OPG would have increased rates of screening with MR angiogram (MRA), and have a higher prevalence of vascular abnormalities in our cohort.

Current NF1 screening guidelines do not routinely recommend brain MRI or MRA unless there is clinical indication.

Methods: We performed a retrospective chart review of NF1 patients who attended a multidisciplinary NF clinic at Children's Colorado Hospital from January 2014 to December 2016. Patient demographics, imaging modality, imaging results, and vascular abnormalities were all assessed.

Results: Of the 130 patients identified, 117 had a MRI scan with only 35 also having a MRA scan to specifically screen for vasculopathy. 25 % (9/35) of patients with MRAs and 7.7 % (9/117) of the NF1 patients in our entire cohort (who received any head imaging) had vascular complications (moyamoya changes or stenosis) noted on their scan. Out of the 9 patients identified, 4 had OPG tumor and 5 were asymptomatic. Overall, 13% (4/31) of children with NF1 and OPG had a vascular abnormality, although screening with MRA was higher in patients with OPG (19/31, 61%) than those without OPG (4/117, 3.4%).

Conclusion: This is the highest ever reported prevalence of vascular abnormalities in a cohort of children with NF, with almost 8% of children having vascular disease. While OPG appears to be a risk factor for vasculopathy in our cohort, the increased rate of screening in this population may be a confounder. Based on the data collected in our series, and support from literature, we propose that NF1 patients with cerebral vasculopathy are under recognized. Further systematic studies of NF1 are needed to better characterize the risk of vasculopathy in these patients. In the meantime, we would consider screening all patients with NF1 who are undergoing brain MRI to have the addition of MRA, particularly those with an OPG.

Timothy Bernard, MD, University of Colorado School of Medicine; Carolyn Green, MD, University of Colorado School of Medicine.

Natural History of NF2-Associated Intracranial Meningiomas

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

Purpose: The lifetime risk of meningioma development in patients with neurofibromatosis 2 (NF2) is around 80%. While several groups have described the natural history of sporadic meningiomas, only one report describes the natural history of NF2-associated meningiomas. In light of new clinical trial efforts targeting NF2-associated meningiomas, we sought to analyze data from the NF2 Natural History Study to provide additional data on the growth patterns of intracranial meningiomas in NF2.

Methods: We analyzed data from the NF2 Natural History study, including newly diagnosed NF2 patients from six sites between 2002 and 2005. Patients who met NIH diagnostic criteria for NF2, and who received no active intervention, were followed longitudinally with cranial magnetic resonance imaging. Only patients with at least two scans and tumors with at least 2 separate measurements were included. Tumor measurements included greatest diameter and volume.

Results: Thirty-seven patients (43% female), and a total of 83 tumors were included in the analysis. Subjects had a median age at study enrollment of 26.2 years (7.4-72.8 years), and were followed a median of 2 years (0.58-3.22 years). Subjects had a median of 2 intracranial meningiomas. At enrollment, the median maximum diameter of individual tumors was 16.4 mm (0-79 mm), and the median volume of individual tumors was 0.92 cc (0.04-79 cc). The median one-dimensional growth was 1.8 mm/year (-39.3 – 19.1 mm), and 33% of tumors grew 1mm or less per year. Volumetrically, the annual median growth rate of tumors was 0.07cc/year (-5.8 – 9.6 cc/year), and only 6% of tumors grew 20% or more per year. Notably, among this cohort of volumetrically faster growing tumors, annual volume increase was 0.66cc/year.

Conclusions: Our data confirms previous evidence of slow or no growth among the majority of NF2-associated meningiomas, notably aligning more closely with Goutagny's report of volumetric growth (7.3% of meningiomas growing $\leq$20% per year) than linear growth (66% of tumors growing $<$1mm/year). Notably, there are a small number of faster growing meningiomas in this population that may be more likely to require intervention. Our data will be an important consideration in designing clinical trials focused on NF2-associated intracranial meningiomas.

Additional Author List: Marlon Seijo: MGH; Vanessa L. Merker, BS: MGH; Monica Sheridan, MS: MGH; William H. Slattery, III, MD: House Clinic; Scott R. Plotkin, MD, PhD: MGH

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Does Neurofibromatosis Type 2 Increase the Risk of Stroke?

Charu Kaiwar, MD, *Center for Individualized Medicine, Mayo Clinic, Scottsdale AZ*

Introduction: There are 4 reports in literature of clinically diagnosed Neurofibromatosis 2 (NF2) patients (of which 3 tested positive for a pathogenic variant in *NF2*) with stroke or intra cranial aneurysms [1-4]. These reports suggest NF2 may be a risk factor for cerebrovascular disease (CVD). We aim to investigate the frequency of stroke in patients with a clinically diagnosis of NF2 by retrospective analysis of Mayo medical records.

Methods: An in-house text search tool, Advanced Cohort Explorer (ACE), was utilized to filter EMRs for Mayo Clinic patients at Rochester with diagnosis of NF2 or acoustic neuromas from January 1999 to January 2017. These records were then manually reviewed to look for patients who fit the clinical criteria for diagnosis of NF2 and those who had a cerebrovascular accident.

Results: There were 202 patient records with a diagnosis of NF2 or acoustic neuromas. Manual review of the records yielded 151 patients who fit the clinical diagnostic criteria for NF2 (81 females and 70 males). The median age at presentation was 30 (range 2-78), and median age at last follow-up was 45.5 and median duration of follow-up was 10 years. Only 1 of the 151 patients had a stroke. The patient was a young female with pontine stroke at age 37. Magnetic resonance angiography was unremarkable.

Conclusions: This is the first retrospective analysis for prevalence of cerebrovascular disease in patients with NF2. Reviewing 18 years of patients records at the Mayo Clinic in Rochester MN, resulted in 151 patients who met clinical criteria for diagnosis of NF2, of whom only 1 had a cerebrovascular accident. These results demonstrate that NF2 is not a risk factor for stroke in young patients.

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Full Author List:
1. Charu Kaiwar MD, Center for Individualized Medicine, Mayo Clinic, Scottsdale, AZ
2. Dusica Babovic-Vuksanovic MD, Department of Clinical Genomics, Mayo Clinic, Rochester, MN
3. Radhika Dhamija MD, Department of Clinical Genomics, Mayo Clinic, Phoenix, AZ

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Programmed Death Ligand 1 Expression and Tumor Infiltrating Lymphocytes in Tumors Associated with Neurofibromatosis Type 1 and 2

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

Background: Immune checkpoint inhibitors targeting programmed cell death 1 (PD-1) or its ligand (PD-L1) have been shown to be effective in treating subsets of patients with a variety of cancers. Biomarker studies have found positive associations between clinical response rates and PD-L1 expression on tumor cells, as well as the presence of tumor infiltrating lymphocytes (TILs). It is currently unknown whether tumors associated with neurofibromatosis types 1 and 2 (NF1 and NF2) express PD-L1. We hypothesized that NF-associated tumors may express PD-L1 and therefore might be amenable to immunotherapy with immune checkpoint inhibitors.

Methods: We performed immunohistochemistry for PD-L1 (Spring Bioscience, clone SP142), CD3, CD20, CD8, and CD68 in 17 NF1-related tumors (11 dermal neurofibromas and 6 plexiform neurofibromas) and 20 NF2-related tumors (10 meningiomas and 10 schwannomas) using archival formalin-fixed paraffin-embedded tissues. Expression of PD-L1 was considered positive in cases with >5% membranous staining of tumor cells, in accordance with previously published biomarker studies.

Results: PD-L1 expression was detected in all tumors, with highest expression levels observed in plexiform neurofibromas and schwannomas. PD-L1 positive tumor cells with >5% membranous staining were observed in 6/6 (100%) of plexiform neurofibromas, 9/11 (82%) of dermal neurofibromas, 7/10 (70%) of schwannomas, and 4/10 (40%) of meningiomas. Sparse to moderate presence of CD68, CD3, or CD8 positive tumor-infiltrating lymphocytes (TILs) was found in 37/37 (100%) of tumor specimens. However, expression of CD20 positive lymphocytes was limited to rare cells detectable in 4/37 (11%) of tumors.

Conclusion: Our findings indicate that adaptive resistance to cell-mediated immunity plays a major role in the tumor immune environment of NF1 and NF2 associated tumors. Expression of PD-L1 on tumor cells and presence of TILs suggests that these tumors may be responsive to immune therapy with immune checkpoint inhibitors, which should be explored in clinical trials for NF patients.

Full List of Authors: Shiyang Wang, MD, PhD; Benjamin Liechty, MD; Seema Patel; Jeffrey Weber, MD, PhD; Matija Snuderl, MD (NYU Langone Medical Center, New York, NY); Matthias Karajannis, MD, MS (Memorial Sloan Kettering Cancer Center, New York, NY)

Neurofibromatosis Type 1 – The Pain Experience in a Common RASopathy

Alanna Kongkriangkai, MS, *Cincinnati Children's Hospital Medical Center*

Objective: Tumor growths, bone deformities, migraine headaches and other health-related complications reported in patients with neurofibromatosis type 1 (NF1) are often associated with substantial pain. However, due to the lack of systematic studies investigating the features of the pain experience, information in the literature regarding pain interference, intensity, chronicity, frequency, and pain characteristics in the NF1 population is limited. Thus, the current study sought to describe and quantify the pain experience in children and adults with NF1.

Methods: A cross-sectional study was conducted via validated and supplemental surveys, which were administered to children and adults between the ages of 8 and 40, at the following locations: Neurofibromatosis (NF) clinic at Cincinnati Children's Hospital Medical Center (CCHMC), CCHMC general genetics clinics, the adult NF clinic at University Hospital, and the annual NF Symposium held at CCHMC. Validated questionnaires include the Numeric Rating Scale 11 (NRS11) to assess pain intensity and the Patient Reported Outcomes Measurement Information System (PROMIS) to assess pain interference. Additionally, we created a supplemental survey to measure pain frequency, chronicity, pain quality, and pain location.

Results: A total of 49 participants (28 children and 21 adults) with NF1 completed the pain survey between August 2016 and December 2016. Most notably, our preliminary study identified pain is present in patients with NF1 (n=27, 55%), and begins at an early age (i.e. as young as 8 years old). Further, pain is chronic in nature for children and adults (n=17, 63%), with 41% of participants (n=20) reporting the need to take over-the-counter and/or prescription medication to manage their pain symptoms. Patients were also likely to report high levels of pain intensity, pain frequency, and pain interference. The most commonly identified sources of pain included migraine headaches and NF-related tumors. Pain was often described as neuropathic (burning, tingling, numbness, or itching types of pain), which was localized to the head, back, and extremities.

Conclusion: Describing the pain experience of a multisystem disorder such as NF1 can begin to provide families and clinicians with anticipatory guidance in regards to recognizing the impact of pain on disease management, therapy and overall quality of life.

Full Author List: Alanna Kongkriangkai, MS, Christopher King, PhD, Lisa J. Martin, PhD, Emily Wakefield, LGC, Carlos Prada, MD, Geraldine Kelly-Mancuso, RN, Elizabeth K. Schorry, MD, and Cincinnati Children's Hospital Medical Center

Clinical and Genetic Features of Korean Patients with NF2

Beom Hee Lee, *Medical Genetics Center, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea*

Neurofibromatosis 2 (NF2) is characterized by uni- or bilateral vestibular schwannomas in combination with schwannomas affecting other cranial and peripheral nerves, meningiomas, ependymomas, or astrocytomas. Its extimated prevalence is much lower than that of NF1, and affects the multi-ethnic group. Herein, we described the clinical and genetic features of Korean patients with NF2. A total of 24 Korean patients (12 males and 12 females) were enrolled in the study. The presentation signs were hearing disturbance (12 pts), increased intracranial pressure signs (4 pts), facial palsies (2 pts), and other neurological signs including seizure, weakness, gait disturbance, abnormal sensory function, diplopia and abnormal eyeball movement. Neurogenic tumors were also incidentally found in two patients. Family history were positive only in three patients. Bilateral vestibular neuromas were detected in most cases (22 pts), and multiple meningiomas, gliomas, ependymomas, and schwannomas were suspected in 15 pts, 1 pt, 3 pts and 16 pts, respectively. Germline *NF2* mutations were found in the genomic DNA, extracted from peripheral leukocytes (WBC), of all the 3 patients with positive family history, whereas the positive rate was only 47% in the genomic DNA from WBCs of the sporadic 19 patients. Genetic testing in the tumor tissue was available only in two patients, in whom *NF2* mutations were all found in tumor tissues but not in WBCs. The current study reports the characteristic clinical features of NF2 similarly observed in Korean patients with NF2. The somatic mosaicism for *NF2* mutations in sporadic cases accounts for the low detection rate of germline mutation in these patients. Further studies are required to determine whether genetic testing using advanced genomic technologies might help to detect low level of somatic mosaicisms.

Beom Hee Lee[1], Hyeji Kim[2], Gu-Hwan Kim[1], Jae-Min Kim[1], In-Hee Choi[1] and Han-Wook Yoo[1]

[1]Medical Genetics Center, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea
[2]Department of Pediatrics, Asan Medical Center Children's Hospital, University of Ulsan College of Medicine, Seoul, Korea

Investigating Tumor Targeted Glutamine Antagonists as a Novel Therapeutic Approach for Neurofibromatosis Type I Peripheral Nerve Tumors

Kathryn M. Lemberg, MD, PhD, *Department of Pediatric Oncology and Johns Hopkins Drug Discovery, Johns Hopkins School of Medicine*

Neurofibromatosis type I (NF1) is a heritable tumor predisposition syndrome characterized by formation of plexiform neurofibromas. These benign, but potentially compressive and disfiguring peripheral nerve tumors have the capacity to transform into malignant peripheral nerve sheath tumors (MPNST). Traditional cytotoxic chemotherapeutic strategies for MPNST that is incompletely surgically resected at diagnosis offer a 5 year event-free survival of less than 40% *(1).* There is need for further understanding of the biology of plexiform neurofibroma and MPNST so that additional therapeutic strategies may be developed. Reprogramming of energy metabolism has emerged as a hallmark of cancer that has not been investigated rigorously in NF1 associated tumors. In particular tumor cells alter their uptake and utilization of the abundant amino acid glutamine, which serves as a source of amine groups and carbon scaffolds for nucleic acid bases, citric acid cycle intermediates, and other amino acids. Preclinical studies have demonstrated that the glutamine antagonist 6-diazo-5-oxo-norleucine (DON) has efficacy against a spectrum of tumor types *(2),* but clinical development was halted due to gastrointestinal toxicity. Our group has recently described DON prodrugs that circulate inert in plasma and are biotransformed in tumor tissues to release DON, thus improving drug delivery while minimizing gastrointestinal toxicity *(3).* On this basis we undertook studies of glutamine metabolism in cell culture models of plexiform neurofibromas *(4)* and MPNST. We found that cells derived from plexiform neurofibroma or MPNST have increased sensitivity to deprivation of glutamine compared to Schwann cells derived from non-tumored nerve. DON treatment inhibited growth of MPNST cells more than immortalized $NF1^{+/+}$ Schwann cells. While DON showed limited partitioning into MPNST cells versus plasma, our novel prodrugs preferentially delivered DON into MPNST with >5-10 fold enrichment versus plasma. Initial prodrug pharmacokinetic studies in a mouse model of plexiform neurofibroma *(5)* demonstrated that the prodrug successfully delivered DON at micromolar concentrations to peripheral nerve, supporting further evaluation of these novel glutamine antagonists as a potential therapeutic strategy in NF1-associated plexiform neurofibroma and MPNST.

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Full list of authors: Kathryn M. Lemberg[1], MD, PhD, Ying Wu[1], Jesse Alt[1], Alexandra J. Gadiano[1], Rana Rais[1], PhD, Pavel Majer[2], PhD, Jaishri Blakeley[1], MD, and Barbara Slusher[1], PhD, MAS
Affiliations: [1]Johns Hopkins University and [2]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences

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Automated Detection of Tumor Borders in NF1 Optic Pathway Gliomas

Awais Mansoor, PhD, *Children's National Health System*

Background: Recent studies have established quantitative size thresholds using volumetric MRI to define the presence or absence of an optic pathway glioma (OPG) secondary to neurofibromatosis type 1 (NF1). In this study, we evaluate an automated MRI analysis algorithm for detecting and localizing the OPG along the anterior visual pathway (AVP; optic nerve, optic chiasm, optic tract) using previously published quantitative criteria as threshold between OPG and non-OPG regions.

Methods: Twenty pediatric patients (10 NF1-OPGs and 10 healthy controls) with T1, T2, and FLAIR-weighted cube MRI sequences [~0.4 x 0.4 x (0.6-4.0) mm^3 resolution] were analyzed. All NF1-OPGs involved at least one optic nerve. AVP measures were acquired using automated segmentation augmented by deep learning techniques. Thresholds for tumor borders separating OPG regions from the non-OPG regions were established using previously published size thresholds (mean + 1.0 SD) derived from healthy controls. Optic nerve and optic tract borders were established using diameter whereas the combined optic chiasm height and width were used as threshold for border separation. Accuracy of tumor detection was evaluated by the number of correctly classified voxels and volume estimation error for OPG and non-OPG regions.

Results: NF1-OPGs were isolated to the optic nerve (N = 5), included the optic nerve and chiasm (N = 2), or the entire AVP (optic nerve, chiasm and tract; N = 3). The tumor border was correctly identified using the automated algorithm in 10/10 NF1-OPG cases. None of the control subjects' measures were identified as OPGs. The automated algorithm correctly identified 97.6% voxels within OPGs and 99.5% of voxels in non-OPG regions. The volume estimation error was found to be 6.87±9.14% and 5.51±7.01% for OPG and non-OPG regions, respectively.

Conclusions: Our automated quantitative MRI analysis algorithm accurately detects and localizes the OPG border along the AVP in children with NF1. Accurate identification of tumor borders is essential for monitoring longitudinal changes in OPG size as well as developing quantitative criteria for tumor progression in NF1.

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Full list of authors: Awais Mansoor, PhD,[1] Roger J. Packer, MD,[1] Marius G. Linguraru, DPhil,[1] Robert A. Avery, DO, MSCE.[2]
[1]Children's National Health System, Washington, DC. [2]Children's Hospital of Philadelphia, Philadelphia, PA.

Acceptance and Commitment Training for Adolescents and Young Adults with NF1, Plexiform Neurofibromas, and Chronic Pain: Preliminary Results from a Randomized Controlled Trial

Staci Martin, PhD, *National Cancer Institute*

Background: Multiple symptoms of NF1 cause chronic pain, including plexiform neurofibromas (PN), yet no randomized studies have examined non-pharmacological interventions for pain, specifically targeting individuals with NF1 and PNs. We conducted a randomized controlled trial to determine the effects of a psychological intervention, Acceptance and Commitment Therapy (ACT), among adolescents and young adults (AYA) ages 16 to 34 years with NF1 and PNs and chronic pain. We hypothesized that pain interference would decrease from pre- to post-intervention.

Methods: Individuals were recruited from the CTF registry and NF1 clinics around the country. Participants were randomized to receive the ACT intervention immediately (ACT group) or after 8 weeks (Wait List group). The intervention was delivered through two, 2-hour in-person sessions on consecutive days, followed by 8 weeks of online training consisting of weekly email assignments and biweekly video chats with an ACT trainer. The trainer taught participants ACT-consistent techniques, such as mindfulness exercises and coping strategies for focusing on their values rather than their pain. Questionnaires assessing pain interference, pain intensity, pain acceptance, pain inflexibility, disease-specific quality of life (QOL), pain anxiety, and depression were administered in clinic before and after the 8-week intervention in both groups.

Results: Thirty-four ACT group participants (M age = 24.2 years, 43% male) and 16 Wait List participants (M age = 24.0 years, 67% male) were included in these analyses. In the ACT group, mean pain interference ($t = 3.1$, $p < .01$) and pain intensity ($t = 3.0$, $p < .01$) scores significantly decreased from baseline to immediately post-intervention. In addition, pain acceptance ($t = 6.2$, $p < .001$) and QOL ($t = 2.5$, $p < .05$) scores increased while inflexibility ($t = 3.6$, $p = .001$), pain anxiety ($t = 3.8$, $p = .01$), and depression decreased ($t = 2.03$, $p < .05$). In the Wait List group, there were no significant differences on any study measures from baseline to 8 weeks ($ps > .05$).

Conclusions: Preliminary results indicate that an ACT intervention is effective in reducing the extent to which pain interferes with daily functioning, and in improving aspects of physical and social-emotional wellbeing. It is noteworthy that these effects were seen after just four hours of in-person training and limited online sessions; future ACT interventions delivered primarily remotely may make this intervention accessible to more patients with NF1, PNs and chronic pain.

Full List Authors: Staci Martin, PhD[1], Taryn Allen, PhD[1], Mary Anne Tamula, MA[2], Kari Struemph, PhD[1], Andrea Baldwin, CRNP[1], Pam Wolters, PhD[1], Brigitte Widemann, MD[1]
[1]National Cancer Institute, National Institutes of Health; [2]Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research Inc.

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Using Health Services Research to Analyze Pathways of Care in Schwannomatosis

Vanessa Merker, BS, *Massachusetts General Hospital; Boston University School of Public Health*

Background: Delay in diagnosis is a critical issue for schwannomatosis (SWN) patients since lengthy delays can lead to sub-optimal care management and psychological distress. We investigated the steps in the diagnostic pathway for SWN in order to identify potential target areas for improvement.

Methods: We conducted a retrospective chart review of patients with definite SWN seen in neurofibromatosis (NF) specialty clinics at two U.S. academic medical centers. We used process-mapping, a business technique increasingly being applied to healthcare quality improvement initiatives, to chart each health care provider (HCP) patients consulted for a SWN-related sign or symptom until time of diagnosis. We recorded the date of first contact with each HCP, the HCP's specialty, and the patients' reason for presentation to care.

Results: Medical records of 27 patients have been analyzed to date. The median time from first HCP visit to SWN diagnosis was 4.8 years (range 2 months-42 years). Patients presenting with a mass (n=13) had a shorter median time to diagnosis than patients presenting with pain or neurological symptoms only (n=14) (3.4 years vs. 5.1 years). Patients saw a median of 5 HCPs (range, 1-10) in a median of 3 specialties (range, 1-6) during the diagnostic process (not including staff at NF clinics). Patients most frequently consulted primary care (70%), followed by neurosurgery (63%), general surgery (44%), orthopedic surgery (41%) and neurology (30%). Of the 21 patients for whom referral source to the NF clinic could be determined, 11 were referred by a neurosurgeon, 5 self-referred, and 5 were referred by other specialists. Only one patient received a definitive SWN diagnosis prior to referral to the NF clinic.

Conclusions: Diagnostic delay is a significant problem in SWN. To improve this we have identified a group of providers, general and orthopedic surgery, that see a large proportion of SWN patients, but do not commonly refer them to NF clinics for evaluation. As such, we recommend targeting these providers with information about SWN.

Additional Authors: Jaishri Blakeley MD, Shannon Langmead NP, Johns Hopkins University; A. Rani Elwy PhD, Martin Charns DBA, Mark Meterko PhD, Boston University School of Public Health; Scott Plotkin MD, PhD, Massachusetts General Hospital.

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Developing the NF2 Initiative at NYU Langone Medical Center

Carole Wind Mitchell, RN, MS, *NYU Langone Medical Center, New York, NY*

NF2 is a progressive genetic disorder which causes tumors to grow on nerves. These tumors originate within the brain and spinal cord and can result in significant disability. Tumors cause loss of hearing, loss of vision, difficulty swallowing, difficulty walking and often cause chronic pain. In September 2016, the Director of the Rusk Institute of Physical Medicine and Rehabilitation, the Director of the NF2 program and the Nurse Coordinator of the NF Center met to discuss partnering to coordinate care for our NF2 patients.

The Rusk Institute at NYU offers a multidisciplinary approach; treating the entire person, body, mind, and spirit, identifying the patient's potential for what they can accomplish. NF2 patients are referred to Rusk once a need is identified. Under a team care approach, an attending physician who is board certified in physical medicine and rehabilitation evaluates the patient. The physiatrist develops an individualized treatment plan that may involve sessions with a number of different specialists. These include physical and occupational therapists, speech and swallowing specialists, vocational therapists, nutritionists, and social workers. Some of the outpatient rehab services include speech language services for patients with cochlear implants, voice therapy to improve the function and quality of patients' voices, vestibular rehabilitation / balance disorders using exercises to address symptoms and deficits such as dizziness, ataxia, and gait disturbances.

Many patients with NF2 are struggling to maintain their independence in spite of these disabling complications. We developed the NF2 Initiative to help our patients who are overwhelmed with multiple medical appointments and are often trying to meet their full time employment obligations. By partnering with The Rusk Institute, we have helped develop new strategies for daily living, including coping with change and uncertainty, new forms of support for patients and their loved ones, and new techniques for managing pain to improve comfort and quality of life.

Full list of authors: Carole Wind Mitchell, NYU Langone Medical Center, New York, NY

The University of Minnesota Experience with Bevacizumab for Treatment of NF2-Associated Vestibular Schwannomas

Christopher L. Moertel, MD, *University of Minnesota Masonic Children's Hospital*

Beginning in 2009, patients with NF2-associated vestibular schwannomas were treated according to a hearing algorithm developed by the Neurofibromatosis Clinic in concert with the neurotology service at the University of Minnesota. The algorithm incorporated hearing preservation surgery and technology (cochlear implant (CI), auditory brainstem implant (ABI)) in addition to bevacizumab (per Plotkin). Radiation therapy was not offered to the NF2 patient group. Patients were segregated into three groups according to best ear word recognition score (WRS): Those with word recognition scores greater than 70% were assigned to observation. Patients with scores between 30 and 69% were assigned to bevacizumab therapy. Those with deafness on presentation were also assigned to the bevacizumab arm. Treatment failures were assigned to evaluation for CI or ABI. A separate algorithm was followed according to tumor size. If a patient presented with a stable CPA tumor less than 1 cm, they were evaluated for surgery. Tumors greater than 1 cm were assigned to the bevacizumab algorithm. Patients who experienced a stable hearing response had the option of continuation therapy with bevacizumab 7.5 mg/kg every 3 weeks. 12/17 patients with evaluable tumors experienced tumor volume reduction. 7/13 with evaluable audiograms exhibited improvement in WRS. 4/13 experienced a change in grade according to AAO-HNSF criteria. 2 patients were enrolled for protection of a CI as recommended by their neurotologist – the CI has been preserved in both cases. An additional two patients with normal hearing initiated therapy for symptoms related to schwannomas at other locations, but were included in the observation arm. Observed toxicity (in decreasing order of frequency) is as follows: hypertension, proteinuria, dysgeusia, ovarian failure, treatment-associated neuropathy, exacerbation of tinnitus/pain, dysfunctional uterine bleeding and anaphylaxis.

Co-authors: Pavlina Sverak, Samuel C. Levine, Meredith E. Adams, Tina C. Huang, Stephen J. Haines, Katherine Sommer; University of Minnesota

Intracranial Non-Pilocytic Astrocytoma Low-Grade Glial Tumors in Children with Neurofibromatosis Type 1 (NF1)

Jeffrey C. Murray, MD, *Cook Children's Medical Center*

Introduction: Mutations of the NF1 tumor suppressor gene predispose children to the development of both benign and malignant tumors of the peripheral and central nervous system (CNS). Pilocytic astrocytomas are by far the most common intracranial neoplasms, most commonly involving the optic-hypothalamic region, though other histologies have been sporadically reported, suggesting that the molecular drivers of NF1-associated tumors may vary. We add to the literature here, reporting two more children with atypical CNS tumors.

Review: We surveyed the medical literature pertaining to optic pathway and non-optic pathway intracranial tumor histologies in children with NF1. The vast majority of variant CNS tumors have been reported in adult NF1 patients, with only rare case reports in children.

Reports: (1) A 5-year-old boy with NF1 presented with headaches and emesis. MRI revealed an enhancing, 2 x 2 x 3 cm tumor arising from the dorsal aspect of the pons. The patient underwent near-total resection. Histopathology revealed *pilomyxoid astrocytoma.* Since neurosurgery, the child remains neurologically stable, without focal neurologic deficits. No adjuvant therapy was given. Serial MRIs reveal probable small amounts of persistent tumor, yet without progression ten years later. (2) A 14-year-old girl with NF1 presented with progressive headaches, nausea and fatigue. MRI revealed a contrast-enhancing mixed solid/cystic 3 x 3 x 2 cm mass in the parietal lobe. The patient underwent gross total resection. Histopathology revealed *pleomorphic xanthoastrocytoma.* Since neurosurgery, the child remains neurologically stable and without signs of tumor recurrence. No adjuvant therapy was given.

Discussion/Conclusions: As the histologic diagnosis criteria for pediatric CNS neoplasms becomes further refined, variant histologies are more commonly reported, even in children with NF1. Recognizing that not all intracranial neoplasms in children with NF1 are pilocytic tumors, there may be potential pre-operative diagnostic and therapeutic implications, particularly as various genetic markers associated with certain low-grade CNS neoplasms in children are better understood and testing more uniformly applied (e.g., V600E BRAF deletion mutations in ganglioglioma and KIAA1549 BRAF fusion mutations in pilocytic astrocytoma). A better understanding of the true landscape of NF1-associated CNS tumors in NF1 children will hopefully permit more targeted therapeutics and perhaps reduce treatment morbidity. We recommend the ongoing reporting of all atypical CNS tumor histologies in children with NF1.

Additional Authors: John Honeycutt, MD, Hayden W. Head, MD, Sandy Cope-Yokoyama, MD

PedsQL Neurofibromatosis Type 1 Module for Children, Adolescents and Young Adults: Feasibility, Reliability, and Validity

Kavitha Nutakki, MBBS, MPH, *Children's Health Services Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN*

Objective: The objective of the present study was to report on the measurement properties of the Pediatric Quality of Life Inventory (PedsQL) Neurofibromatosis Type 1 Module for pediatric patients ages 5-25 from the perspectives of patients and parents.

Methods: The 104-item PedsQL NF1 Module and 23-item PedsQL Generic Core Scales were completed in a multi-site national study by 323 patients and 335 parents (343 families). Patients were diagnosed with NF1 using the National Institutes of Health diagnostic criteria.

Results: In addition to a Total Scale Score, 18 unidimensional scales were derived measuring skin itch bother, skin sensations, pain, pain impact, pain management, cognitive functioning, speech, fine motor, balance, vision, perceived physical appearance, communication, worry, treatment anxiety, medicines, stomach discomfort, constipation, and diarrhea. The PedsQL NF1 Module Scales evidenced excellent feasibility, excellent reliability for the Total Scale Scores (patient self-report $\alpha = 0.98$; parent proxy-report $\alpha = 0.98$), and good to excellent reliability for the 18 individual scales (patient self-report $\alpha = 0.71$-0.96; parent proxy-report $\alpha = 0.73$-0.98). Intercorrelations with the Generic Core Scales supported construct validity. Known-groups validity was supported by patients with plexiform neurofibromas demonstrating more NF1-specific symptoms and problems than patients without plexiform neurofibromas. Factor analysis supported the unidimensionality of the 18 individual scales.

Conclusions: The PedsQL NF1 Module Scales demonstrated acceptable to excellent measurement properties, and may be utilized as standardized metrics to assess NF1-specific symptoms and problems in clinical research and practice in children, adolescents, and young adults.

Kavitha Nutakki, MBBS, MPH, [a] James W. Varni, PhD, Nancy L. Swigonski, MD, MPH
[a]Children's Health Services Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN

Health-Related Quality of Life in Children, Adolescents and Young Adults with Neurofibromatosis Type 1: A Multisite National Study

Kavitha Nutakki, MBBS, MPH, *Children's Health Services Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN*

Background: Health-related quality of life (HRQOL) is a subjective and multi-dimensional construct that includes physical, psychological (including emotional and cognitive), and social health dimensions as defined by the World Health Organization. Generic HRQOL instruments can be used to compare HRQOL across patient groups and benchmarking with healthy populations.

Objective: To compare HRQOL in children, adolescents and young adults with Neurofibromatosis Type 1 (NF1) with an age-, sex- and race/ethnicity-matched healthy control sample from the existing Pediatric Quality of Life Inventory™ (PedsQL™) database.

Study Design: The 23-item PedsQL™ 4.0 Generic Core Scales were completed by 320 children, adolescents and young adults with NF1 ages 5-25 and their parents living across the United States as a part of the field test of the PedsQL™ NF1 Module. The matched healthy control sample was derived from an overall sample of 5480 children and 9430 parents.

Results: Children with NF1 reported significantly lower PedsQL™ scores on all domains in comparison to healthy children with larger effect sizes. Parents of children with NF1 also reported lower PedsQL™ scores, with statistically significant differences in all domains.

Conclusion: Children, adolescents and young adults with NF1 demonstrate significantly lowered HRQOL in comparison to healthy controls, and comparable to newly-diagnosed pediatric cancer patients receiving chemotherapy and radiation therapy. This study strongly supports the use of the PedsQL™ 4.0 Generic Core Scales in the field test as a standardized benchmark instrument of generic HRQOL.

Authors: Kavitha Nutakki, MBBS, MPH, James W. Varni, PhD, Nancy L. Swigonski, MD, MPH Children's Health Services Research, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN

Age Dependent Differences in Cognition and Behaviour of Children with NF1

Neeltje Obergfell, MSc, *Medical University of Vienna (Austria)*

Neurofibromatosis type 1 (NF1) is a common autosomal dominant single-gene disorder with a very complex course that affects various fields of the children's lives and their environment. In addition to physical impairments, NF1 also entails significant impairments on a behavioural as well as on a cognitive level. The progression of the resulting developmental deficits is still unclear. Therefore, this study aims to examine age-dependent differences regarding cognition and behaviour of children with NF1. Concerning performance measurements (IQ, vocabulary, visual spatial processing and motor construction, inductive reasoning, attention, processing speed, working memory, fine and gross motor skills, facial expression recognition) and parental ratings (attention, peer relationship, physical complaints, quality of life) the retrospective data of 140 children with a defined diagnosis of NF1 within the age range 3 to 18 years will be analysed using a comprehensive test battery assessing the above mentioned domains to identify the natural history of delays. Around 140 assessments were administered to children with NF1 as part of their routine clinical care at the Medical University of Vienna between January 2007 and March 2017. Of 140 assessments, around 80 first assessments, 40 second assessments and 20 third assessments were performed. The patients were segregated into 4 age-defined cohorts: Preschool (3 – 5 years of age), Young School-Age (6 – 8 years of age), Older-School-Age (9 – 11 years of age) and Teen (12 – 18 years of age) groups. To determine significant group differences, analysis of variance (ANOVA) were calculated. In addition total areas of delay were evaluated for each cohort and compared. From the records of about 40 subjects with longitudinal data available, t-tests were calculated to identify differences in developmental achievement. Initial analysis showed significant differences in behaviour and cognition between the different age groups as well as in longitudinal assessment. In conclusion, our findings demonstrate a relation between age and NF1 associated deficits and therefore guidelines for age-dependent neuropsychological assessments and therapy are needed.

List Authors: Neeltje Obergfell MSc, Medical University of Vienna, Alexander Haselgruber BSc, University of Vienna, Amedeo A. Azizi MD, Medical University of Vienna, Irene Slavc MD, Medical University of Vienna, Ulrike Leiss PhD, Medical University of Vienna, Thomas Pletschko PhD, Medical University of Vienna.

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The Use of Dietary Supplements, Nutraceuticals and Dietary Modification by Persons with Neurofibromatosis

Melissa Peires-Hughes, *NYU Langone Medical Center*

More than 100,000 people in the United States are living with neurofibromatosis 1 (NF1), neurofibromatosis 2 (NF2), or schwannomatosis (SWN). Medical therapies for treatment of specific symptoms of the neurofibromatoses (NF) are limited and patients may seek complementary and alternative therapies. We are interested in assessing what supplements and dietary modifications are being used by people with NF.

We searched a variety of known NF-related websites, blogs, and forums for terms related to diet, dietary supplements, nutraceuticals, and alternative medicine. We developed a list of supplements and dietary modifications that were mentioned and/or discussed as being utilized for prevention or treatment of various aspects of NF. Specifically, we identified 37 supplements that were mentioned in multiple threads/sites. We conducted a search of the literature for each of these supplements in general and in relation to NF1, NF2, and SWN. Additionally, we used the list of identified supplements to create an on-line questionnaire, distributed to persons with NF to better understand the use of supplements and dietary modifications, including which symptoms these supplements and modifications are being used to address.

It is important for clinicians caring for persons with NF to understand the various alternative modalities being used by patients, as these modifications may impact their health and health care management decisions.

Full List Authors: Kaleb Yohay, MD, Director, Comprehensive Neurofibromatosis Center, NYU Langone Medical Center, Celia Engelson, MS, FNP-C, NYU Langone Medical Center, Kara Anstett, MS, CGC NYU Langone Medical Center

Pediatric Cancers in NF1

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

Neurofibromatosis type 1 (NF1) is the most common monogenic cancer predisposition syndrome. Our recent report on a population based cohort of 1404 NF1 persons showed an increased cancer risk and mortality < 20 years of age. Children with NF1 are known to have an increased risk of brain tumors, especially optic gliomas, but epidemiologic studies on the risk of other brain tumors, malignant peripheral nerve sheath tumors (MPNSTs), leukemia and other malignancies are sparse.

We have used the Finnish NF1 cohort to analyze the incidence, risk and survival of malignancies in NF1 patients < 20 years of age. Persons born in 1987-2011 were included in the analysis. A total number of 524 persons, 238 females and 286 males, were followed through files of Finnish Cancer Registry from birth up to age 20 years, death, emigration or December 31st, 2014, yielding 8,353 person years.

Cancer Registry search revealed 55 patients who had malignancy < 20 years of age. Standardized incidence ratios (SIR) and excess absolute risks (EAR) per 100,000 person years for different cancers were calculated. SIR of 37.7 and EAR of 688.1 was demonstrated for all malignancies. SIR (51.9) and EAR (895.9) for females was significantly higher than for males ($P=0.015$). The total standardized mortality ratio (SMR) for cancer deaths was 81.34.The most frequent location of malignancies was the central nervous system with 47 cases (median age 7.2 years) and SIR of 123.7. Optic gliomas were registered only if biopsied or treated. Thus, this study mainly reports other brain tumors than optic gliomas. The survival of NF1 patients with brain tumors did not differ from general population. The results demonstrated 9 malignant peripheral nerve sheath tumors (MPNSTs), with median diagnosis age of 15.2 years. Cumulative risk of having an MPNST by age 20 was 2.8%. Although leukemia has been reported to be associated with NF1, our study did not reveal an increased risk.

In conclusion, brain tumors are the first NF1-associated malignancies in children. The MPNSTs are a major concern in the adolescence. The risk for myeloid malignancies may not be as high as suggested in the literature.

Author list: Matti Rantanen MSc, Finnish Cancer Registry, Roope A Kallionpää MSc (pharm), University of Turku, Elina Uusitalo MSci, University of Turku, Minna Pöyhönen, Helsinki University Hospital, Janne Pitkäniemi PhD, Finnish Cancer Registry, Juha Peltonen MD, PhD, University of Turku

Funding: Cancer Society of Finland and Turku University Hospital

Longitudinal Academic and Executive Functioning Profiles of Children with Neurofibromatosis Type 1 (NF1) and Plexiform Neurofibromas (PNs)

Marie Claire Roderick, PsyD, *Pediatric Oncology Branch, National Cancer Institute*

Background: Youth with NF1 experience neurocognitive dysfunction, particularly in attention and executive functioning (EF), which may negatively affect learning and academic achievement. Cross-sectional studies indicate reading, math, and spelling difficulties and underperformance relative to their siblings or unaffected peers. However, the specific long-term academic and EF profiles of youth with NF1 have yet to be established due to a lack of longitudinal research. The current study examined the academic and EF of youth with NF1 over time.

Methods: Individuals, ages ≤35 years, with NF1 and PNs were eligible to enroll on a NF1 Natural History protocol. Participants completed comprehensive neuropsychological assessments, including academic testing (Woodcock Johnson-III), and their teachers completed an EF questionnaire (BRIEF-T) at baseline and 3 years later. Correlations examined relationships between academic, cognitive, and teacher scores selected apriori; t-tests evaluated change in academic scores over time; and regression analyses determined if baseline cognitive variables (i.e., cognitive flexibility, working memory, processing speed, and attention) and teacher ratings predicted academic skills at 3 years.

Results: Thirty-six participants (mean age=13 years, range 7-17, 61% males, 72% in special education) with NF1 and PNs had the selected cognitive data. Mean scaled scores (ss) of cognitive variables were below average to average (ss range=4-8) at baseline and stable at 3 years (ps>.05). Mean teacher ratings of day-to-day EF, while stable over time (ps>.05), revealed clinically significant problems with organization of materials (T-score=>65) at baseline and 3 years. Mean composite academic standard scores (SS) in reading, math, and written language at both time points were in the low end of the average range (SS range=85-93). However, mean math (94 to 85; p<.01) and written language (93 to 87; p<.05) scores declined significantly at 3 years. Additionally, about 25% of individuals performed below average (SS≤85) in these academic domains at baseline and over 40% at 3 years. Baseline cognitive variables, except attention, were associated with 3-year academic skills (ps<.05). The model examining cognitive flexibility and processing speed at baseline was significant (p<.05) and predicted 19% of the variance in math functioning at 3 years. Models exploring other academic domains were not significant. Baseline teacher EF ratings did not predict 3-year academic functioning (ps>.05).

Conclusions: While the academic functioning of most youth with NF1 and PNs is in the low end of average, a higher proportion relative to the normative sample exhibit below average skills. Over time, math and written language functioning declined significantly. Longitudinal research is needed to examine other factors besides cognitive functioning that could possibly contribute to this population's academic difficulties, such as ongoing medical problems (e.g., pain, disease severity), family functioning, and social-emotional wellbeing. The development and implementation of interventions are needed to help attenuate cognitive and academic problems in NF1.

Full Author List: Marie Claire Roderick, PsyD[1], Mary Anne Toledo-Tamula, MA[2], Pam Wolters, PhD[1], Staci Martin, PhD[1], Andrea Maria Baldwin, CRNP[1], Andy Gillespie, RN[1], Trish Whitcomb, BS, BA[1], Anne Goodwin, RN[1], and Brigitte Widemann, MD[1]

[1]Pediatric Oncology Branch, CCR, National Cancer Institute, [2]Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, Inc., NCI Campus at Frederick, Frederick, Maryland, 21702

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Longitudinal Evaluation of Pain in Schwannomatosis Patients

Monica Sheridan, BA, *Massachusetts General Hospital*

Background: The defining clinical feature of schwannomatosis is chronic pain. This patient population lacks high-quality prospective data, needed to understand the natural history of this condition and for the development of clinical trials on long-term pain management.

Methods: We prospectively recruited patients with schwannomatosis from the International Schwannomatosis Registry. Patients completed surveys at baseline and at 6 months on worst pain intensity (Numerical Rating Scale-11); pain type (ID Pain); physical functioning, anxiety, depression, and pain interference (PROMIS short forms) using a recall period of 1 week. Patients also reported pain medication usage and the presence of chronic pain, defined as pain lasting ≥3 months. T-scores were reported for all PROMIS measures, for which the US general population mean=50 (SD=10). To assess change from baseline, we used a minimal clinically important difference of ±6 for PROMIS measures and ±2 for pain intensity.

Results: 37 subjects (ages 32-78, mean age=52, 57% female) have completed the baseline and 6 month follow up survey as of March 2017. 33 of 37 subjects reported chronic pain, with two subjects having developed new chronic pain in the last 6 months. Most subjects (70%) are on pain medications, with 70% continuing the same medications from baseline. At 6 months, participants' worst pain intensity over the past week was a mean of 5/10; a majority of participants (57%) had stable scores and 22% of subjects had improved and worsened pain respectively. 73% of participants showed no change in pain type (as either predominantly neuropathic or nociceptive). At baseline, a minority of subjects scored significantly worse on PROMIS measures in physical function (30%), anxiety (32%), depression (22%), and pain interference (37%) than the general population. After 6 months, PROMIS measures remained stable in a majority of participants (78%, 59%, 76%, and 66% respectively).

Conclusion: The quality of life of schwannomatosis patients is severely impacted by chronic pain. In our sample, over half of subjects continue to report ≥5/10 pain, with decrements in anxiety, depression, pain interference, and physical functioning lasting more than 6 months. This cohort represents a target population for clinical trials in pain management.

Additional Authors: Vanessa Merker, BS, Justin Jordan, MD, Ana-Maria Vranceanu, PhD, Massachusetts General Hospital; Jaishri Blakeley, MD, Shannon Langmead, CRNP, Johns Hopkins Medical Institute; Kaleb Yohay, MD, NYU Langone Medical Center; and Scott Plotkin, MD, PhD, Massachusetts General Hospital

Long-term Results for NF2 Patients Treated With Bevacizumab

Monica Sheridan, BA, *Massachusetts General Hospital*

Background: Bevacizumab treatment has been shown to improve hearing and reduce tumor volume in a minority of NF2 patients. However, long term data on outcomes for these patients has not been reported. We report long term follow up data on clinical outcomes across a longer time period (up to 9 years).

Methods: We retrospectively reviewed clinical reports, radiologic measurements and audiologic evaluations of 31 previously reported patients with NF2 treated with bevacizumab. 18 cases, followed for ≥5 years, were used for reporting of long-term tumor growth and hearing preservation. Tumor size was determined by volumetric analysis and tumor growth is calculated as percent change from baseline (before first treatment). Hearing was monitored using word recognition scores (WRS) and pure tone audiometry. WRS were obtained using standard 50-item recorded lists of monosyllables delivered at levels selected for maximum performance. Standard pure tone threshold measures at 500, 1000, 2000 and 4000Hz were combined into a Pure Tone Average (PTA). Pre-established 95% critical difference intervals were used to define WRS stability; a minimal clinically important difference of ±10dB was used to define PTA stability.

Results: 18 of 31 subjects (ages 12-73, mean age=33, 67% female) have continued bevacizumab for more than 5 years (mean=6.8, range 5-9). Five patients (28%) showed tumor growth >20%, 4 patients (22%) were stable throughout the follow-up period, and 9 patients (50%) showed sustained reduction in tumor volume >20%. Two patients (11%) had sustained improvement in WRS from baseline through the entire follow-up period. A majority of patients (13; 72%) had stable WRS at the last available score; 3 patients (17%) had significantly worse WRS from baseline to the end of follow-up. Sustained improvement was not seen for PTA results, which were characterized by steady decline across the years in most cases. PTA worsened (>10dB) in 67% of cases (12/18) and remained stable in the remaining 6 (33%).

Conclusions: Sustained clinical benefit was observed in some patients treated with bevacizumab over a 5-9 year period. While clinical progression is typically expected over this time period, a majority of these patients receiving bevacizumab showed stability in hearing and stability or reduction in tumor volume.

Additional Authors: Vanessa Merker, BS, Chris Halpin, PhD, Justin Jordan, MD, MPH, and Scott Plotkin, M.D, PhD, Massachusetts General Hospital

Meta-analysis of Neurofibromatosis 1 (NF1) and Cognitive and Academic Achievement Outcomes

Taylor Smith, PhD, *California Polytechnic State University, San Luis Obispo*

Background: Neurofibromatosis 1 (NF1) puts individuals at risk for a wide range of maladaptive cognitive and academic outcomes. While many cognitive outcomes, for example intelligence, tend to fall within normal limits, research indicates a characteristic "downward shift" in intelligence. Additionally, specific deficits in attention, executive functioning, visual-spatial functioning, and basic reading are commonly reported. To our knowledge, however, there is no published meta-analysis examining the relationship between NF1 and cognitive and academic outcomes. Therefore, the purpose of this meta-analysis is to investigate the strength of the association between NF1 and cognitive and academic outcomes and to determine whether results vary systematically based on study and sample-level characteristics.

Methods: This meta-analysis is part of a larger, ongoing meta-analysis examining a range of neuropsychological, academic, and behavioral outcomes in NF1 samples. Random effects meta-analysis will estimate the mean of all relevant true effects. Subgroup analysis and meta-regression analyses will examine the influence of sample and methodological variables on observed effect size. Primary articles were retrieved from PubMed, Science Direct and Web of Science databases. Preliminary inclusion criteria include case-control studies with at least cognitive, intellectual and/or academic achievement outcome. Cases are individuals with NF1. Controls are typically developing controls or community sampled controls.

Results: The literature search yielded 4,578 unique studies. A total of 336 articles were included in the full text review. We are currently completing data extraction and analysis and expect that approximately 50 studies will be included in this meta-analysis.

Conclusion: This meta-analysis will inform our understanding of cognitive and academic risk in the NF1 population. This will help parents and professionals with educational and treatment planning for individuals with NF1.

Full List Authors: Michael Vargas B.S., California Polytechnic State University, San Luis Obispo; Leah Thomas, Student, California Polytechnic State University; Jacklyn Engle, Student, California Polytechnic State University, San Luis Obispo; Alan Aung, Student, California Polytechnic State University, San Luis Obispo; Maria T. Acosta MD, Children's National Health System & George Washington University; Jessica A. Kaczorowski Ph.D., California Polytechnic State University, San Luis Obispo
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Amusia is a Common Feature in Neurofibromatosis Type 1: Impairment in Musical Perception and its Electrophysiological Correlates

Juliana Souza, *Neurofibromatosis Outpatient Reference Center (CRNF)*

Background: Some cognitive disturbances, such as language disorder and learning difficulties are frequent in neurofibromatosis type 1 (NF1). It is postulated that this 'cognitive profile' is related to alterations in the cortical white matter, characterizing it as a possible cerebral disconnection disorder. Some experts claim they have observed frequent musical difficulties in people with NF1, such as the difficulty to sing in tune or play any instrument. In light of the recent verification of Auditory Processing Disorder (APD) in the disease, it is considered as probable the existence of some damage in the musical perception of such individuals. This difficulty in noticing and executing music is described in literature as amusia, and in its congenital form is also considered as a result of a possible cerebral disconnection disorder.

Objective: Investigate the occurrence of amusia in NF1; evaluate its characteristics and its connections to the subjects previous musical training; analyze their electrophysiological correlates and compare these findings with what has been previously described in the literature for congenital amusia.

Methods: 18 volunteers with NF1 (*cases*) and 22 healthy ones (*control*), without auditory deficiency, paired by sex, age and schooling were evaluated through the application of the reduced version of the Montreal Battery Evaluation of Amusia (MBEA), after answering a questionnaire about their musical background. The integrity of the primary cortical areas of auditory processing was evaluated through the registry of evoked potential Mismatch Negativity (MMN).

Results: Amusia was much more prevalent amongst the cases than amongst the controls (67% *vs.* 4.5%), and strong association with NF1 (*$P = 0{,}001$ OR = 42 IC: 4.5-39.6*). For their diagnosis it was defined as a cutoff in the MBEA the score value inferior to two standard deviations in the controls' average. It was detected in individuals with NF1 the impairment in all of the subgroups of tasks in the test (*melodic organization, temporal organization and memory*), with significant damage in the temporal processing. The occurrence and latency of MNN were similar in both groups; however, amongst the people with NF1 it was observed a higher average value of latencies in those with worse performance in the MBEA.

Discussion: Amusia showed more frequent in NF1, associated with an important impairment of the temporal processing. It was not observed a consistent connection between the musical background and its occurrence. Since most people with NF1 present APD it is likely that there is an association between this disorder and musical perception deficit. The occurrence of MNN showed itself preserved in these individuals, although with higher average value of the latencies amongst the non-musical. The alteration of these evoked potential and the important impairment of the temporal musical processing made evident in the present study are not characteristically present in congenital amusia, which suggests different neurophysiologic substrates for amusia in NF1.

Conclusion: Amusia is a common disturbance in people with neurofibromatosis type 1. It presents itself in this disease with different characteristics than those in congenital amusia, related to an important dysfunction in the temporal processing and the alteration in the MMN.

Key words: Neurofibromatosis type 1; Amusia; tone deafness; musical perception; musical abilities; Montreal Battery of Evaluation of Amusia; auditory tests; evoked potentials; Mismatch Negativity.

Authors: Cota BCL[1], Resende LM[2], Fonseca JGM[3], Souza JF[1], Rodrigues LOC[1], Rezende NA[1].

Authors affiliations: Neurofibromatosis Outpatient Reference Center (CRNF), School of Speech and Hearing Sciences and School of Medicine of Federal University of Minas Gerais, Brazil.

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Magnetic Resonance Spectroscopic Assessment of Signal Abnormalities in Children with NF1

Stavros M Stivaros, BSc, MBChB, FRCR, PhD, *University of Manchester, UK*

Background: NF1 is a tumour predisposition syndrome where optic pathway glioma (OPG) is the most prominent tumour type. Affected individuals can also develop low grade gliomas throughout the neuro-axis. Standard magnetic resonance imaging (MRI) is complicated by overlap in appearances between focal areas of signal intensity (FASI) related to myelin-vacuolation and tumour. The most common non-invasive technique used to acquire measures of neurochemistry in vivo is proton Magnetic Resonance spectroscopy (H^1 MRS). Spectral profiles in both normality and tumour are well recognised. We have systematically imaged a cohort of children using standard MRI as well as H^1 MRS to evaluate spectroscopy in both initial diagnosis as well as follow-up in these patients.

Methods: We followed 244 children referred to the NF1 nationally commissioned service imaged with both standard MRI and H^1 MRS assessment of any areas of FASI. We determined asymptomatic or symptomatic patients according to their clinical progression.

Results: Thirty nine patients had imaging evidence of OPG (16%) on the basis of standard MRI with 11 requiring intervention. Assessment of FASI in patients with no OPG showed a normal spectral profile. In patients with an OPG, tumour profile was seen in 8 (73%). In patients with no OPG, 16 FASI were identified as being tumour by spectroscopy. Progression to treatment was linked with worsening spectral profile in all cases with treatment related improvement in spectroscopy seen in all responders.

Conclusion: This is the first study to assess H^1 MRS for NF1 FASI imaging surveillance in cases of known OPG, showing tumour profile, which we hypothesise, is linked to infiltration along the optic radiations. We have shown that standard MRI is not sensitive enough to differentiate between myelin-vacuolation and sporadic tumours in non OPG NF1. We recommend that H1 MRS is an important adjunct in the assessment of NF1.

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Full List Authors: Grace Vassallo MD DCH MRCPCH, Royal Manchester Children's Hospital, Eileen Hupton RGN RSCN, Royal Manchester Children's Hospital, Lauren Lewis BSc, Royal Manchester Children's Hospital, Ian Kamaly MBChB MD FRCS, Royal Manchester Children's Hospital, JP-Kilday MBChB PhD, Royal Manchester Children's Hospital, D Gareth Evans MD, University of Manchester

Development of Patient-reported Outcomes (PROs) to Assess Pain in Individuals with Neurofibromatosis 1 (NF1) and Plexiform Neurofibromas (PNs) for Clinical Trial Endpoints: Results from Adult Participants

Kari Struemph, 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 validated 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: 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 Clinical Outcome Assessment program. As part of a larger study, individuals with NF1, ages >5 years, and PN-related pain participated in focus groups or individual interviews to review these measures for use with patients with NF1, PNs and pain. Male and female groups were conducted separately. For the current project, focus groups and interviews from the adult participants (ages 25+ years) were transcribed and qualitative content analysis was used to systematically review the patient responses. Initial categories were developed using a deductive technique and emergent categories were added after the initial transcript review. Secondary inductive analysis for predominant themes was then completed.

Results: Seven adult focus groups (n=22; 9 male and 13 female) and 8 individual interviews (4 male and 4 female) were completed (patient age range 19-62 years). Recurrent themes include how pain varies over time and in intensity; the ability to rate the pain of specific tumors and distinguish between the pain associated with different tumors; confusion about the meaning of "overall" pain; limited knowledge of PNs; generation of new items to assess pain interference; and openness to completing electronic ratings more frequently from home.

Conclusions: This qualitative research is obtaining critical information from patients about their pain and how best to measure it for clinical trials. Adult data indicates that the current questionnaires assessing pain intensity and pain interference require some modification to more accurately evaluate the chronic and variable pain experienced by patients with NF1 and PNs. Results indicate a need for more targeted questions, simplification of measures, more frequent ratings, and tailoring to issues specifically experienced by this population.

Full Author List: Kari Struemph, PhD[1], Pam Wolters, PhD[1], Staci Martin, PhD[1], Jim Tonsgard, MD[2], Cynthia MacKenzie, RN[2], Elizabeth K. Schorry, MD[3], Sara Manning, MPH[3], Karin Walsh, PsyD[4], Tess Kennedy, BA[4] & Brigitte Widemann, MD[1].
[1]POB, NCI, NIH, [2]University of Chicago, [3]Cincinnati Children's Hospital Medical Center & [4]Children's National Health System.

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Onset Symptoms and Germline Mutation Variation in Neurofibromatosis Type 2

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

Background: Neurofibromatosis type 2 (NF2) is autosomal-dominant tumor predisposition syndrome characterized by not only various multiple tumors, but also various clinical manifestations. Onset symptoms are also known to be hearing impairment, gait disturbance, ocular symptoms, skin tumors etc. Although previous reports cited onset symptoms and onset age, there are few reports regarding the correlation between onset symptoms and germline NF2 mutation type.

Objectives: The aim of this study is to clarify the correlation between onset symptoms and germline NF2 mutation type in NF2 patients.

Methods: We conducted a retrospective analysis of 44 patients who were diagnosed as NF2 between 2000 and 2016. Germline mutation analysis was performed with Sanger sequence and multiple ligation-dependent probe amplification (MLPA). We statistically evaluated the correlation between onset symptom and germline mutation variation.

Results: Onset symptoms of 44 patients were hearing impairment (17 cases, 38.7%), symptoms of skin tumors (12 cases, 27.3%), that of spinal tumors (5 cases, 11.4%), other symptoms (2 cases, 4.5%), unknown (8 cases, 18.2%). Germline mutation types were nonsense (7 cases, 15.5%), frame shift (1 case, 2.2%), deletion (4 cases, 9.1%), splice site (8 cases, 18.2%), missense (5 cases, 11.1%), and undetected (20 cases, 44.4%). Among patients with germline NF2 mutation of nonsense, frame shift, and deletion, no one presented hearing impairment as onset symptom. On the other hand, patients with other mutation types (missense mutation, undetected cases) presented frequently with hearing impairment as onset symptom.

Conclusions: Onset clinical manifestation of NF2 patients varies depending on germline NF2 mutation type.

Author list: Satoru Miyawaki MD,PhD, Atsushi Okano MD, Shunsaku Takayanagi MD,PhD, Akitake Mukasa MD,PhD, Hirofumi Nakatomi MD,PhD, and Nobuhito Saito MD,PhD; Department of Neurosurgery, Faculty of Medicine , The University of Tokyo, Japan

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Gastrointestinal Stromal Tumors in NF1

James Tonsgard, MD, *University of Chicago*

Gastrointestinal stromal tumors or GISTS are the most common mesenchymal tumor in the GI tract. These tumors are believed to be driven by mutations in KIT, PDGFRA, succinate dehydrogenase complex or BRAF. The incidence of GISTs in NF1 is unclear with reports suggesting an incidence of anywhere between 1 to 30%. GISTs in NF1 are reported to be negative for mutations in KIT and PDGFRA suggesting an alternative molecular mechanism driving these tumors. We report 9 patients with GISTs in a total of 127 adults greater than 40 years of age seen in our clinic since 2011, for an incidence of 7%. The age of presentation ranged from 42 to 59 years of age. All of the tumors were present in the small bowel. The tumors were multiple in all the patients. All but one of the tumors showed a low grade of malignancy (< 5 mitotic figures per hpf). All patients were treated with surgical excision. Only two of the patients were treated with imatinib. The single patient with a high grade malignancy died within a year of presentation. The remainder (89%) are alive and have been symptom free for 5 years without adjuvant therapy. Symptoms in some patients were subtle including anemia and were seen only with abdominal imaging. Three of the 9 patients had a preceding history of an MPNST. Our findings indicate that GISTs occur in adults with NF1 between 40-60 years of age and are usually treatable by simple surgical excision.

Peripheral Nerve Ultrasound in Pediatric Patients with Neurofibromatosis Type 1 (NF1)

Pia Vaassen, MD, *Department of Pediatrics, Sana Kliniken Duisburg, 47055 Duisburg, Germany*

Background: Peripheral nerve sheath tumors (neurofibromas) are the hallmark finding in neurofibromatosis type 1 (NF1) and can mostly be detected by clinical examination. However, volumetry or characterization of the growth pattern of neurofibromas usually requires magnetic resonance imaging. Recent studies demonstrated that ultrasound is an alternative and non-invasive procedure to characterize peripheral nerve sheath tumors. We therefore tried to characterize neurofibromas by performing an ultrasound examination of peripheral nerves in NF1 patients. Since it has been shown previously that fascicle formation is altered in a cell culture model of neurofibroma development we also tried to determine if structural changes of peripheral nerves can be detected by ultrasound prior to neurofibroma formation.

Methods: Since there are no reference values for the diameter of the median nerve in children we first applied ultrasound to measure the diameter of the median nerve in a cohort of healthy children (aged 4-17 years). Then we compared the obtained values with measurements in an age- and sex-matched group of children with NF1 (with and without a clinically identified neurofibromas). In this setting, transversal and longitudinal sections of the median nerve were obtained with a 12-15 Mhz ultrasound transducer on the inside of the forearm. The peripheral nerve was traced in caudal and cranial direction. Wide base and depth of the median nerve were measured in selected positions and the cross sectional area was calculated.

Results: In single cases changes of peripheral nerve morphology could be detected by ultrasound prior to clinically detectable neurofibroma formation. However, in the majority of NF1 patients morphological changes of peripheral nerves could not be detected. In addition, there was no significant difference between the peripheral nerve diameter in NF1 patients and the control group.

Discussion: Ultrasound can identify slight morphological changes of peripheral nerves in some NF1 patients prior to neurofibroma formation. In general, significant morphological or morphometric changes of peripheral nerves in NF1 patients could not be detected. However, ultrasound plays an important role for follow-up examinations of NF1 patients with superficial neurofibromas since it is a fast, easy and readily available method to characterized the size, structure and topography of these tumors.

Author list: Nele Segarra MD, Axel Feldkamp MD, Thorsten Rosenbaum MD PhD, Department of Pediatrics, Sana Kliniken Duisburg, Germany

An Investigation of the Brain Morphologies of Youth with Neurofibromatosis Type 1

Ariana Vajdi, BS, *UCLA*

Introduction: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder caused by a mutation of the *neurofibromin* gene on chromosome 17. It is associated with a range of both physical complications and cognitive deficits. Individuals with NF1 present with particular challenges in executive function, which are understood to be mediated by the frontal and parietal regions of the brain. However, the brain alterations underlying these deficits are not well understood. We conducted a cross-sectional investigation of regional brain morphometry in subjects with NF1 compared to typically developing controls (TD); notably, we decomposed global volume measures into cortical thickness and surface area, which are believed to have distinct neurodevelopmental origins.

Methods: T1-weighted scans were collected from 42 NF1 subjects (mean age=12.1, 38% male) and 29 TD control participants (mean age=12.1, 48% male). We processed the scans using the FreeSurfer image analysis suite to extract measures of cortical thickness, surface area, and volume, globally and across 12 regions of interest (ROIs) in the frontal and parietal lobes. We then examined group differences using an ANOVA, covarying for gender and age.

Results: NF1 subjects showed significantly increased total white matter volume across the left and right hemispheres compared to TD controls (both $p=.001$). NF1 subjects also presented with significant increases in cortical thickness and/or surface area in 8 out of the 12 regions examined. In particular, subjects exhibited increased thickness and surface area in the lateral orbitofrontal cortex bilaterally ($p=.040$, $p=.015$, respectively) and increased surface area in the superior and inferior frontal gyri across both hemispheres (all $p=.05$). Overall, these increases were more prominent in frontal (vs. parietal) regions.

Conclusions: Neuroimaging findings suggested that youth with NF1 exhibit both increased cortical thickness and surface area in fronto-parietal regions of the brain with few exceptions, as well as increased total white matter volume, which may play a role in the characteristic cognitive impairments. Future analyses will relate neuroimaging findings to variability in cognition and behavior.

Tena Rosser MD, Children's Hospital Los Angeles/University of Southern California; Rachel Jonas PhD, UCLA; Laura Hansen MA, UCLA; Leila Kushan-Wells MS, UCLA; Alcino Silva PhD, UCLA; Carrie E. Bearden PhD, UCLA

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Crowe's Sign in Neurofibromatosis Type 1

David Viskochil, MD, PhD, *Division of Medical Genetics, University of Utah, Salt Lake City, UT*

Experienced practitioners have come to appreciate the emergence of intertriginous freckling as a reliable and key clue in diagnosing NF1 in early childhood. It is such a distinctive finding that we recently clinically diagnosed mosaic NF1 in an 8-yo girl with <6 café-au-lait spots who had right axillary freckling and left inguinal freckling. On blood lymphocyte analysis, she had low-level mosaicism for a 2-bp *NF1* deletion in exon 37 [=/4982_4983delGT];[=]. Even though recognized as a diagnostic aid in NF1 by Frank W. Crowe in 1964, the pathophysiology of NF1-melanocytes in intertriginous zones, especially the axillary vault, to alter its melanin synthesis in a patch-like pattern consistent with lentigo simplex is not known.

We reviewed a cohort of children in our NF Clinic for the natural history of freckling in NF1. In a retrospective cross-sectional study, we assessed 348 pediatric patients (age range 3 months to 18 years) with NF1 between 2000 and 2016 who had at least 1 evaluation at the University of Utah NF Clinic. Freckling in at least 1 site was present in 318 subjects (91%); 292 in the axillae, 307 in the groin, 282 in both sites. Neck freckling was noted in 36, and more extensive truncal freckling was noted in 17. Consecutive exam forms were available in 104 subjects. Seventy-five subjects were evaluated with sequential exams, and the median age of onset of freckling was 3 years, 5 months (range 9 months – 10 years). Absence of intertriginous freckling in someone with multiple café-au-lait macules approaching puberty raises suspicion regarding the diagnosis of NF1.

Why melanocytes in the intertriginous zones are more susceptible to develop pigment changes as distinctive freckles is still unknown. Increased skin temperature, absence of light exposure, skin secretions, and friction have all been proposed. To assess the *NF1* mutation status of axillary freckling, a 21-year old volunteer with generalized NF1 underwent skin biopsy of 3 freckles in the left axilla. Samples were processed and both melanocyte and fibroblast cultures were obtained from each of the 3 freckles. His germline *NF1* mutation (c.2842delC) was shared by all 3 freckles, and each freckle had an acquired second pathogenic *NF1* mutation in melanocyte cultures that were not present in fibroblasts from the same biopsy sample (freckle A: c.6579+2T>A; freckle B: c.998dupA; freckle C: c.7181delC). These data indicate double inactivation of *NF1* by independent somatic mutation in melanocytes derived from clustered axillary freckles occurs post-embryologically, likely after neural crest stem cells have differentiated to melanocytic lineage.

Angela Peron[2], David A. Stevenson[3], Alicia Gomes[4], Tom Callens[4] and Ludwine Messiaen[4]
[2]Child Neurology Unit – Epilepsy Center, San Paolo Hospital, Department of Health Sciences, Universita' degli Studi di Milano, Milan, Italy; [3]Division of Medical Genetics, Stanford University, Stanford, CA; [4]University of Alabama, Birmingham, Medical Genetics and Genomics, Birmingham, AL

The Impact of a Mind-Body Program on Multiple Dimensions of Resilience among Patients with Neurofibromatosis

Ana-Maria Vranceanu, PhD, *Massachusetts General Hospital and Harvard Medical School*

The neurofibromatoses (NF) comprise of a group of genetic disorders that predispose patients to develop nerve sheath tumors that often cause significant morbidity including disfiguring cutaneous tumors (NF1); complete hearing loss, facial weakness, and poor gait (NF2); and chronic disabling pain (schwannomatosis). Currently there is no cure for NF. Symptom management provided by surgery and palliative measures are primary means of treatment. Prior research has shown that patients with NF have impaired quality of life and heightened psychological distress. Resiliency – the ability to bounce back when faced with chronic stressors – is a multidimensional construct particularly relevant to patients with NF who have to adjust to living with this chronic incurable condition. A recent randomized controlled trial (Vranceanu et al., 2016) demonstrated that the 8 session live video Relaxation Response Resiliency Program adapted for patients with NF (3RP-NF) produced sustained increases in both physical and psychological quality of life, relative to an attention placebo control. This secondary analysis further examines the effects of the 3RP-NF versus control on multiple dimensions of resilience.

Sixty-three patients (46 female, 56 White) were randomized to 3RP-NF ($N = 32$, Mage $= 43$) or control ($N = 31$, Mage $= 40$), and completed pre- and post-intervention measures. Of these, 52 completed a 6-month follow-up assessment. The multidimensional assessment of resilience included measures of coping, social support, gratitude, optimism, spirituality, and mindfulness.

Repeated measures ANOVA with linear contrasts indicated that the 3RP-NF produced sustained increase in multiple measures of resilience. Participants randomized to 3RP-NF demonstrated greater improvements from pre- to post-intervention in coping (6.68; 95% CI: 1.78–11.58; $p = .008$), social support (9.16; 95% CI: 0.82-17.50; $p = .032$), and mindfulness (2.23; 95% CI: 0.16–4.29; $p = .035$), relative to control. These improvements were maintained at 6-month follow-up. On average, participants in 3RP-NF showed improvements at in optimism and spirituality.

The 3RP-NF improved multiple dimensions of resilience, and produced increases in coping, social support, and mindfulness that were over and above those observed in the control condition. Improvements were sustained at six-month follow-up. Results suggest that psychosocial intervention can promote resilience among patients with NF and that 3RP-NF may be efficacious in targeting multiple dimensions of resilience.

Catherine Pierre-Louis, BA[1], Emily L. Zale, MS[1,2,3], Eric Ricklin[1,4], Eric A. Macklin, PhD[1], Ana-Maria Vranceanu, PhD[1,2]

[1]Massachusetts General Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Syracuse University, Syracuse, NY; [4]Fordham University, Bronx, NY

Promoting Resilience among Parents of Youth with NF: Using Qualitative Data to Inform Intervention Development

Ana-Maria Vranceanu, PhD, *Massachusetts General Hospital and Harvard Medical School*

Neurofibromatosis (NF1 and 2) are multisystemic, complex and rare diseases with many still unrecognized features in pediatric patients. Parents of children with NF are at an increased risk of emotional and physical health problems due to the demands associated with carrying for a child with NF, including guilt associated with passing on or potential causes for the NF, dealing with multiple medical appointments, uncertainty of disease management and progression, cognitive, social and emotional difficulties experienced by the child, and trouble around self prioritization. Anecdotal information and clinical experience has shown that often children sense their parents' level of stress and worry, and shy away from sharing potential concerns, as to not add to their parents' distress; ultimately, this leads to increased stress for children. However, no psychosocial interventions targeting parental stress are available for this population.

We aimed to determine parents' perceptions of stressors associated with parenting a child with NF in order to inform the development of a resiliency intervention. We conducted three live video semi structured focus groups with parents of youth with NF (N=40), which were subsequently transcribed and coded using grounded theory.

Parents reported heightened stress associated with the child's educational, medical and social needs, as well as concerns about their child's physical and mental health. They also reported stress associated with managing finances, multiple medical appointments, role challenges (i.e. being a parent or partner), and managing uncertainty/unpredictability around their child's NF diagnosis. These stressors reportedly affected employment status (i.e. work scale backs), relationships (i.e. social, familial, with partner, other children), and the self (i.e. negative effects on parents' physical and mental health). Few parents reported engaging in adaptive coping such as mindfulness, exercise, or use of social support.

All participants expressed interest in a mind body program aimed at improving resiliency by teaching coping skills (e.g., mindfulness, adaptive thinking, positive psychology skills) and providing support. Additional topics initiated by parents included facilitating healthy social interactions for the child, resource sharing, managing financial stressors, and parental role challenges. Barriers to participation included travel and time of intervention sessions, and all participants were in favor of the intervention being delivered via a web-based platform (i.e. Skype).

Results show parent's enthusiasm for a resiliency intervention targeting stress associated with parenting a child with NF, and provide valuable information for the content of the intervention and its delivery modality.

Pierre-Louis, Catherine[1]; Rugamas, Carmen[1]; Riklin, Eric[2]; Vranceanu, Ana-Maria[1, 3]

[1]Psychiatry, Massachusetts General Hospital, Boston, MA, United States; [2]Forham University, Bronx, NY, United States; [3]Harvard Medical School, Boston, MA, United States.

Improvements in Quality of Life from Baseline to One-Year Follow; Long Term Results from a Live Video RCT

Ana-Maria Vranceanu, PhD, *Massachusetts General Hospital and Harvard Medical School*

Background: NF1, NF2, and schwannomatosis are incurable tumor suppressor syndromes, associated with significant psychological distress and impaired quality of life (QoL). We previously reported that participation in a mind body group program adapted for patients with NF, the Relaxation Response Resiliency program (3RP-NF) results in significantly more improvement in QoL compared to participation in an attention placebo control, the Health Enhancement Program for NF (HEP-NF), and that improvements maintain at a 6 month follow up. We now report on: 1) overall changes between baseline and 1 year follow up, and 2) changes between 6 months and 1 year follow-up.

Methods: Sixty-three patients were enrolled, randomized and completed the post intervention measures. Fifty-two completed the 6 months follow up and 53 completed the 1 year follow-up. Participants completed the WHOQOLBref to assess 4 aspects of QoL at all time points.

Results: Patients were English-speaking without significant hearing loss recruited from across the world though advertisements sent by CTF. The majority of patients had NF1 (46), 10 had NF2, and 6 had schwannomatosis. Patients in the 3RP-NF group showed significantly greater improvement between baseline and the 1 year follow-up than the control group in Physical Health QoL (12.68; 95% CI: 1.76-23.59; P=.02] and Social Relations QoL [16.81; 95% CI: 3.03-30.59; P=.02]. Although patients in the 3RP-NF improved more than those in HEP-NF on Psychological QoL [8.36; 95% CI: -2.63-19.36; P =.13] and Environmental QoL [8.39; 95% CI: -1.70-18.48; P=.10], results did not reach the significance level with this small sample. All changes were above the minimally clinical importance difference (MCID =6.5). There were no significant differences in changes between 6 months and 1 year between those in the 3RP-NF and HEP-NF on any of the QoL domains ($p<.1$).

Conclusion: The 3RP-NF delivered via live video is associated with sustained improvement in physical and social QoL over 1 year period. Improvement in psychological and environmental QoL, although not significantly higher in the 3RP-NF versus HEP-NF, were clinically meaningful to patients. Results should be replicated in a fully powered RCT, to allow comparisons among NF type and assess interventionist effects.

Full Author List: Ana-Maria Vranceanu, PhD; Emily L. Zale; Chris Funes; Scott R. Plotkin.

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Beyond Autism: Understanding Social Dysfunction in Children with NF1 Through an Integrative Model of Social Skills Development and Dysfunction

Karin S. Walsh, PsyD, *Children's National Health System (CNHS) & The George Washington University School of Medicine (GWU)*

Social dysfunction in NF1 has been broadly acknowledged for some time, with more recent investigations focusing specifically on the prevalence of Autism Spectrum Disorders (ASD) in this patient population. While this approach provides some opportunities to understand social dysfunction in NF1, the scope of investigation is limited to diagnostic categorization. It is unclear if children with NF1 map completely on to the ASD phenotype. Development of social skills begins in infancy and continues into adolescence, relying on appropriate development linked to many internal and external factors.

We will present the integrative Socio-Cognitive Integration of Abilities Model (SOCIAL; Beauchamp & Anderson, 2010) as an approach to more fully examining and understanding the social dysfunction observed in NF1 and consider multiple areas where disruption may occur. The SOCIAL model highlights multiple integrative factors including cognitive, emotional, and environmental that are associated with disrupted social development. We will present data from several studies within our research program demonstrating deficits in multiple key domains within the model. Specifically, children with NF1 demonstrate significant disruptions in attention and executive function (working memory, planning, organization, and monitoring primarily), deficits in social awareness and social cognition (facial affect recognition), and elevated rates of internalizing psychological difficulties (anxiety), all of which are associated with poorer social development and functioning in children and adolescents with NF1, as the SOCIAL model suggests.

This multi-factorial, theoretically-based approach to examining the social deficits observed in children and adolescents with NF1 will allow for broader opportunities for identifying the complex inter-relationship between cognitive and psychological factors involved in social dysfunction, which allows for more individualized intervention over a wider developmental span.

Full list authors: Kristina K Hardy PhD[1,2]; Stephany Cox PhD[1]; Tess Kennedy BA[1]; Anthony Gioia BA[1]; Claire Semerjian MA[1]; Maria T Acosta MD[1,2]; Roger J Packer MD[1,2]

[1]CNHS [2]GWU

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Somatic Genomic Copy Number and Histological Features of Breast Cancer from Women with NF1

Xia Wang, MD, PhD, *Moffitt Cancer Center*

Women affected with Neurofibromatosis type 1 (NF1) have moderately increased risk of breast cancer. This study characterized NF1 breast cancer by histology and somatic genomic copy number analysis. Investigating these features can help to understand the mechanisms of breast cancer development and progression in women with NF1.

NF1 women with a history of breast cancer were recruited by three CTF affiliated NF clinics: Henry Ford Health System, Johns Hopkins University, and Children's National Medical Center. Additional recruitment advertisements were distributed by CTF newsletters as well as among NF patient support groups. Their breast cancer tumor specimens were collected. Immunohistochemistry (IHC) assay for HER2/ErbB2 was conducted using antibodies from Cell Signaling Technology. Fifteen archived breast cancer tumor specimens were collected from 14 women with NF1. Nine (9/15) stained strongly positive for HER2. Sufficient genomic DNA was obtained from 11 tumor specimens. Affymetrix OncoScan® FFPE Assay with Nexus Express V3 was applied to assess genomic copy number variation (CNV) and allelic status. Nine datasets of breast cancer from women with NF1 (NF+BrCa) were considered of sufficiently high-quality to be analyzed. To provide control samples for analysis, 61 high-quality sporadic breast cancer OncoScan datasets (BrCa) were selected from multiple sources including NCBI. Among these, 13 datasets were identified as HER2 positive samples. Comparing NF+BrCa datasets with BrCa datasets, CN losses on 1p and 4p in NF+BrCa appear to be significant ($p<0.05$). In addition, NF+BrCa lacks the CN gains of BrCa samples on 10q, Xp, and Xq, ($p<0.05$); however, none of these regions have passed multiple test, $q=1$. *ERBB2,* the gene encoding HER2, does not appear significantly more amplified in NF+BrCa than in sporadic breast cancer. The results suggest HER2 overexpression is a prominent feature of breast cancer in women with NF1. Other mechanisms of mRNA overexpression have likely contributed to the overexpression besides *ERBB2* genomic amplification. Cancer related gene *PHOX2B* is within region 4p13-p12. *FGFR2* is within 10q26.12-q26.13. *MSN* overlaps region Xq11.1-q12. *MTCP1* is within Xq26.2-q28. Excluding 13 datasets of HER2 over-expressed sporadic breast cancers, comparative analysis results remain very similar. The unique CNV profiles of NF+BrCa would need to be confirmed with more samples.

Patrick Gonzales PhD, University of Alabama at Birmingham; Dhananjay Chitale MD PhD, Henry Ford Hospital; Renee Tousignant MS, GeneDx; Jamie Teer PhD, Moffitt Cancer Center; Jaishri Blakeley MD, Johns Hopkins University; Maria Acosta MD, Children's National Medical Center; Bruce Korf MD PhD, University at Alabama at Birmingham; Michael Tainsky PhD, Wayne State University; Ludwine Messiaen PhD, University at Alabama at Birmingham

Grant - US Army Medical Research

Indications and Outcomes of Children Treated with Radiation Therapy for Central Nervous System Tumors in Neurofibromatosis Type 1

David S. Wolf, MD, PhD, *Emory University School of Medicine/Children's Healthcare of Atlanta*

Introduction: Brain tumors occur in approximately 20% of children with neurofibromatosis type 1 (NF1). Most of these tumors involve the optic pathway and are presumed to be pilocytic astrocytomas (WHO grade 1). Malignant tumors are uncommon. For tumors requiring treatment, chemotherapy is the main option. Though radiation therapy is an effective treatment for gliomas, it is avoided in children. This is especially true in NF1 due to concerns for long term effects, including vasculopathy and secondary malignancy (malignant peripheral nerve sheath tumor or malignant glioma).

Methods: This is a retrospective review of patients seen at Children's Healthcare of Atlanta from 2010 – 2016 for neurofibromatosis type 1 and a central nervous system tumor who also received radiation therapy.

Results: 298 patients were identified as having NF1 and central nervous system tumor. Seven were treated with radiation therapy (2.7 %). Seven of 8 had biopsy prior to initiation of treatment. Three patients had chiasmatic/hypothalamic gliomas (2/3 with pilocytic astrocytoma on biopsy), one had high grade pleomorphic xanthoastrocytoma (felt to be secondary to prior alkylator therapy), and three had high grade gliomas (one in spine, two in thalamus). Four of 7 patients received proton beam therapy. Indications for receiving radiation therapy were chemotherapy failure (2), primary malignancy (4), and initial treatment for low grade tumor (1). Pseudoprogression occurred in three cases. One patient developed cavernomas. No instances of secondary malignancy were identified. One patient died due to disease progression, 3 are currently in hospice care, 2 are survivors >10 years and were lost to follow-up.

Conclusion: Radiation therapy was primarily utilized in children with a primary malignant central nervous system tumor or those who failed chemotherapy. Pseudoprogression was common. There were long term survivors. No secondary malignancies occurred.

Full authors: David S. Wolf MD, PhD, Emory University School of Medicine; Ben Siegel BS, Emory University School of Medicine

Using Patient-Reported Outcomes (PROs) to Assess Pain and Classify Pain Morbidity in Neurofibromatosis 1 (NF1) Clinical Trials for Plexiform Neurofibromas (PNs): Baseline Data from the Pediatric Phase II Selumetinib Trial

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

Background: A phase II registration trial of the MEK1/2 inhibitor selumetinib is underway to treat inoperable PNs in children with NF1. For drug approval, the FDA is requiring clinically significant improvement in symptoms, such as pain, in addition to reduction in tumor volume. No validated methods exist for classifying pain morbidities and evaluating changes in pain in NF1 trials. This analysis describes and compares the baseline clinical and PRO pain data from the selumetinib trial to examine their use in assessing pain and classifying pain morbidity.

Methods: Children, 3-18 years, with NF1, inoperable PNs, and PN-related morbidity, were eligible for stratum 1. Pain morbidity was defined separately using 1) clinical information and 2) PRO data (self-report for ages ≥ 8 years; parent report for ages ≥5 years), and outcomes were compared. Clinical pain morbidity was defined as pain reasonably related to PN location that: a) limits patients' daily living activities, or b) requires regularly scheduled pain medication (neuropathic, NSAIDs), or c) requires as-needed narcotic medication. PRO pain morbidity was defined as: 1) Numeric Rating Scale (NRS-11) worst tumor pain (physician-selected or self-selected) self-report rating from 4-10 (0-10 scale) or 2) Pain Interference Index (PII) self or parent report mean total score ≥1.0 with at least 2 scores ≥3 (0-6 scale).

Results: Fifty children (mean age=10.36 years; range=3-17; 60% male) were identified for stratum 1; 47 had PRO data (2 were too young for PROs; 1 had missing data). Of these 47, 25 (53%) had pain morbidity determined by clinical symptoms, and 24 (51%) were classified with pain morbidity using PRO criteria (in total 41/47 [87%] classifications agree). When pain medication was considered with PRO data for 3 patients, agreement increased to 44/47 (94%); the other 3 discrepancies were all for young children (<8 years) who only had parent PII ratings that met criteria. The clinical pain morbidity group's baseline median NRS-11 rating=5 (range 0-10), Child PII rating=1.25 (range 0-5.5), and Parent PII rating=2.5 (range 0-4.8); the non-pain morbidity group's baseline median NRS-11 rating=0 (range 0-3), Child PII rating=0.09 (range 0-1.5), and Parent PII rating=0 (range 0-1.8). The tumor chosen separately by the patient and physician for rating pain matched in 75% of cases.

Conclusions: Clinical and PRO data are both necessary for defining pain morbidity in NF clinical trials, leading to high agreement (94%). These data suggest that current pain PROs are clinically valuable, supporting their use as endpoints in NF trials, but also confirm the need for further modifications and validation of these tools to assess tumor pain, specify the target tumor to rate, and develop PROs for young children, which are underway.

Full Author List: Pam Wolters, PhD[1], Andrea Gross, MD[1], Staci Martin, PhD[1], Marie Claire Roderick, PsyD[1], Andrea Baldwin, CRNP[1], Marielle Holmblad, RN, BSN[2], Trish Whitcomb, BS, BA[1], Mary Anne Toledo-Tamula, MA[2], Kari Struemph, PhD[1], Michael Fisher, MD[3], Brian Weiss, MD[4], Aerang Kim, MD[5], Brigitte Widemann, MD[1]
[1]National Cancer Institute, [2]Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research Inc., NCI Campus at Frederick, Frederick, MD 21702, [3]Children's Hospital of Philadelphia, [4]Cincinnati Children's Hospital and Medical Center, [5]Children's National Medical Center

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Access to Care for the Neurofibromatosis Type 1 Pediatric Population of South Carolina

Lesli Woodall, PA-C, *Medical University of South Carolina*

Background/Rationale: The purpose of the study was to measure the current access to care in the state of South Carolina for children and adolescents with a diagnosis of neurofibromatosis type 1 (NF1) and to determine the need for an NF Clinic (meeting the criteria for an NF Clinic Network per the Children's Tumor Foundation) in South Carolina, as none currently exists.

Methods/Methodology: The nature of the investigation was non-experimental and used a prospective reference period. Data collection was cross-sectional using purposeful, judgmental sampling of pediatric and family practice clinicians (Medical Doctors, Doctors of Osteopathic Medicine, Physician Assistants, and Nurse Practitioners) located in the state of South Carolina. On-line accessible surveys, administered through Survey Monkey, were made available to these clinician types.

Results: A large majority of respondents reported being unfamiliar with the NF Clinic Network and more than a quarter are unfamiliar with the diagnostic criteria for NF1, despite having an average of more than seven patients with NF1 and greater than sixteen years of experience in family medicine and pediatrics. An alarming majority reported being unfamiliar with treatment protocols, clinical trials, and the use of off-label treatments for manifestations of NF. In addition, referrals to NF clinics were minimal and were not made to the nearest NF Clinics.

Conclusions: Results of the study indicate a critical lack of access to care in South Carolina due to both the lack of provider awareness and absence of an NF Clinic in the state. The patient population size and NF Clinic billable services model warrant a financial analysis by pediatric hospital systems in South Carolina in support of the establishment of an NF Clinic.

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NF CLINIC NETWORK

CHILDREN'S TUMOR FOUNDATION
ENDING NF THROUGH RESEARCH

All NF Clinics Breakout Session

Monday, June 12, 2017 4:15 pm-7:15 pm
Mount Vernon meeting room

4:15-4:30	**CTF Welcome**	***Annette Bakker, PhD*** ***Children's Tumor Foundation***
		Dave Viskochil, MD, PhD ***University of Utah***
4:30-4:45	**NF Clinic Network Updates**	***Heather Radtke, MS, CGC*** ***Children's Tumor Foundation***
4:45-5:10	**NF Clinic Outliers - How to Approach the Challenge**	***Dusica Babovic-Vuksanovic, MD*** ***Mayo Clinic Rochester***
5:10-5:20	**Case Presentations**	
5:20-5:35	**REiNS and Cutaneous NF Working Group**	***Ashley Cannon, PhD, MS, CGC*** ***University of Alabama Birmingham***
5:35-5:50	**Break**	
5:50-6:10	**Rare Disease Registries: NF Registry in Context**	***Pamela Knight, MS*** ***Children's Tumor Foundation***
6:10-6:30	**Cancer Risks in NF1**	***Juha Peltonen, MD*** ***Institute of Biomedicine Finland***
6:30-6:50	**NF Care Guidance – Pediatric and Adult Efforts**	***David Miller, MD, PhD*** ***Boston Children's Hospital***
		Doug Stewart, MD ***National Cancer Institute***
6:50-7:10	**Improving Access to NF Care**	***Vanessa Merker, BS*** ***Massachusetts General Hospital***
		Justin Jordan, MD, MPH ***Massachusetts General Hospital***
7:10-7:15	**Program Conclusion**	

International NF1-Autism Consortium Team (INFACT)

CTF Meeting
Washington D.C.
June 12, 2017
4:15pm – 6:45pm

Organizers:

Stephanie M. Morris, MD
Instructor
Division of Pediatric Neurology
Department of Neurology
Washington University

David H. Gutmann, MD, PhD
Donald O. Schnuck Family Professor
Vice Chair for Research Affairs
Department of Neurology
Director, Washington University NF Center

John N. Constantino, MD
Blanche F. Ittleson Professor
Psychiatry and Pediatrics
Direction, William Greenleaf Eliot
Division of Child & Adolescent Psychiatry
Washington University

Maria T. Acosta, MD
Clinical Director, Gilbert Family NF Institute
Associate Professor
Department of Pediatrics and Neurology
George Washington University

Overview and Objectives: We have successfully leveraged the collective expertise of six major centers with expertise in NF1 and autism to analyze the ASD phenotype in over 500 patients with NF1, and publish the first collaborative INFACT manuscript in *JAMA Psychiatry*. The objective of this meeting is to discuss next-phase consortium research endeavors.

Workshop Program:

4:15pm **Introduction**
Stephanie Morris **(15 minutes)**

4:30pm **Behavioral Impairments in Children with NF1 – A Standardized Approach to Screening and Treatment**
Maria Acosta/Stephanie Morris **(45 minutes)**

a) Adopt standardized screening for behavioral problems in children with NF1
   - Guideline/Recommendations paper from INFACT

b) Strategize a uniform treatment protocol of behavioral problems in children with NF1
   - Future prospective study through NF Clinical Trials Consortium

5:15pm **The Next Wave of INFACT Research**
Group Discussion **(80 minutes)**

Action Items:
- Creation of centralized database (Sage Bionetworks)
  - Non-INFACT site contribute to collection of cases/data
- Effort to enrich existing database
  - Registry spreadsheet from each site
  - Addition of interventions (drugs/dev therapies) that have been tried
- Advancing a Genotype-Phenotype Grant Application

6:35pm **Wrap-Up (10 minutes)**

INFACT Participating Sites and Members

Children's National Medical Center
Maria T. Acosta, MD
Clinical Director, Gilbert Family NF Institute
Medical Director, ADHD Genetics Study (NGRI)
macosta@childrensnational.org

Washington University
John N. Constantino, MD
Blanche F. Ittleson Professor
Psychiatry and Pediatrics
Director, William Greenleaf Elliot
Division of Child & Adolescent Psychiatry
Co-Director, IDDRC
Washington University
constanj@psychiatry.wustl.edu

David H. Gutmann, MD, PhD
Donald O. Schnuck Family Professor
Vice Chair for Research Affairs
Department of Neurology
Director, Washington University NF Center
gutmannd@neuro.wustl.edu

Stephanie Morris, MD
Instructor
Division of Pediatric Neurology
morris.s@wustl.edu

University of Manchester (UK)
Susan Huson, MD, FRCP
Oxford Regional Genetics Service
Manchester Center for Genomic Medicine
Susan.huson@cmft.nhs.uk

Shruti Garg, PhD
Clinical Senior Lecturer in Translational Child Psychiatry
Shruti.garg@manchester.ac.uk

Jonathan Green, FRCPsych
Professor of Child and Adolescent Psychiatry
Jonathan.green@manchester.ac.uk

University of Leuven (Belgium)
Eric Legius, MD, PhD
Professor and Chair of Human Genetics
Head, Center for Human Genetics
Catholic University of Leuven
Eric.legius@uzleuven.be

Ellen Plasschaert, PhD
Psychology
Center for Human Genetics
Ellen.plasschaert@uzleuven.be

Murdoch Children's Research Institute
Jonathan Payne, PhD
Senior Research Fellow
Child Neuropsychology
The Royal Children's Hospital
jonathan.payne@mcri.edu.au

Kathryn North, MD, PhD
Director, Murdoch Children's Research Institute
University of Melbourne
kathyn.north@mcri.edu.au

Belinda Barton, PhD
Department Head, CHERI
Pediatrics & Child Health
Children's Hospital at Westmead
Belinda.barton@sydney.edu.au

University of California, San Francisco
Laurie Weiss, PhD
Genetics of Autism Research Program
Department of Psychiatry
Institute for Human Genetics
lauren.weiss@ucsf.edu

Cleveland Clinic Foundation
Thomas Frazier, PhD
Director, Center for Autism
Center for Pediatric Behavioral Health
Children's Hospital for Rehabilitation
FrazierT2@ccf.org

Attendees List – 2017 NF Conference

LAST	FIRST	INSTITUTION	EMAIL
Aguilar-Bonilla	Ana	Arnold Palmer Hospital for Children	ana.aguilar-bonilla@orlandohealth.com
Ahmed	Sherif	Massachusetts General Hospital	sherif.ahmed@mgh.harvard.edu
Alcantara	Krizelle Mae	University of the Philippines Diliman	kmalcantara@pgc.up.edu.ph
Aldape	Kenneth	Princess Margaret Cancer Centre	kaldape@gmail.com
Allaway	Robert	Sage Bionetworks	robert.allaway@sagebase.org
Allen	Taryn	National Cancer Institute	taryn.allen@nih.gov
Alston	Jessica	F-Prime Capital	jalston@fprimecapital.com
Ammoun	Sylwia	Plymouth University	sylwia.ammoun@plymouth.ac.uk
Anastasaki	Corina	Washington University in St Louis	korinaanastasaki@wustl.edu
Anderson	Sabrena	Clinic 24	sabrenap@hotmail.com
Anstett	Kara	NYU Langone Medical Center	kara.anstett@nyumc.org
Artt	Anita	Exelixis, Inc	aartt@exelixis.com
Ayter	Sükriye	Faculty of Medicine	sayter@hacettepe.edu.tr
Babovic-Vuksanovic	Dusica	Mayo Clinic	dbabovic@mayo.edu
Bakker	Annette	Children's Tumor Foundation	abakker@ctf.org
Baldwin	Andrea	National Cancer Institute	baldwinam@mail.nih.gov
Banine	Fatima	Oregon Health & Science University	baninef@ohsu.edu
Barclay	Paul	N/A	pbarclay417@gmail.com
Barnes	Bianca	Cupid Charities	Bianca@Cupids.org
Barnett	Carolina	University of Toronto	carolina.barnett@gmail.com
Barton	Belinda	The Children's Hospital at Westmead	belinda.barton@health.nsw.gov.au
Beach	Paul		pbinbox2@cs.com
Beauchamp	Roberta	Massachusetts General Hospital	rbeauchamp@partners.org
Bergner	Amanda	Ambry Genetics	abergner@ambrygen.com
Berman	Yemima	Royal North Shore Hospital	Yemima.berman@health.nsw.gov.au
Bertsch	Nicole	Mayo Clinic	Bertsch.Nicole@mayo.edu
Bessler	Waylan	Indiana University	wbessler@iu.edu
Betesh	Lisa	CHLA	lbetesh@chla.usc.edu
Bielamowicz	Kevin	Arkansas Children's Hospital	kjbielamowicz2@uams.edu
Blatner	Nichole	Exelixis, Inc	nblatner@exelixis.com
Bocquet	Arnaud	Institut de Recherche Pierre Fabre	arnaud.bocquet@pierre-fabre.com
Bokstein	Felix	Tel Aviv Sourasky Medical Center, Tel Aviv Univercity	fbokstein@gmail.com
Bora	Naba	DoD US Army	nabajyoti.bora.civ@mail.mil
Bornhorst	Miriam	Children's National Health System	Mbornhorst@childrensnational.org
Bouley	Stephanie	Geisel School of Medicine at Dartmouth	Stephanie.J.Bouley.GR@dartmouth.edu
Brainin	Rob	Children's Tumor Foundation	robbrainin@gmail.com
Brault	Jennifer	Vandewrbilt Children's Hospital	jennifer.a.brault@vanderbilt.edu
Brems	Hilde	KU Leuven Center for Human Genetics	Hilde.Brems@kuleuven.be
Brenner	Gary	Massachusetts General Hospital & Harvard Medical School	gjbrenner@partners.org
Brosseau	Jean-Philippe	University of Texas Southwestern Medical Center	drzed79@hotmail.com
Browder	Vidya	Children's Tumor Foundation	vbrowder@ctf.org
Brown	Rebecca	Mount Sinai Icahn School of Medicine	rbxbrown@gmail.com
Bugiolacchi	Ana Maria Laura	University of Buenos Aires	laurabugio@gmail.com
Burks	Ciersten	Indiana University	ciburks@iupui.edu
Campen	Cynthia	Stanford University	ccampen@stanford.edu
Cannon	Ashley	UAB	acannon@uab.edu
Cao	Xinwei	St Jude Children's Research Hospital	xinwei.cao@stjude.org
Casnar	Christina	University of Wisconsin - Milwaukee	clcasnar@uwm.edu
Ciavarelli	Patricia	University of Buenos Aires	pciavare@gmail.com
Chang	Long-Sheng	Nationwide Children's Hospital and The Ohio State University	Long-Sheng.Chang@nationwidechildrens.org
Chew	Lindsey	University of Arizona	lindseychew522@gmail.com
Clapp	David	Indiana University, School of Medicine	dclapp@iu.edu
Clark	Alyssa	Connecticut Children's	aclark@connecticutchildrens.org
Colson	Kara	Florida Hospital for Children	kara.colson@flhosp.org
Constantino	John	Washington University in St. Louis	constantino@wustl.edu
Cooper	Jonathan	UT Southwestern Medical Center	jonathan.cooper@utsouthwestern.edu
Coover	Robert	Cincinnati Children's Hospital	Robert.Coover@cchmc.org
Cornelius	Albert	DeVos Children's Hospital/Spectrum Health	albert.cornelius@helendevoschildrens.org
Côté	Lucie	CHU Sainte-Justine	lucie.cote19@icloud.com
Cox	Stephany	Children's National Health System	scox@childrensnational.org
Craigen	William	Baylor College of Medicine	wcraigen@bcm.edu
Cunha	Karin	Universidade Federal Fluminense	karingcunha@gmail.com
Cutter	Gary	Univerisity of Alabama at Birmingham	kayvon@uab.edu
Dabscheck	Gabriel	Doctor	gabriel.dabscheck@gmail.com
Dalvi	Nagma	Nemours AI Dupont Children's Hospital	nagma.dalvi@gmail.com
Dang	Mai	Children's Hospital of Philadelphia	dangm@email.chop.edu
de Blank	Peter	Cincinnati Children's Hospital Medical Center	peter.deblank@cchmc.org
De Raedt	Thomas	Harvard Medical School	tderaedt@rics.bwh.harvard.edu
Denayer	Ellen	UZ Leuven	ellen.denayer@uzleuven.be
DiMario	Francis	CT Children's Medical Center	fdimari@connecticutchildrens.org
Dinel Pond	Dinel	Children's of Minnesota	dinel.pond@childrensmn.org
Dodd	Rebecca	University of Iowa	rebecca-dodd@uiowa.edu
Doergeloh	Beate	Hannover Medical School, Hannover, Germany	doergeloh.beate@mh-hannover.de
Dombi	Eva	National Cancer Institute	dombie@mail.nih.gov
Doser	Karoline	Danish Cancer Society Research Center	kdoser@cancer.dk
Dowdy	Carla		cdowdy30@yahoo.com
Dunn	Aaron	Cupid Charities	chad@cupids.org
Dyer	Michael	St. Jude Children's Research Hospital	michael.dyer@stjude.org
Eelloo	Judith	Central Manchester University Hospitals NHS Foundation Trust	judith.eelloo@cmft.nhs.uk
Elgersma	Ype	Erasmus MC University Medical Center	y.elgersma@erasmusmc.nl
Engel	Julie	Cardinal Glennon Children's Hospital	julie.engel@ssmhealth.com
Engel	Elizabeth	Saint Louis University	engelm2@slu.edu
Engelson	Celia	NYU Langone Medical Center	cengelsonfnp@gmail.com
Evans	D Gareth	UNIVERSITY OF MANCHESTER	gareth.evans@cmft.nhs.uk
Evans	Matthew	Guy's and St Thomas' NHS Foundation Trust	matthew.evans@gstt.nhs.uk

Attendees List – 2017 NF Conference

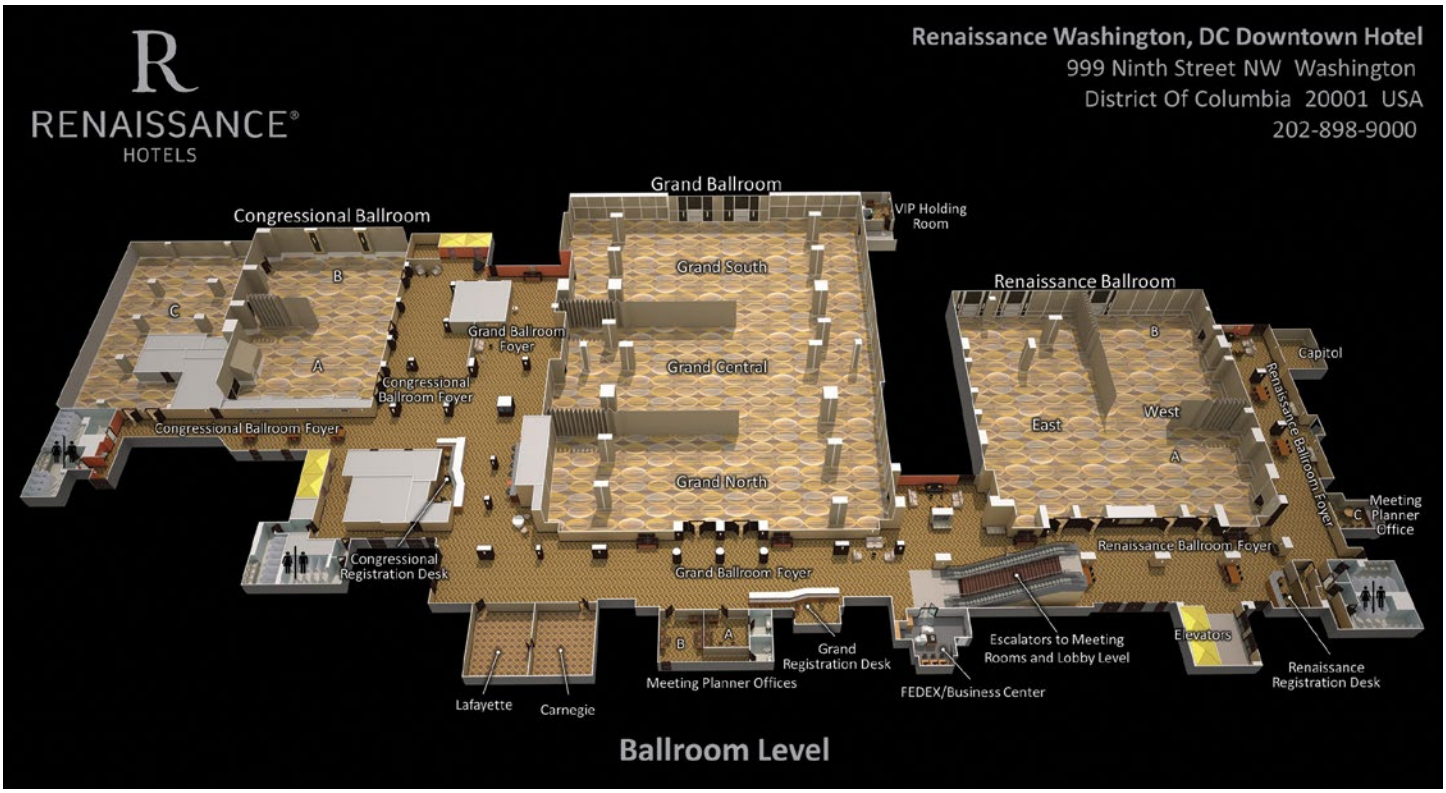
LAST	FIRST	INSTITUTION	EMAIL
Fenckova	Michaela	Radboud University Medical Center	michaela.fenckova@radboudumc.nl
Fernandez Rodriguez	Juana	IDIBELL	juanifernandez@gmail.com
Fernandez-Valle	Cristina	University of Central Florida	cfv@ucf.edu
Ferner	Rosalie	Guy's and St. Thomas' NHS Foundation Trust and King's College London	rosalie.ferner@gstt.nhs.uk
Ferreira de Souza	Juliana	Federal University of Minas Gerais	ju_souza@hotmail.com
Field	Jeffrey	University of Pennsylvania/Perelman School of Med	jfield@upenn.edu
Fisher	Michael	Children's Hospital of Philadelphia	fisherm@email.chop.edu
Fong	Sylvia	BioMarin Pharmaceutical	sfong@bmrn.com
Franklin	Barbara	AdvocureNF2	barbarafranklin144@gmail.com
Fuchs	Elaine	Rockefeller University	clong@mail.rockefeller.edu
Fuse	Marisa Ann	University of Central Florida	mfuse@knights.ucf.edu
Gardner	Kathy	University of Pittsburgh	kathyg@pitt.edu
Geller	Thomas	St Louis University	gellertj@slu.edu
Giancotti	Filippo	UT MD Anderson Cancer Center	FGGiancotti@mdanderson.org
Gibbs	Jackson	George Washington Univ. SMHS	jaygibbs2003@yahoo.com
Gillespie	Andrea	NCI	gillesan@mail.nih.gov
Giovannini	Marco	UCLA	Mgiovannini@mednet.ucla.edu
Goetsch	Allison	Ann and Robert H. Lurie Children's Hospital of Chicago	AGoetsch@luriechildrens.org
Gomes	Alicia	UAB Department of Genetics	agomes@uabmc.edu
González Muñoz	Teresa	Spanish National Cancer Research Centre	mtgonzalezm@cnio.es
Goodwin	Anne	NCI/CCR	goodwina@mail.nih.gov
Graveel	Carrie	Van Andel Research Institute	carrie.graveel@vai.org
Green	Carolyn	Childrens Hospital Colorado	carolyn.green@childrenscolorado.org
Greenwood	Robert	University Of North Carolina School of Medicine	greenwor@neurology.unc.edu
Griffis	Cristin	Medical College of Wisconsin	cgriffis@mcw.edu
Grit	Jamie	Van Andel Research Institute	jamie.grit@vai.org
Gross	Andrea	National Cancer Institute	andrea.gross@nih.gov
Gruen	Jamie	Children's Hospital Los Angeles	
Guo	Jianman	University of Pennsylvania	jianman@mail.med.upenn.edu
Gupta	Punita	St. Josephs Childrens Hospital	punita181@gmail.com
Gusella	James	Harvard Medical School	gusella@helix.mgh.harvard.edu
Gutmann	David	Neurology	gutmannd@wustl.edu
Hahn	Cindy	Texas Neurofibromatosis Foundation	chahn@texasnf.org
Hall	Amanda	CHOP	HallA5@email.chop.edu
Hardy	Kristina	Children's National Medical Center	kkhardy@childrensnational.org
Harris	Gordon	Massachusetts General Hospital	gjharris@partners.org
Harris	Rebecca	Children's Tumor Foundation	rharris@ctf.org
Haws	Tina	Children's Hospital Colorado	tina.haws@childrenscolorado.org
Healy	Aileen	Cydan Development	rgeekie@cydanco.com
Hemenway	Molly	Univ of Colorado/Children's Hospital Colorado	molly.hemenway@childrenscolorado.org
Higham	Christine	NIH	christine.higham@nih.gov
Hirbe	Angela	Washington University	hirbea@wustl.edu
Hockenberry	Christen	CHOC Children's	thistle39@gmail.com
Holmblad	Marielle	Leidos Biomedical Research	marielle.holmblad@nih.gov
Holmes	Paul	Guy's & St. Thomas' NHS Trust	Paul.Holmes@gstt.nhs.uk
Horovitz	Reid	Children's Tumor Foundation	rhorovitz@ctf.org
Horvitz	Richard	Children's Tumor Foundation BOD Chair	rah@mmcohio.com
Hsiao	Meng-Chang	Department of Genetics, University of Alabama at Birmingham	mchsiao@uab.edu
Hughes	JoAnna	Hood College	joannahughes28@gmail.com
Hupton	Eileen	Central Manchester Foundation Trust	eileen.hupton@cmft.nhs.uk
Iavarone	Antonio	Columbia University Medical Center	ai2102@columbia.edu
Ingerick	Samantha	Lucile Packard Children's Hospital at Stanford	singerick@stanfordchildrens.org
Inniss	Rona	GSTT	rona.inniss@gstt.nhs.uk
Isakson	Sara	University of Minnesota	isaks047@umn.edu
Jacobsen	Chad	Carolinas Healthcare System	chad.jacobsen@carolinashealthcare.org
Jacobson	Mona	University of Colorado, Children's Hospital Colorado	mona.jacobson@childrenscolorado.org
Janusz	Jennifer	Children's Hospital Colorado	jennifer.janusz@childrenscolorado.org
Jarnagin	Kurt	BioPharm	kjarnagin@earthlink.net
Jones	Georgette	Hood College	jonesg@hood.edu
Jordan	Justin	Massachusetts General Hospital	jtjordan@mgh.harvard.edu
Jumbe	Shasha	Bill & Melinda Gates Foundation	shasha.jumbe@gatesfoundation.org
Kahn	Itamar	Technion – Israel Institute of Technology	kahn@technion.ac.il
Kalamarides	Michel	Hopital Pitie-Salpetriere, AP-HP	michel.kalamarides@aphp.fr
Kallionpää	Roope	University of Turku	roope.kallionpaa@utu.fi
Karajannis	Matthias	Memorial Sloan Kettering Cancer Center	karajanm@mskcc.org
Kehrer-Sawatzki	Hildegard	University of Ulm	hildegard.kehrer-sawatzki@uni-ulm.de
Keller	Joyce	Children's Tumor Foundation	joycekeller@quickenloans.com
Keller	Bryant	University of Minnesota	kell1503@umn.edu
Kelly-Mancuso	Geraldine	CCHMC	Geraldine.Kelly-Mancuso@cchmc.org
Kelts	Kate	Children's Tumor Foundation	kkelts@ctf.org
Kesterson	Bob	University of Alabama at Birmingham	kesterso@uab.edu
Khanna	Rajesh	University of Arizona	rkhanna@email.arizona.edu
Ki	Dong Hyuk	Harvard Medical School/Dana-Farber Cancer Institute	dongh_ki@dfci.harvard.edu
Kim	AeRang	Children's National Medical Center	AeKim@childrensnational.org
King	Candace	Van Andel Institute Graduate School	candace.king@vai.org
Kirby	Amelia	Saint Louis University	akirby@slu.edu
Kissil	Joseph	Scripps Research Institute	jkissil@scripps.edu
Klein-Tasman	Bonita	University of Wisconsin-Milwaukee	bklein@uwm.edu
Klesse	Laura	UT Southwestern	laura.klesse@utsouthwestern.edu
Knight	Pamela	Children's Tumor Foundation	pknight@ctf.org
Koczkowska	Magdalena	University of Alabama at Birmingham	mkoczkowska@uab.edu
Koneazny	Lisa	Children's Hospital of WI	LKoneazny@chw.org
Konsitzke	Charles	University of Wisconsin - Madison	charles.konsitzke@wisc.edu
Korf	Bruce	University of Alabama at Birmingham	bkorf@uabmc.edu
Kuninaka	Shinji	Keio University	skuninak@keio.jp
Langmead	Shannon	Johns Hopkins Comprehensive NF Center	slangme2@jhmi.edu

Attendees List – 2017 NF Conference

LAST	FIRST	INSTITUTION	EMAIL
Largaespada	David	University of Minnesota	larga002@umn.edu
LaRosa	Salvatore	Children's Tumor Foundation	slarosa@ctf.org
Laughton	Stephen		stephenl@adhb.govt.nz
Leathers	Chad	Cupid Charities	chad@cupids.org
Lee	Joanna	Lurie Children's Hospital	JoaLee@luriechildrens.org
Lee	Beomhee	Asan Medical Center	bhlee@amc.seoul.kr
Legius	Eric	Center for Human Genetics	cme@uzleuven.be
Lemberg	Katie	Johns Hopkins University	klember1@jhmi.edu
Li	Veetai	Women and Children's Hospital of Buffalo	vli@kaleidahealth.org
Lightner	Donita	Neurology/Neuro-Oncology	donita.lightner@uky.edu
Lin	Andrew	Memorial Sloan Kettering Cancer Center	lina1@mskcc.org
Lin	Carol	CHOC Children's Hospital	clin@choc.org
Listernick	Robert	Ann & Robert H. Lurie Children's Hospital of Chicago	Rlisternick@luriechildrens.org
Liu	Grant	Children's Hospital of Philadelphia	gliu@mail.med.upenn.edu
Lloyd	Alison	University College London	alison.lloyd@ucl.ac.uk
Lu	richard	Cincinnati Children's hospital Medical Center	richard.lu@cchmc.org
Ma	Rufeng	Rensselaer Polytechnic Institute	mar6@rpi.edu
Manso	Simone		s.manso@yahoo.it
Mansouri	Sheila	University of Toronto	mansourisheila@gmail.com
Mansouri	Alireza	University of Toronto	alirezamansouritoronto@gmail.com
Martin	Staci	National Cancer Institute	martins@mail.nih.gov
Masgras	Ionica	University of Padua	ionica.masgras@gmail.com
Matsumoto	Steven	Oregon Health & Science University	matsumos@ohsu.edu
Mattingly	Ray	Wayne State University	r.mattingly@wayne.edu
Mautner	Victor-Felix	University Medical Center Hamburg Eppendorf, Germany	nfambulanz@uke.de
McGoldrick	Patricia	Mount Sinai Health Systems	patricia.mcgoldrick@mountsinai.org
Merker	Vanessa	Massachuetts General Hospital	vmerker@partners.org
Messiaen	Ludwine	UAB Medical Genomics Laboratory	lmessiaen@uabmc.edu
Miller	David	Boston Children's Hospital	david.miller2@childrens.harvard.edu
Mills	Kristen	Rensselaer Polytechnic Institute	millsk2@rpi.edu
Mo	Juan	UT Southwestern Medical Center	juan.mo@utsouthwestern.edu
Mody	Pari	Arnold & Porter Kaye Scholer LLP	Pari.Mody@apks.com
Moertel	Christopher	University of Minnesota	moert001@umn.edu
Mokhtari	Sepideh	Moffitt Cancer Center	Sepideh.mokhtari@moffitt.org
Moots	Paul	Vanderbilt University Medical Center	paul.moots@vanderbilt.edu
Moots	Skyler	NF Tennessee	skylermoots@gmail.com
Morris	Stephanie	Washington University, St. Louis	morris.s@wustl.edu
Morris	Jill	NIH	jill.morris@nih.gov
Morrison	Helen	Leibniz Research Institute for Aging	Helen.Morrison@leibniz-fli.de
Moutal	Aubin	University of Arizona	aubinmoutal@email.arizona.edu
Murray	Jeffrey	Cook Children's Hospital	jeffrey.murray@cookchildrens.org
Nievo	Marco	Children's Tumor Foundation	mnievo@ctf.org
Nikrad	Julia	University of Minnesota	nikr0006@umn.edu
Nishida	Yoshihiro	Nagoya University Graduate School of Medicine	ynishida@med.nagoya-u.ac.jp
Obergfell	Neeltje		neeltje.obergfell@meduniwien.ac.at
Oblinger	Janet	Nationwide Children's Hospital & The Ohio State University	Janet.Oblinger@nationwidechildrens.org
Oetgen	Matthew	Children's National Health System	moetgen@childrensnational.org
Oh	Jungmi	Seoul National University	jmoh@snu.ac.kr
Osborne	Kimberly	University of Kentucky - Neurology Department	KimOsborne@uky.edu
Ostrow	Kimberly	Johns Hopkins School of Medicine	kostrow3@jhmi.edu
Ota	Arihito		arimushi@jikei.ac.jp
Packer	Roger	Childrens National Medical Center	rpacker@childrensnational.org
Pancza	Patrice	Children's Tumor Foundation	ppancza@ctf.org
Panzer	Karin	University of Iowa Hospitals & Clinics	karin-panzer@uiowa.edu
Papi	Laura	University of Florence, Italy	l.papi@dfc.unifi.it
Patel	Neha	University of Wisconsin	npatel@pediatrics.wisc.edu
Patil	Dipak	The Scripps Research Institute - Florida	dpatil@scripps.edu
Payne	Jonathan	Murdoch Childrens Research Institute	jonathan.payne@mcri.edu.au
Peltonen	Juha	Department of Cell Biology and Anatomy	juha.peltonen@utu.fi
Peltonen	Sirkku	University of Turku and Turku University Hospital	sipelto@utu.fi
Perreault	Sébastien	University of Montreal	s.perreault@umontreal.ca
Petrilli	Alejandra	University of Central Florida	Alejandra.Petrilli@ucf.edu
Piculell	Katherine	Boston Children's Hospital - Genetics	katherine.piculell@childrens.harvard.edu
Pillay Smiley	Natasha	Ann and Robert H. Lurie Children's Hospital	npillaysmiley@luriechildrens.org
Pivnick	Eniko	UTHSC, Memphis TN	ekarman54@gmail.com
Place	Emily	FDA	emily.place@fda.hhs.gov
Plotkin	Scott	Massachusetts General Hospital	splotkin@partners.org
Pope	Kimberly	CDMRP	kimberly.b.pope2.ctr@mail.mil
Prakash	Vikram	Cardinal Glennon Children's Hospital	vprakash@slu.edu
Pridgeon	Matthew	Helen DeVos Children's Hospital	matthew.pridgeon@grmep.com
Radhika	Dhamija	Mayo Clinic	dhamija.radhika@mayo.edu
Radtke	Heather	Children's Tumor Foundation	hradtke@ctf.org
Ramesh	Vijaya	Massachusetts General Hospital	ramesh@helix.mgh.harvard.edu
Rasola	Andrea	University of Padova	andrea.rasola@unipd.it
Ratner	Nancy	Cincinnati Children's Hospital	Nancy.Ratner@cchmc.org
Reilly	Karlyne	NIH/NCI	reillyk@mail.nih.gov
Rezende	Nilton	Federal University of Minas Gerais	narezende@terra.com.br
Riccardi	Vic	The Neurofibromatosis Institute	vmriccardi@charter.net
Richards	B. Stephens	Texas Scottish Rite Hospital	steve.richards@tsrh.org
Robinson	John Elliott	California Institute of Technology	jelliottrobinson@gmail.com
Roderick	Marie Claire	National Cancer Institute	marieclaire.roderick@nih.gov
Röhl	Claas	NF Kinder	claas.roehl@nfkinder.at
Rosenbaum	Thorsten	Sana Kliniken Duisburg	thorsten.rosenbaum@gmx.de
Rubenstein	Allan	NYU Langone Medical Center	arubenstein@cns-pc.com
Sarnoff	Herb	Infixion	herbsarnoff@yahoo.com
Schindeler	Aaron	University of Sydney	aaron.schindeler@sydney.edu.au
Schmitt	Anne Marie	Institut de Recherche Pierre Fabre	anne.marie.schmitt@pierre-fabre.com

Attendees List – 2017 NF Conference

LAST	FIRST	INSTITUTION	EMAIL
Schorry	Elizabeth (Betty)	Cincinnati Children's Hospital Medical Center	elizabeth.schorry@cchmc.org
Seidlin	Mindell	Seidlin Consulting LLC	ms@seidlinconsulting.com
Seijo	Marlon		marlonseijo@gmail.com
Serra	Eduard	Institute for Health Science Research Germans Trias I iPujol	eserra@imppc.org
Shabason	Emily	The Children's Hospital of Philadelphia	shabasone@email.chop.edu
Sharafi	Parisa	TOBB University, Faculty of Medicine	psharafi@etu.edu.tr
Sharp	Gregory	Arkansas Children's Hospital	sharpgregoryb@uams.edu
Shea	Stephanie	Children's Hospital Colorado	stephanie.shea@childrenscolorado.org
Sheridan	Monica	Massachusetts General Hospital	mrsheridan@mgh.harvard.edu
Sherman	Lawrence	Oregon National Primate Research Center	shermanl@ohsu.edu
Shibuya	Peter	Children's National Health System	pshibuya@childrensnational.org
Shih	Chie-Schin	Riley Hospital for Children	Chieschin@gmail.com
Shofty	Ben	The Gilbert Israeli NF Center	shoftyben@gmail.com
Siqveland	Elizabeth	Children's - Minnesota	elizabeth.siqveland@childrensmn.org
Skefos	Catherine	UAB	cskefos@uab.edu
Slobogean	Bronwyn	Johns Hopkins University	bslobog1@jhu.edu
Smith	Miriam	University of Manchester	miriam.smith@manchester.ac.uk
Smith	Taylor	California Polytechnic State University, San Luis Obispo	tsmith82@calpoly.edu
Sondergaard	Krista	Johns Hopkins University Institute of Genetic Medicine	ksonder1@jhmi.edu
St. Jacques	Leslie	University Health Network	leslie.stjacques@mail.utoronto.ca
Sosa-Longo	Melissa	Children's Tumor Foundation	msosa-longo@ctf.org
Stansfield	Brian	Augusta University	bstansfield@augusta.edu
Stathis	Marigo	NTAP at Johns Hopkins University	mstathi1@jhmi.edu
Steensma	Matthew	Van Andel Research Institute	matt.steensma@vai.org
Stemmer-Rachamimov	Anat	Massachusetts General Hospital	astemmerrachamimov@mgh.harvard.edu
Stevenson	David	Stanford University	dasteven@stanford.edu
Stivaros	Stavros	University of Manchester/ Royal Manchester Children's Hospital	stavros.stivaros@manchester.ac.uk
Struemph	Kari	National Cancer Institute	kari.struemph@nih.gov
Sullivan	Lara	Pfizer	lara.sullivan@pfizer.com
Sommer	Katherine	University of Minnesota	ksommer10@umphysicians.umn.edu
Sun	Daochun	Memorial Sloan Kettering Cancer Center	sund@mskcc.org
Sverak	Pavlina	University of Minnesota	svera004@umn.edu
Tahaei	Seyedmohammad Ebrahim	Vanderbilt University	ebrahim.tahaei@vanderbilt.edu
Taylor	Ashley	The University of Oklahoma Health Sciences Center	ashley-c-taylor@ouhsc.edu
Teranishi	Yu	Department of Neurosurgery, University of Tokyo Hospital	eugeneterra@me.com
Thompson	Heather	California State University, Sacramento	heather.thompson@csus.edu
Tomchik	Seth	The Scripps Research Institute	stomchik@scripps.edu
Tonsgard	James	University of Chicago	tonsgard@midway.uchicago.edu
Topilko	Piotr	IBENS	topilko@biologie.ens.fr
Trapane	Pamela	University of Iowa Hospitals & Clinics	pamela-trapane@uiowa.edu
Tritz	Rebekah	Augusta University	rtritz@augusta.edu
Turner	Ashley	University of Alabama at Birmingham	anturner@uab.edu
Ullrich	Nicole	Boston Children's Hospital	Nicole.Ullrich@childrens.harvard.edu
Upadhyaya	Meena	Cardiff University	upadhyaya@cardiff.ac.uk
Vaassen	Pia	Sana Kliniken Duisburg gmbH	pia.vaassen@googlemail.com
Vajdi	Ariana	Children's Hospital Los Angeles	trosser@chla.usc.edu
Van Mater	David	Duke University	david.vanmater@duke.edu
Vassallo	Grace	Central Manchester Foundation Trust	grace.vassallo@cmft.nhs.uk
Vélez Reyes	Germán	University of Minnesota	velez044@umn.edu
Verma	Sharad	Johns Hopkins University	sverma20@jhmi.edu
Viskochil	David	University of Utah	dave.viskochil@hsc.utah.edu
Vitte	Jeremie	UCLA	jvitte@mednet.ucla.edu
Vranceanu	Ana-Maria	MGH/Harvard Medical School	avranceanu@mgh.harvard.edu
Vukelj	Simon	Children's Tumor Foundation	svukelj@ctf.org
Walker	James	Center for Genomic Medicine - Massachusetts General Hospital	jwalker@helix.mgh.harvard.edu
Wallace	Peggy	University of Florida	peggyw@ufl.edu
Wallis	Deeann	UAB Genetics	dwallis@uab.edu
Walsh	Karin	Children's National Health System	kwalsh@childrensnational.org
Wang	Xia	Moffitt Cancer Center	xia.wang@moffitt.org
Watson	Adrienne	Recombinetics Inc.	adrienne@recombinetics.com
Wei	Christina	Cincinnati Children's Hospital	Christina.Wei@cchmc.org
Weimer	Jill	Sanford Research - Children's Health Research Center	jill.weimer@sanfordhealth.org
Weintraub	Lauren	Albany Medical Center	weintrl@mail.amc.edu
Weiss	Brian	CCHMC	brian.weiss@cchmc.org
Welling	D. Bradley	Harvard Department of Otolaryngology	brad_welling@meei.harvard.edu
Whitcomb	Patricia	NCI / POB	whitcomt@mail.nih.gov
White	Shannon	Georgetown University	smw248@georgetown.edu
Williams	Kyle	University of Minnesota	kbwillia@umn.edu
Williams	Victoria	Guy's and St Thomas' NHS Trust	victoria.williams@gstt.nhs.uk
Wind Mitchell	Carole	NYU Langone Medical Center	carole.mitchell@nyumc.org
Wolf	David	Emory University/CHOA	dwolf03@emory.edu
Wolkenstein	Pierre	Henri-Mondor Hospital, UPEC, APHP, Créteil, France	pierre.wolkenstein@aphp.fr
Wolters	Pam	National Cancer Institute	woltersp@mail.nih.gov
Wong	Ling	NIN / NINDS	wonglm@ninds.nih.gov
Wong	Ling	National Institute of Neurological Disorders and Stroke	ling.wong@nih.gov
Woodall	Lesli	Medical University of South Carolina	woodalll@musc.edu
Wootton	Michael	Vie Capital Partners	michaelawootton@gmail.com
Wu	Lai Man Natalie	CCHMC	laiman.wu@cchmc.org
Wu	Jianqiang	Cincinnati Children's Hospital	Jianqiang.wu@cchmc.org
Xing	Lei	UNC Chapel Hill	lxing@email.unc.edu
Xu	Lei	MGH/Harvard Medical School	lei@steele.mgh.harvard.edu
Yates	Charles	Indiana University School of Medicine	cwyates@iupui.edu
Yi	Chunling	Georgetown University	cy232@georgetown.edu
Zadeh	Gelareh	Toronto Western Hospital, University Health Network	Gelareh.Zadeh@uhn.ca
Zhu	Yuan	Gilbert Neurofibromatosis Institute	yzhu@childrensnational.org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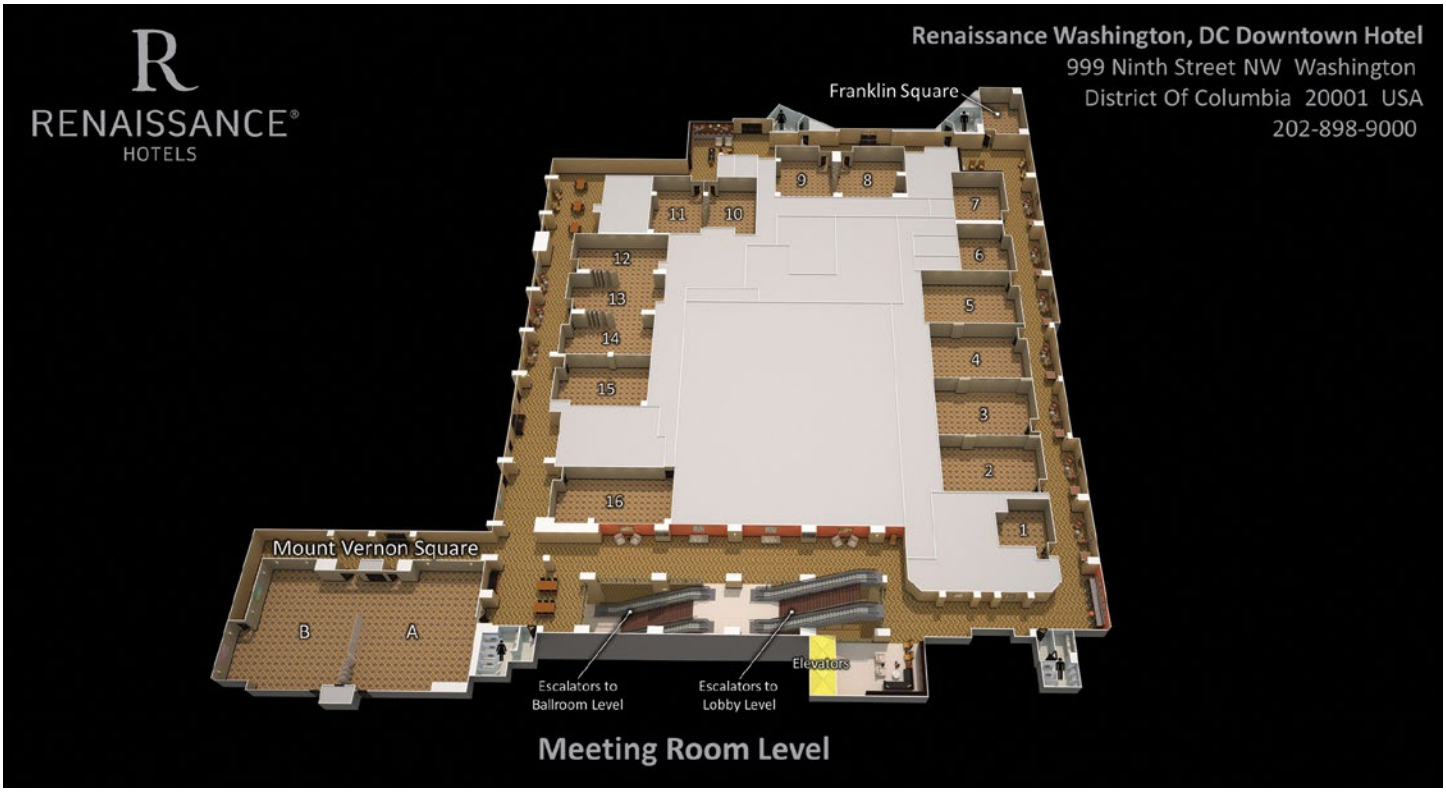
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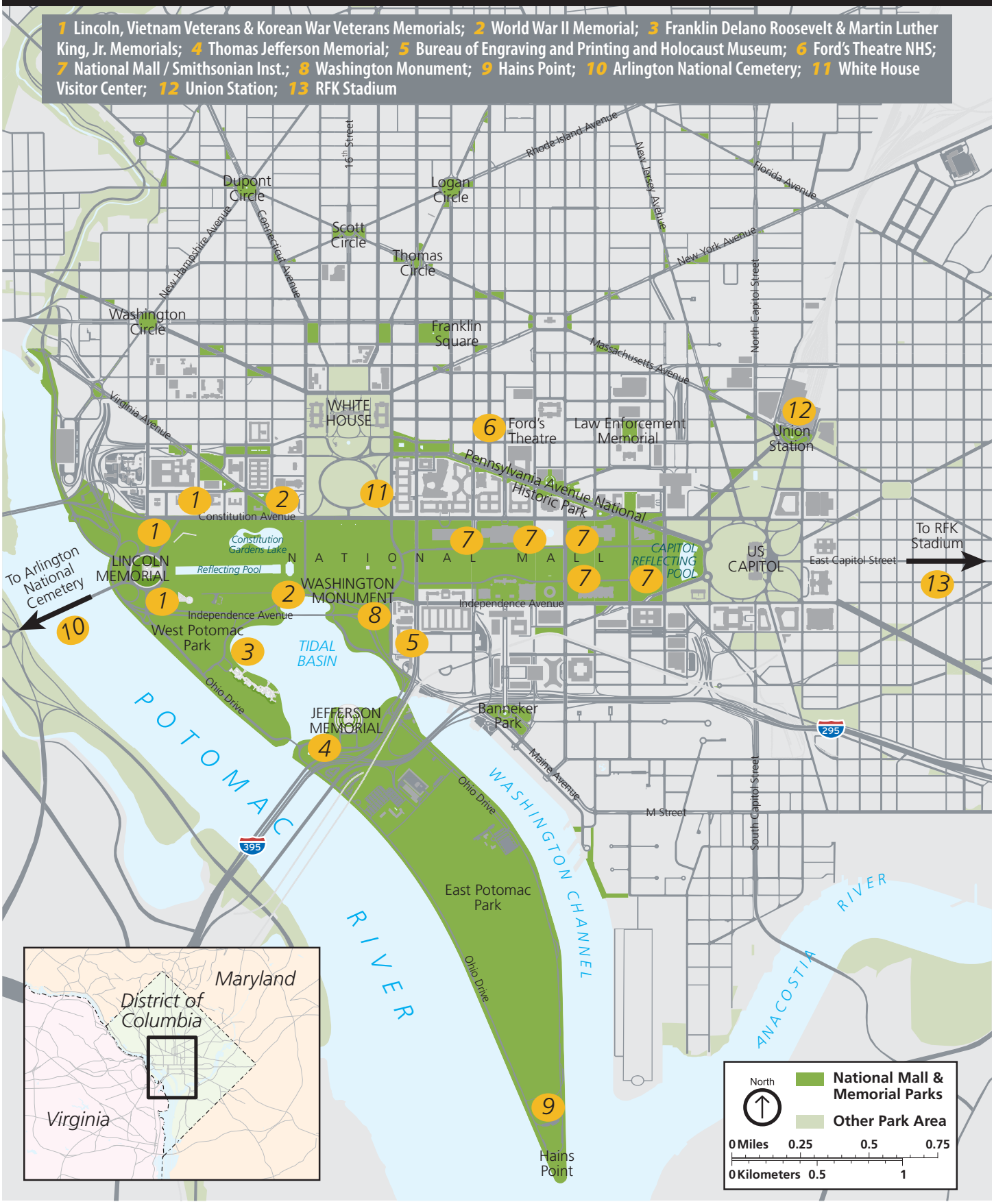

Nearby Restaurants: Notable Dining Within a Short Walk or Taxi Ride Away

	AMERICAN FARES:		
1	***15 Squares	Renaissance Hotel Lobb	Ext: 4131
2	Acadiana	901 New York Ave. NW.	202-408-8848
3	Corduroy	1122 9th. Street nw	202-589-0699
4	Kinship	1015 7th. Street nw	202-737-7700
5	The Source	575 Pennsylvania Ave. nw	202-637-6100
6	Clyde's	707 7th. Street nw	202-349-3700
7	Matchbox	713 H Street nw	202-289-4441
8	The Dabney	122 Blagden Alley nw	202-450-1015
9	Convival	801 O Street nw	202-525-2870
10	Pennsylvania 6	1350 Eye Street nw	202-796-1600
11	Georgia Brown's	950 15th. Street nw	202-393-4499
12	City Tap House	901 I Street nw	202-644-9433
13	District Chophous	509 7th. Street nw	202-347-3434
14	Old Ebbit Grille	675 15th. Street nw	202-347-4800
15	701 Restaurant	701 Pennsylvania Ave. nw	202-393-0701
16	The Smith	901 F Street nw.	202-868-4900
17	Sixth Engine	438 Mass. Ave. nw	202-506-2455
	COFFEEHOUSES / GOURMETS & BREAKFAST:		
18	***Starbucks	Hotel Lobby	EXT:3464
19	Fruitive	1094 Palmer Alley nw	202-836-7749
20	New World Café	8th. & I Street nw	202-842-0045
21	Lincoln House Res	504 10th. Street nw	202-638-4008
22	Farmers & Distille	600 Mass. Ave. nw	202-464-3001
23	Brueggers Bagel	509 9th. Street nw	202-393-1663
24	Luke's Lobster	624 E Street nw	202-347-3355
25	Five Guys	808 H Street nw	202-932-9000
26	Taylor Gourmet	485 K Street nw	202-289-8001
27	Hard Rock Café	999 E Street nw	202-737-7625
28	Ben's Chili Bowl H	1001 H Street nw	202-733-1895
29	Ella's Pizza	9th. & F Street nw.	202-638-3434
	CARIBBEAN/ LATIN/ ASIAN-LATINO:		
30	Chaplin	1501 9th. Street nw	202-644-8806
31	Cuba Libre	801 9th. Street nw	202-408-1600
32	Texas De Brazil	455 Mass. Ave. nw	202-898-1413
33	Fogo De Chao	1101 Pennsylvania Ave. nw	202-347-4668
	CHINESE/ JAPANESE/ PAN-ASIAN/ THAI:		
34	Tony Cheng Mong	619 H Street nw	202-371-8669
35	Ming's	617 H Street nw	202-289-1001
36	Grand Trunk	641 Indiana Ave. nw	202-347-3293
37	Sushi Aoi	1100 New York Ave. nw	202-408-7770
38	Haad Thai	1100 New York Ave. nw	202-682-1111
39	Wok And Roll	604 H Street nw	202-347-4656
40	Ping Pong Dim Sur	900 7th. Street nw	202-506-3740
	FRENCH / GERMAN / SUSHI:		
41	Sax Rest. & Loung	734 11th. Street nw.	202-737-0101
42	Brasserie Beck	1101 K Street nw.	202-408-1717
43	Le Diplomate	1601 14th. Street nw	202-332-3333
44	DBGB	931 H Street nw	202-695-7660
45	Montmartre	327 7th. Street sw	202-544-1244
46	Central(French/Americ)	1001 pennsylvania Ave. nw	202-626-0015

	ITALIAN:		
47	Bibiana	12th. & H Street nw	202-216-9550
48	Casa Luca	1099 New York Ave. nw	202-628-1099
49	Carmines	425 7th. Street nw	202-737-7770
50	Graffiato	707 6th. Street nw	202-289-3600
51	Alba Osteria	425 I Street nw	202-733-4454
52	Catch 15	1518 K Street nw	202-969-2858
53	I Ricchi	1220 19th. Street nw	202-835-0459
	INDIAN/ VEGETARIAN FRIENDLY:		
54	Rasika	663 D Street nw	202-637-1222
55	Busboys And Poet:	1025 5th Street nw	202-789-2227
56	Bombay Club	815 Connecticut Ave. nw	202-659-3727
57	Shouk	655 K Street nw	202-652-1464
58	Maoz Vegetarian	1817 M Street nw	202-290-3117
59	Taj Of India	2809 M Street nw	202-965-4266
60	Elizabeth's Gone F	1314 L Street nw	202-347-8349
	MEDITERRANEAN/ MIDDLE EASTERN:		
61	Etete	1942 9th. Street nw.	202-232-7600
62	Dukem Ethiopan	1116 U Street nw	202-667-8735
63	Taberna Del Alaba	1776 I Street nw	202-429-2200
64	Fig & Olive	934 Palmer Alley nw.	202-559-5004
65	Zaytinya	701 9th. Street nw	202-638-0800
66	Kellari	1700 K Street nw	202-535-5274
67	***BREWERIES & BARS:***		
	***President's Bar	Hotel Lobby	EXT:4126
68	Capitol City Brewi	11th. & H Street nw	202-628-2222
69	Gordon Biersch	900 F Street nw	202-783-5454
70	Fado Irish Pub	808 7th. Street nw	202-789-0066
71	R.F.D Wash.DC	810 7th. Street nw	202-289-2030
72	***MEXICAN/ TEX-MEX/ SOUTHWESTERN/ SPANISH:***		
	Jaleo	480 7th. Street nw	202-628-7949
73	Oyamel	401 7th. Street nw	202-628-1005
74	La Tasca	722 7th. Street nw	202-347-9190
75	Rosa Mexicano	575 7th. Street nw	202-783-5522
76	Toro Toro	1300 I Street nw	202-682-9500
	SEAFOOD:		
77	Legal Seafood	704 7th. Street nw	202-347-0007
78	Hank's Oyster Bar	1624 Q Street nw	202-462-4265
79	Oceanaire	1201 F Street nw	202-347-2277
80	Pearl Dive Oyster	1612 14th. Street nw	202-319-1612
81	Joe's Seafood	750 15th. Street nw.	202-489-0140
	STEAK / AMERICAN STYLES:		
82	BLT Steak	1625 I Street nw	202-689-8999
83	Ruth Chris	724 9th. Street nw	202-393-4488
84	Bobby Vans	1201 New York Ave. nw	202-589-1504
85	Capitol Grille	601 Pennsylvania Ave. nw	202-737-6200
86	Social Reform	401 9th. Street nw	202-393-1300
87	Charlie Palmer	101 Constitution Ave. nw	202-547-8100
88	The Palm	1225 19th. Street nw	202-293-9091
89	Del Frisco's Steak	950 I Street nw	202-289-0201
90	Del Campo	777 I Street nw	202-289-7377

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Guy's and St. Thomas' NHS Foundation Trust and King's College, London, UK

Larry S. Sherman, PhD

Oregon National Primate Research Center, Oregon Health and Sciences University

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Jaishri Blakeley, MD
D. Wade Clapp, MD
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Ype Elgersma, PhD
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Michael Fisher, MD
Marco Giovannini, MD, PhD
Matthias Karajannis, MS, MD
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Robert Listernick, MD
Alison Lloyd, PhD
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Mentors

Jaishri Blakeley, MD
Thomas De Raedt, PhD
Ype Elgersma, PhD
Michael Fisher, MD
Eric Legius, PhD
Robert Listernick, MD
Alison Lloyd, PhD
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