Dear NF Conference Attendees:

On behalf of the Children’s Tumor Foundation, welcome to the 2009 NF Conference: New Frontiers. The theme references the meeting content and also Portland itself, historically a gateway port of the Pacific North West. The urban setting offers a ‘new frontier’ in comparison to the mountain and beach locales of past NF Conferences, but one which we feel you will enjoy. Portland is an easy-going city offering history, beauty and relaxation – features encapsulated in our host hotel The Nines, itself a part of the tapestry of Portland history, renovated from the former landmark Meier & Frank department store.

The last year has seen major NF research advances. The dovetailing of discovery, translation and the clinic can be seen throughout the meeting. We are firmly in the age of NF clinical trials and proud that the Children’s Tumor Foundation is part of this advance: in 2009 we funded our first two pilot Clinical Trial Awards. We continue to build a pipeline of candidate NF drug therapies through the Foundation’s multi-center NF Preclinical Consortium and the seed grant Drug Discovery Initiative (DDI) program. Through these translational initiatives we are cultivating NF collaborations with the biotechnology and pharmaceutical sector, a critical factor in moving NF research forward to the clinic.

At the same time, basic research advances continue, such as in the unraveling of schwannomatosis. Through our Schwannomatosis Awards program, the Foundation has committed around $500,000 to schwannomatosis since INI-1 was identified as a candidate gene in 2007. This includes discovery research, genesis of schwannomatosis mouse models and now, establishing a Schwannomatosis Natural History Database.

Underpinning the bench-to-bedside pathway is the Foundation’s NF Clinic Network, now 38 Affiliate Clinics in the United States and growing. NFCN strives to improve NF clinical care and to build bridges with the NF patient community. In planning is the addition of an NF BioBank and Patient Registry, to facilitate researcher access to NF tissue and clinical data, and empower NF patients to participate in accelerating NF research to the clinic.

We still face many frontiers on the road to ending neurofibromatosis, but let’s celebrate the path we have carved so far.

Enjoy the meeting!

Sincerely,

Kim Hunter-Schaedle, Ph.D.  
Chief Scientific Officer

John W. Risner  
President
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The Children’s Tumor Foundation is dedicated to ending neurofibromatosis (NF) through research. For over 25 years, the Foundation has funded NF research with the goal of identifying NF drug therapies and improving the lives of those living with the disorder. To date, the Foundation has committed over $30M to NF research initiatives. The Foundation also provides resources for NF patients and their families, and endeavors to increase public awareness of NF.

The Foundation has been a long-time key advocate for federal support of NF research. The Foundation works closely with the National Institutes of Health and has been instrumental in the inception and continuation of the United States Army’s Congressionally Directed Medical Research Program.

The research programs of the Children’s Tumor Foundation support discovery and translational research, clinical trials and clinical care.

Open Programs: Call for Applications

- **Young Investigator Awards:** Our longest running research program provides two year grants for pre- and post-doctoral researchers in NF research. YIA continues as a cornerstone program of the Foundation. Many of today’s NF community leaders are former YIA recipients. Next deadline: early 2010.

- **Drug Discovery Initiative Awards:** Grants of up to $50,000 for pilot preclinical drug screening. To date, this program has funded 25 drug screens. Multiple deadlines: see www.ctf.org.

- **Clinical Trial Awards:** Offer $125,000 to conduct small-scale pilot clinical trials of candidate NF therapeutics. Deadline: Letter of Intent required. Contact the Foundation first.

- **NF Clinic Network (CTF-NFCN):** Recognizes those clinics offering comprehensive NF clinical care. Now 38 Affiliate Clinics. Any clinic may apply for participation. U.S. Clinics eligible to receive seed funding to expand clinic activities. Deadlines: February 28th and August 31st.

Ongoing Programs (no current call for applications)

- **The NF Preclinical Consortium:** part of a $5M effort to advance NF research in the clinics. Research teams at six centers collaborating in screening the most promising candidate NF therapeutics in multiple preclinical NF tumor models in parallel: UCSF, Washington University, Cincinnati Children’s Hospital Medical Center, House Ear Institute, Harvard Medical School’s Brigham & Women’s Hospital and Massachusetts General Hospital.

- **Schwannomatosis Awards:** An ad hoc initiative that has invested $500,000 since 2007 to expand our understanding of the disorder. Includes discovery research, animal model development, and a Natural History Database.
Foundation Staff at the 2009 NF Conference

Research & Medical Programs

Kim Hunter-Schaedle, Ph.D  Chief Scientific Officer ....................... khs@ctf.org
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John W. Risner  President .............................................. jrisner@ctf.org
Tom Malone  Chief Financial Officer  tmalone@ctf.org

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Kelly McGowan  Chapter Coordinator  kmcgowan@ctf.org

Communications

Garrett Gleeson  Communications Associate  ggleeson@ctf.org
Get Involved in the Children’s Tumor Foundation’s National Programs!

The Children’s Tumor Foundation’s mission is dedicated to the health and well-being of the 100,000 Americans who suffer from neurofibromatosis (NF), through medical research funding, patient support services, improved clinical care, and raising public awareness. The Children’s Tumor Foundation’s National Programs help raise money for these important services by providing structured, fun, and easy-to-manage events that will encourage a broad level of participation.

Racing4Research

Racing4Research is a unique national program that raises money year round to fund our Drug Discovery Initiative1 (DDI) through corporate sponsorship, NF Heroes, and individual pledges. The Children’s Tumor Foundation and Farnbacher Loles Racing joined forces in 2006 and developed this exceptional fundraising initiative built around the greatest endurance race in North America, the Rolex 24 Hours At Daytona. CTF Farnbacher Loles racing team drives to help raise awareness and find a cure for neurofibromatosis (NF). At the this year’s Daytona 24 the Racing4Research team raised over $300K for NF research and finished the race by logging 604 laps. As the #85 car races NF families and Heroes around the country follow on the television or online and watch as we get one lap closer to our goal.

A very special component of the program is the NF Heroes; it provides a great opportunity for individuals to share their story about living with NF and travel to Daytona to be part of race weekend and meet the drivers. This year we recruited twenty-four NF Heroes nationwide, and although some were unable to attend the race, they cheered the car along from their homes and lounges across the country where they held race day parties. We always are looking for NF Heroes across the country to assist in R4R fundraising.

To learn more about the program please visit: www.racing4research.org
For further information contact: Jill Beck, beck@ctf.org or Traceann Adams, tadams@ctf.org

1 The Drug Discovery Initiative program increases the number of candidate NF drugs being tested, and therefore increases the chances that we will find therapies to treat the tumors, bone abnormalities, learning disabilities and other manifestations of NF.
The Children’s Tumor Foundation’s NF Endurance Team offers the opportunity for individuals to participate in team endurance sports to raise money for neurofibromatosis research, promote awareness about NF, and provide a network of caring support for those suffering with NF and their families. We participate in over forty-one annual endurance events including marathons, half marathons, relay races, long bike rides, and triathlons. Our team is made up of over 3,400 participants from across the United States and around the world. Many members have an “NF touch,” but a growing number do not.

Our program provides opportunity and support to run or walk marathons and half marathons or participate in triathlons and long-distance bike rides.

- Contact with an experienced coach to offer training tips and advice
- Lodging, airfare, entry, and apparel incentives
- Your own personal Web site for online fundraising
- Team encouragement and friendship
- A connection with a local “NF Hero”
- An invitation to a team pasta dinner (at staffed events)
- Updates on research progress our team has enabled

To learn more about the program please visit: www.ctf.org/endurance

For further information contact: Steve Kendra, skendra@ctf.org

The NF Walk is a national program for the Children’s Tumor Foundation, whose mission is to raise awareness about NF and funds for NF research. Throughout the year, the Foundation works with volunteers to organize and participate in established walk events in communities and cities across the country. We encourage individuals to mobilize teams of friends, families, co-workers, and corporate sponsors to join in and raise awareness and funds for NF. NF Walk events are fun, simple, and can easily be organized at parks, schools or tracks in your community with anywhere from 30-300 individuals. The Foundation will work with individuals to coordinate their walk, provide T-shirts, wristbands, additional walk collateral, and online fundraising needs.

To learn more about the program please visit: www.ctf.org/walk

For further information contact: Traceann Adams, tadams@ctf.org
The Tea Party for NF is an easy, elegant and heartwarming way to celebrate those living with NF and raise awareness about NF. “Virtual” (by mail only) or Actual Tea Parties are held anytime throughout the year. Many hostesses have held a party in May to celebrate “May is NF Awareness” month.

Still, the Tea Party for NF concept is flexible. It may be a High Tea held at a local tea house, a Mother/Daughter Tea with separate activities for each, or iced tea and lemon bars in the backyard with friends on a hot summer day. You can even combine the party with special occasions like birthdays and anniversaries.

Regardless of your preference, the Children’s Tumor Foundation will help make your party fun, easy and successful by providing you with online fundraising tools and host/ess kit that includes the following items for your party: invitation cards, response cards and envelopes, keepsake bookmarks, tea bags. All you have to do is mail to your friends, family, and guests and they’ll enjoy a cup of tea and make a donation to help end NF!

To learn more about the program please visit: www.ctf.org/teaparty
For further information contact: Traceann Adams, tadams@ctf.org

The NF Camp is held annually in two one-week sessions at Camp Kostopulos in Emigration Canyon, Utah, serving up to 80 children, ages 12 to 21 who are afflicted with neurofibromatosis (NF). Roughly 85% of those who attend do so with financial assistance from the Children’s Tumor Foundation, as these children come from predominately low-to-middle income families who are also, in many cases, shoudering the burden of out-of-pocket medical expenses because of insufficient or no health insurance to cover treatments for the many complications of NF. We make certain that every child who wants to attend camp will have the opportunity, no matter their financial circumstances.

The NF Summer Camp plays a vital role within the Foundation’s varied patient support programs. Adolescence can be a difficult time for anyone, but young people with neurofibromatosis face a unique set of medical and psychosocial challenges putting them at risk for anxiety, depression, low self-esteem and social isolation. The purpose of the NF Summer Camp is to introduce these children, many for the first time in their lives, to others with NF who share a common experience in living with the disorder. Teens benefit immensely from connecting with peers who share their needs, questions and concerns. The camp engages children in group activities, many requiring physical skills and exertion, without the fear of ridicule or intimidation from peers who are not limited by disabilities and do not understand what it’s like to live with NF.

To learn more about the program please visit: www.ctf.org/camp
For further information contact: Patrice Pancza, ppancza@ctf.org
To: Attendees of the 2009 Neurofibromatosis Conference

From: NYU Post-Graduate Medical School

Date: June 13, 2009

Re: 2009 Neurofibromatosis CME Credits

Welcome to the CME program 2009 Neurofibromatosis Conference sponsored by the NYU Post-Graduate Medical School.

To obtain CME credit, you must completely fill out an online evaluation and attestation by June 26, 2009. Certificates will be mailed out the week of July 3, 2009 and should be received in 1-2 weeks. Please note that the evaluations should be completed after the course ends. Anyone attesting to the full 22.25 hours of the course before it is completed may not receive CME credits until their attendance can be completely verified.

Here’s the link to the evaluation: http://cme.med.nyu.edu/nf2009

Also, please be advised that you will be receiving, via e-mail, a Course Outcome Measurement Survey, 3-6 months after this meeting. We appreciate your cooperation in answering this survey which will help us to ascertain what new competencies or changes in clinical skills you were able to apply to your practice as a result of this activity.
Target Audience
The 2009 Neurofibromatosis [NF] Conference is a professional conference whose target audience includes:
- Scientific researchers focused on or interested in learning about NF research, including those in the academic and commercial sector
- Pediatricians, geneticists and neurologists who provide primary care for NF patents
- Specialist physicians who provide care for NF patients including but not limited to neurosurgeons, oncologists, orthopedic surgeons, neuropsychologists, etc.
- Professional research management staff from federal and private agencies with an interest in NF research (e.g. attendees from NIH, DOD program staff)

Course Description
The Children’s Tumor Foundation 2009 Neurofibromatosis Conference is the premier annual gathering of the international neurofibromatosis research and clinical communities. The 2009 NF Conference offers 12 sessions in total and endeavors to provide the most current updates on neurofibromatosis clinical trials, clinical management and translational and basic research as follows:
- NF Clinical Trials Updates
- Correlating NF Phenotype and Genotype
- Clinical Features of NF
- Molecular Basis of Tumors in NF
- NF1 in Context: A View of Related Ras-MAPK Disorders
- Basic Unraveling the Signaling Pathways of NF
- Basic: Unraveling the Signaling Pathways of NF (continued)
- Clinical: Cases that taught me something new
- Recent Advances in Understanding NF1 Cognitive Deficits
- NF2 and Schwannomatosis: What’s New?
- Novel cellular and animal models of NF
- Progress in Management of NF1 Bone Abnormalities
- NF Therapeutic Targets and Preclinical Drug Screening

Statement of Need
Neurofibromatosis (NF) is an orphan disorder that affects 13,000 individuals. NF requires multidisciplinary care because its progress is unpredictable and can include brain and peripheral nerve tumors, which can be benign or malignant; bone abnormalities; learning disabilities; and severe pain. The majority of pediatricians, geneticists and neurologists will see cases of NF during their career and should be familiar with the diagnosis and care guidelines for this disorder. As we progressively learn more about the natural history of this disorder, NF clinical diagnosis and care guidelines are being refined. Pediatricians, geneticists and neurologists should be familiar with the most updated current core guidelines and those recommended to Affiliate Clinics of the CTF NF Clinic Network. Additionally, attendees need to be updated on how to manage the manifestations of NF clinically, including detailed guidelines for specific manifestations such as bone abnormalities or learning disabilities as well as surgical management and emerging drug therapies.

Learning Objectives
Attendees of the 2009 NF Conference will be able to:
- List the core NF clinical diagnosis guidelines recommended to Affiliate Clinics of the CTF NF Clinic Network as well as the most recent updates for these recommendations from physician/research workshop groups focused on particular manifestations of NF e.g., NF1 bone dysplasia
- Describe recent advances in guidelines for specific manifestations of NF, such as bone abnormalities or NF2 tumor management, as well as therapeutic options now available.
- Summarize advances in clinical care, management and surgical/drug treatment for NF
- Incorporate into practice the standard SPRED-1 genetic test and other developments from clinical trials and drug screenings
Jointly Sponsored by
NYU Post-Graduate Medical School and
the Children’s Tumor Foundation

Accreditation Statement
This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of NYU Post-Graduate Medical School and Children’s Tumor Foundation. NYU Post-Graduate Medical School is accredited by the ACCME to provide continuing medical education for physicians.

Credit Designation Statement
The NYU Post-Graduate Medical School designates this educational activity for a maximum of 22.25 AMA PRA Category 1 Credits™. Physicians should only claim credits commensurate with the extent of their participation in the activity.

Disclosure Statement
The NYU Post-Graduate Medical School adheres to ACCME Essential Areas and policies, including the Standards for Commercial Support regarding industry support of continuing medical education. In order to resolve any identified Conflicts of Interest, disclosure information is provided during the planning process to ensure resolution of any identified conflicts. Disclosure of faculty and commercial relationships as well as the discussion of unlabeled or unapproved use of any drug, device or procedure by the faculty is listed on the following pages.
FULL DISCLOSURE POLICY

2009 Neurofibromatosis Conference - June 13-16, 2009

It is the policy of the NYU Post Graduate Medical School to insure balance, independence, objectivity, and scientific rigor in all its individually sponsored or jointly sponsored activities. Because CME activities are conducted in the public interest, it is important to assure the public that education received by physicians and other health care professionals through whom patient care decisions are made is conducted with the highest integrity, scientific objectivity and in the absence of bias. A conflict of interest exists when individuals have both a financial relationship with a commercial interest and the opportunity to affect the content of CME about the product or services of that commercial interest. The Accreditation Council for Continuing Medical Education (ACCME) holds providers of CME responsible for collecting information from its instructors, planners and managers of CME content and resolving those conflicts prior to the commencement of the CME activity. The intent of the conflict of interest resolution process is to assure that provider, faculty and planner financial relationships with commercial interests and resultant loyalties do not supersede the public interest in the design and delivery of continuing medical education activities for the profession.

The following have indicated whether or not they have received financial support for consultation, research or evaluation, have a financial interest relative to their presentation or will include the discussion of the unlabeled or unapproved use of a drug, device or procedure:

Course Directors, Planners & Presenters:

Dr. Jeffrey Allen, Planner - Reports no relevant financial relationships
Kathleen Berentsen, Planner - Reports no relevant financial relationships
Joseph Kissil, Planner & Presenter - Reports no relevant financial relationships
Kathryn North, Planner & Presenter - Reports no relevant financial relationships
Kim Hunter Schaedle, Course Director - Reports no relevant financial relationships
Min Wong, Planner - Reports no relevant financial relationships
Norman Sussman, Associate Dean for Postgraduate Programs and Planner - Reports no relevant financial relationships
Sheila Alcantara, Presenter - Reports no relevant financial relationships
Allan Balmain, Presenter - Reports no relevant financial relationships
Allan Belezberg, Presenter – Reports no relevant financial relationships
Yemima Berman, Presenter - Reports no relevant financial relationships
Andre Bernards, Presenter - Reports no relevant financial relationships
Jaishri Blakeley, Presenter - Reports no relevant financial relationships. This presenter’s talk will reference the drugs lapatinib, bevacizumab, and PTC-299.
Johanna Buchstaller, Presenter - Reports no relevant financial relationships
John Carey, Presenter - Reports no relevant financial relationships
Lou Chang, Presenter - Reports no relevant financial relationships
Ruihong Chen, Presenter - Reports no relevant financial relationships
Betty Chow, Presenter - Reports no relevant financial relationships
Karen Cichowski, Presenter - Reports no relevant financial relationships
Jonathan Cooper, Presenter - Reports no relevant financial relationships
Emmanuelle di Tomaso, Presenter - Reports no relevant financial relationships
Madeleine Duvic, Presenter - Reports no relevant financial relationships
Florent Elefteriou, Presenter - Reports no relevant financial relationships
Ype Elgersma, Presenter – Reports no relevant financial relationships
D. Gareth Evans, Presenter – Reports no relevant financial relationships
Faris Farassati, Presenter - Reports no relevant financial relationships
David Feldman, Presenter - Reports no relevant financial relationships
Rosalie Ferner, Presenter - Reports no relevant financial relationships
Michael Fisher, Presenter - Reports no relevant financial relationships
Andrew B. Gladden, Presenter – Reports no relevant financial relationships
Ms. Nikole Hadley, Patient Advocate & Presenter - Reports no relevant financial relationships
C. Oliver Hanemann, Presenter – Reports no relevant financial relationships
Marlan Hansen, Presenter - Reports no relevant financial relationships
Gordon Harris, Presenter - Reports no relevant financial relationships
Janet Hock, Presenter - Reports no relevant financial relationships
Pablo Hollstein, Presenter - Reports no relevant financial relationships
Rachael Hornigold, Presenter - Reports no relevant financial relationships
Sue Huson, Presenter - Reports no relevant financial relationships
Walter J. Jessen, Presenter - Reports no relevant financial relationships
Kimberly Jett, Presenter - Reports no relevant financial relationships
Michel Kalamardes, Presenter - Reports no relevant financial relationships
Geoffrey Killili, Presenter - Reports no relevant financial relationships
Bruce Korf, Presenter - Reports no relevant financial relationships
David Kwiatkowski, Presenter - Reports no relevant financial relationships
Da Yong Lee, Presenter - Reports no relevant financial relationships
Eric Legius, Presenter - Reports no relevant financial relationships
Bob Listernick, Presenter - Reports no relevant financial relationships
Jan Manent, Presenter - Reports no relevant financial relationships
Brendan Manning, Presenter - Reports no relevant financial relationships
Ronen Marmorstein, Presenter - Reports no relevant financial relationships
Sarah Marugg, Presenter - Reports no relevant financial relationships
Viktor-Felix Mautner, Presenter - Reports no relevant financial relationships
Andrea McClatchey, Presenter - Reports no relevant financial relationships
Frank McCormick, Presenter - Reports no relevant financial relationships
Mila McCurrrach, Presenter - Reports no relevant financial relationships
Ian McCutcheon, Presenter - Reports no relevant financial relationships
Kevin McHugh, Presenter - Reports no relevant financial relationships
Ludwine Messiaen, Presenter - Reports no relevant financial relationships
Harry Miao, Presenter - Reports no relevant financial relationships
Helen Morrison, Presenter - Reports no relevant financial relationships
John Mulvihill, Presenter - Reports no relevant financial relationships
Roger Packer, Presenter - Reports no relevant financial relationships
Luis Parada, Presenter - Reports no relevant financial relationships
Jonathan Payne, Presenter - Reports no relevant financial relationships
Juha Peitonen, Presenter - Reports no relevant financial relationships
Scott Plotkin, Presenter - Reports no relevant financial relationships. This presenter’s talk will reference the drug bevacizumab.
Vijaya Ramesh, Presenter - Reports no relevant financial relationships
Nancy Ratner, Presenter - Reports no relevant financial relationships
Katherine Rauen, Presenter - Reports no relevant financial relationships
Karlyne Reilly, Presenter - Reports no relevant financial relationships
Sheryl Rimrodt, Presenter - Reports no relevant financial relationships
Amy Roberts, Presenter – Reports no relevant financial relationships
Steven Scherer, Presenter - Reports no relevant financial relationships
Aaron Schindeler, Presenter - Reports no relevant financial relationships
Mindell Seidlin, Presenter - Reports no relevant financial relationships
Larry Sherman, Presenter - Reports no relevant financial relationships
Alcino Silva, Presenter – Reports no relevant financial relationships
Ms. Lora Stradley, Patient Advocate & Presenter - Reports no relevant financial relationships
Michelle N. Strecker, Presenter - Reports no relevant financial relationships
Anat Stemmer-Rachaminov, Presenter - Reports no relevant financial relationships
Ueli Suter, Presenter - Reports no relevant financial relationships
Nicole Ullrich, Presenter - Reports no relevant financial relationships
Meena Upadhya, Presenter - Reports no relevant financial relationships
Mikka Vilkkula, Presenter - Reports no relevant financial relationships
Dave Viskochil, Presenter - Reports no relevant financial relationships
Linnea R. Vose, Presenter - Reports no relevant financial relationships
James A. Walker, Presenter - Reports no relevant financial relationships
Weixi Wang, Presenter - Reports no relevant financial relationships
Brigitte Widemann, Presenter - Reports no relevant financial relationships
Chunling Yi, Presenter - Reports no relevant financial relationships
Yuan Zhu, Presenter - Reports no relevant financial relationships

The Independent Reviewer reports no relevant financial relationships
2009 Neurofibromatosis Conference
June 13-16, 2009

This course is supported in part by independent medical education grants from:

BIOTECHNOLOGY INDUSTRY ORGANIZATION

PTC THERAPEUTICS, INC.

NOVARTIS INSTITUTES FOR BIOMEDICAL RESEARCH

NATIONAL INSTITUTES OF HEALTH (NIH)
# Schedule At-A-Glance

<table>
<thead>
<tr>
<th>TIME</th>
<th>EVENT</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00 AM</td>
<td>Registration</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>2:00 PM</td>
<td>Welcome and Keynote Presentation</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>3:00 PM</td>
<td>Session 1: NF Clinical Trials Updates</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>7:00 PM</td>
<td>Welcome Dinner aboard the “Portland Spirit”</td>
<td>See Map A at back of Program Book</td>
</tr>
<tr>
<td>8:00 AM</td>
<td>Continental Breakfast</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>8:30 AM</td>
<td>Keynote Presentation</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>9:30 AM</td>
<td>Session 2: Correlating NF Phenotype &amp; Genotype</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>11:00 AM</td>
<td>Break</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>11:20 AM</td>
<td>Session 3A: Clinical Features of NF</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>11:20 AM</td>
<td>Session 3B: Molecular Basis of Tumors in NF</td>
<td>Culture, 6th Fl.</td>
</tr>
<tr>
<td>12:50 PM</td>
<td>Lunch (box lunches provided)</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>1:50 PM</td>
<td>Session 4A: NF1 in Context - A View of Related Ras-MAPK Disorders</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>1:50 PM</td>
<td>Session 4B: Basic - Unraveling the Signaling Pathways of NF</td>
<td>Culture, 6th Fl.</td>
</tr>
<tr>
<td>3:40 PM</td>
<td>Break</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>4:25 PM</td>
<td>Session 5A: Clinical - Cases That Taught Me Something New</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>4:00 PM</td>
<td>Session 5B: Basic - Unraveling the Signaling Pathways of NF (continued)</td>
<td>Culture, 6th Fl.</td>
</tr>
<tr>
<td>8:30 PM</td>
<td>Kells Irish Restaurant and Pub</td>
<td>See Map B at back of Program Book</td>
</tr>
<tr>
<td>8:00 AM</td>
<td>Continental Breakfast</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>8:30 AM</td>
<td>Session 6: Recent Advances in Understanding NF1 Cognitive Deficits</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>10:30 AM</td>
<td>Break</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>11:00 AM</td>
<td>Session 7: NF2 and Schwannomatosis: What’s New?</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
<tr>
<td>12:55 PM</td>
<td>Lunch (on your own)</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>2:00 PM</td>
<td>Experiences and Challenges in the NF Clinic</td>
<td>Culture, 6th Fl.</td>
</tr>
<tr>
<td>4:00 PM</td>
<td>Poster Presentation - Session 1 (odd numbers)</td>
<td>Studio &amp; Gallery, 6th Fl.</td>
</tr>
<tr>
<td>3:30 PM</td>
<td>Poster Presentation - Session 2 (even numbers)</td>
<td>Studio &amp; Gallery, 6th Fl.</td>
</tr>
<tr>
<td>7:30 AM</td>
<td>Continental Breakfast</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>8:00 AM</td>
<td>Session 8B: Progress in Management of NF1 Bone Abnormalities</td>
<td>Culture, 6th Fl.</td>
</tr>
<tr>
<td>10:15 AM</td>
<td>Break</td>
<td>The Nines Ballroom Prefunction, 6th Fl.</td>
</tr>
<tr>
<td>10:30 AM</td>
<td>Session 9: NF Therapeutic Targets and Preclinical Drug Screening</td>
<td>Design &amp; Fashion, 6th Fl.</td>
</tr>
</tbody>
</table>
NOTE TO SESSION CHAIRS

• You must be by the podium 30 minutes before start of session you are chairing to ensure speakers have arrived, go through audiovisual setup, etc.

• It is your responsibility to convene your session PROMPTLY per the schedule!

• Introduce speakers by name and affiliation; and whether they are keynotes, invited speakers or selected abstract speakers. If they are CTF awardees (indicated on the agenda), please mention so in the introduction.

• Introduce the keynote speaker in more detail, by current affiliation, career, etc. (Their biosketch can be found on their abstract page.)

• Patient advocates need only a brief introduction by name as they will tell their own ‘story’.

• It is your responsibility to keep your speakers ON TIME. Visual prompts (clock, lights) will be given; 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 a microphone.

• At the close of the session, please briefly summarize what you see as the key ‘take home’ points of the session.

NOTE TO SPEAKERS

• Bring your slides to the meeting on a flash drive. DO NOT bring your own laptop. You will be notified by Foundation staff at registration as to when to bring your slides to the audiovisual staff.

• Be available by the podium 30 minutes before the start of the session in which you are speaking to understand audiovisual setup and make sure your slideshow is running smoothly.

• Check your time allotment on the agenda. Be prepared to complete your talk in the timeframe given on the agenda! If you run over time you may be ‘cut off’.

NOTE TO POSTER PRESENTERS

• Posters will be on display throughout the Conference from Monday, June 15th to Tuesday, June 16th. Odd number poster presenters will need to stand by their posters on Monday (June 15th) 2:30 - 4:30PM. Even number poster presenters will need to stand by their posters on Monday (June 15th) 4:30 - 6:30PM. Push pins will be provided! You can mount your poster on Monday morning; it should be on display from Sunday until after Session 9 on Tuesday.

Questions?

Please contact a Foundation staff member!
VOTE FOR YOUR FAVORITE BASIC RESEARCH AND CLINICAL POSTERS

Top-voted posters win a prize – and you could too!

ALL ATTENDEES at the 2009 NF Conference are invited to vote for your favorite poster in two categories:

- Basic research
- Clinical research

Please be sure to place your votes in the correct ballot boxes at registration by 7:00 PM on Monday, June 15th (after the poster session)

- Top-voted basic research poster wins a prize
- Top-voted clinical research poster wins a prize
- If YOU voted for a winning poster – you could win a prize too!

Winners announced at 1:00 PM on Tuesday, June 16th at close of the 2009 NF Conference
**WELCOME AND OPENING KEYNOTE**  
2:00 PM – 2:05 PM  
Welcome from the 2009 NF Conference Chairs  
**Kathryn North**, *Children’s Hospital at Westmead, Australia* and **Joe Kissil**, *The Wistar Institute*  

**SESSION 1: NF CLINICAL TRIALS UPDATES**  
Chairs: **Roger Packer**, *Children’s National Medical Center*  
**Jaishri Blakeley**, *Johns Hopkins University*  

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker</th>
<th>Institution/Location</th>
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</thead>
<tbody>
<tr>
<td>3:00 PM</td>
<td>NF1 Clinical Trials of the CDMRP NFRP Clinical Trials Consortium</td>
<td><strong>Roger Packer</strong>, <em>Children’s National Medical Center</em></td>
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<tr>
<td>3:15 PM</td>
<td>Towards a Treatment of the Cognitive Deficits Associated with NF1</td>
<td><strong>Ype Elgersma</strong>, <em>Erasmus University, The Netherlands</em></td>
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<tr>
<td>3:30 PM</td>
<td>NF1 Optic Pathway Glioma Trials</td>
<td><strong>Nicole Ullrich</strong>, <em>Children’s Hospital Boston</em></td>
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</tr>
<tr>
<td>3:45 PM</td>
<td>Trials for NF1 Plexiform Neurofibromas and MPNSTs</td>
<td><strong>Brigitte Widemann</strong>, <em>National Institutes of Health, National Cancer Institute</em></td>
<td></td>
</tr>
<tr>
<td>4:00 PM</td>
<td>Development of Therapies for Cutaneous T-Cell Lymphoma: Pipeline for Dermal Neurofibromas?</td>
<td><strong>Madeleine Duvic</strong>, <em>University of Texas M.D. Anderson Cancer Center</em></td>
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<tr>
<td>4:15 PM</td>
<td>NF1 Dermal Neurofibromas – Ongoing Trials</td>
<td><strong>Scott Plotkin</strong>, <em>Harvard Medical School/Massachusetts General Hospital</em></td>
<td></td>
</tr>
<tr>
<td>4:45 PM</td>
<td>NF2 Clinical Trials: An Overview</td>
<td><strong>Jaishri Blakeley</strong>, <em>Johns Hopkins University</em></td>
<td></td>
</tr>
<tr>
<td>5:00 PM</td>
<td>Phase 2 Study of PTC299 in NF2</td>
<td><strong>Harry Miao</strong>, <em>PTC Therapeutics: Abstract Platform</em></td>
<td></td>
</tr>
<tr>
<td>5:15 PM</td>
<td>Children’s Tumor Foundation NF Preclinical Consortium: An Update</td>
<td><strong>Mila McCurrach</strong>, <em>NFPC Project Manager</em></td>
<td></td>
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<tr>
<td>5:35 PM</td>
<td>Developing the Children’s Tumor Foundation BioBank and Patient Registry</td>
<td><strong>Mindell Seidlin</strong>, <em>Consultant, Children’s Tumor Foundation</em></td>
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**Jazz Cruise Welcome Dinner aboard the “Portland Spirit”**  
(5-10 min. walk - see Map A at back of Program & Abstract Book for directions)  
**EMBARK at 7:00 PM**  
**BOAT DEPARTS at 7:30 PM SHARP!**
Sunday • June 14, 2009

8:00 AM  8:30 AM  BREAKFAST AVAILABLE

KEYNOTE PRESENTATION  .............................................................. Design & Fashion, 6th Fl.
8:30 AM  9:30 AM  A Decade of Modeling NF1 in the Mouse
Luis Parada, University of Texas Southwestern Medical Center
2009 Friedrich von Recklinghausen Award Recipient

SESSION 2: CORRELATING NF PHENOTYPE AND GENOTYPE  ........... Design & Fashion, 6th Fl.
Chair: Susan Huson, University of Manchester, United Kingdom

9:30 AM  9:50 AM  Defining the NF1 Phenotype
Bruce Korf, University of Alabama at Birmingham

9:50 AM  10:10 AM  NF1 in Old Age
John Mulvihill, University of Oklahoma

10:10 AM  10:50 AM  Genetic Modification of NF1 Phenotypes
Andre Bernard, Harvard Medical School/Massachusetts General Hospital: Abstract Platform

10:50 AM  11:00 AM  Whole Body MRI Evaluation in NF1, NF2, and Schwannomatosis
Scott Plotkin, Harvard Medical School/Massachusetts General Hospital

11:00 AM  11:20 AM  Break

*** NOTE: Sessions 3A and 3B run concurrently ***

SESSION 3A: CLINICAL FEATURES OF NF  ..................................... Design & Fashion, 6th Fl.
Chair: Viktor-Felix Mautne, University Medical Center Hamburg-Eppendorf, Germany

11:20 AM  11:35 AM  Clinical, Pathological and Molecular Variables Predictive of MPNST Outcome
Ian McCutcheon, University of Texas M.D. Anderson Cancer Center: Abstract Platform

11:35 AM  11:50 AM  Photodynamic Therapy in Children with NF1 and Plexiform Neurofibromas
Michael Fisher, Children’s Hospital of Philadelphia

11:50 AM  12:05 PM  Velopharyngeal Insufficiency in NF1- A Clinical Sign of Underestimated Clinical Significance?
Yemima Berman, Children’s Hospital at Westmead, Australia: Abstract Platform

12:05 PM  12:20 PM  Vascular Endothelial Cell Dysfunction in NF1
Kimberly Jett, University of British Columbia: Abstract Platform

12:20 PM  12:35 PM  Digital Image Archiving and Analysis for patients with NF1 and NF2
Gordon Harris, Harvard Medical School/Massachusetts General Hospital: Abstract Platform
SESSION 3B: MOLECULAR BASIS OF TUMORS IN NF ..................................................Culture, 6th Fl.
Chair: Helen Morrison, Leibniz Institute For Age Research, Belgium

11:20 AM  11:35 AM  Cell of Origin and Mechanisms of Tumor Initiation in a Mouse Model of Malignant Astrocytoma
Sheila Alcantara, University of Texas Southwestern Medical College (2007 Young Investigator Award Recipient): Abstract Platform

11:35 AM  11:50 AM  Tumor Initiating Cells in Malignant Peripheral Nerve Sheath Tumors
Johanna Buchstaller, University of Michigan, (2008 Young Investigator Award Recipient): Abstract Platform

11:50 AM  12:05 PM  Natural Product Schweinfurthin Inhibits NF1 Tumor Growth Through an NF1-dependent Pathway Affecting Rho Signaling and Cytoskeletal Alteration
Karlyne Reilly, National Institutes of Health, National Cancer Institute at Frederick: Abstract Platform

12:05 AM 12:20 PM  Differential Impact of Nf1 Loss on Neural Stem Cells From Different Brain Regions
Da Yong Lee, Washington University School of Medicine, (2007 Young Investigator Award Recipient): Abstract Platform

12:20 PM  12:35 PM  Tumor-derived Merlin Mutations Abrogate Binding to a Novel Cul4 E3 Ubiquitin Ligase
Jonathan Cooper, Memorial Sloan Kettering Cancer Center: Abstract Platform

12:35 PM  12:50 PM  Functional Relevance of the merlin/RasGAP Interaction
Helen Morrison, Leibniz Institute of Age Research, Germany: Abstract Platform

12:50 PM  1:50 PM  LUNCH (box lunches provided)

*** NOTE: Sessions 4A and 4B run concurrently ***

SESSION 4A: NF1 IN CONTEXT - A VIEW OF RELATED RAS-MAPK DISORDERS....Design & Fashion, 6th Fl.
Chair: Katherine Rauen, University of California, San Francisco

1:50 PM  1:55 PM  Ras-MAPK Pathway: An Overview
Katherine Rauen, University of California, San Francisco

1:55 PM  2:15 PM  Clinical and Mutational Spectrum of Legius Syndrome
Ludwine Messiaen, University of Alabama at Birmingham

2:15 PM  2:30 PM  SPRED1 Mutations in Legius Syndrome: Another Clinically Useful Genotype for Dissecting the Nf1 Phenotype
Meena Upadhyaya, Institute of Medical Genetics, United Kingdom: Abstract Platform

2:30 PM  2:50PM  Cognition in Mice and Man with SPRED1 Mutations
Eric Legius, Catholic University of Leuven, Belgium

2:50 PM  3:10 PM  RASA1 (a RasGap) and CM-AVM, Capillary Malformation-Arteriovenous Malformation
Mikka Viikkula, Universite Catholique de Louvain, Belgium

3:10 PM  3:30 PM  Noonan’s Syndrome
Amy Roberts, Children’s Hospital Boston

3:30 PM  3:50 PM  CFC and Costello Syndrome
Katherine Rauen, University of California, San Francisco
Sunday • June 14, 2009

3:50 PM  4:05 PM  A Pathway to Comprehensive Care: The UCSF NF/Ras Pathway Genetics Clinic
Michelle N. Strecker, University of California, San Francisco: Abstract Platform

4:05 PM  4:25 PM  BREAK

SESSION 4B: BASIC - UNRAVELING THE SIGNALING PATHWAYS OF NF….. Culture, 6th Fl.

Chairs: Frank McCormick, University of California, San Francisco
Vijaya Ramesh, Harvard Medical School/Massachusetts General Hospital

1:50 PM  2:10 PM  Rare Diseases, Common Pathway: Signal Integration by the TSC-mTOR Network
Brendan Manning, Harvard School of Public Health

2:10 PM  2:30 PM  Merlin Modulates Rac-Pak, Downstream MAPK Signaling Through Tight Junction-Associated Proteins
Chunling Yi, The Wistar Institute: Abstract Platform

2:30 PM  2:50 PM  How Basic Research Promotes Insight into NF1 Disease Pathogenesis and Therapeutic Design
Karen Cichowski, Harvard Medical School/Brigham & Women’s Hospital

2:50 PM  3:10 PM  erbB Receptor Signaling in Vestibular Schwannomas
Marlan Hansen, University of Iowa

3:10 PM  3:25 PM  Analysis of p21-activated Kinase Function in NF2 Signaling in vivo
Betty Chow, Fox Chase Cancer Center: Abstract Platform

3:25 PM  3:40 PM  E3 Ubiquitin Ligase Controls Ras Signaling Through Degradation of the NF1 Tumor Suppressor
Pablo Hollstein, Harvard Medical School/Brigham and Women’s Hospital: Abstract Platform

3:40 PM  4:00 PM  BREAK

*** NOTE: Sessions 5A and 5B run concurrently ***

SESSION 5A: CLINICAL - CASES THAT TAUGHT ME SOMETHING NEW …… Design & Fashion, 6th Fl.

Chairs: Rosalie Ferner, Guy’s and St Thomas’ NHS Trust Hospital, United Kingdom
Kathryn North, Children’s Hospital at Westmead, Australia

4:25 PM  5:45 PM  Case Discussions
Casey Discussions
Clinical Case Quiz with Expert Panel: USA vs. Rest of World

RoW:  Sue Huson - St. Mary’s Hospital, Manchester, D. Gareth Evans - St. Mary’s Hospital, Manchester, Eric Legius - Catholic University of Leuven, Belgium
USA:  Dave Viskochil - University of Utah, Bob Listerick - Children’s Memorial Hospital, Bruce Korf - University of Alabama at Birmingham

Discussion
Award of Prizes to Winning Team!
SCHEDULE

Sunday • June 14, 2009

SESSION 5B: BASIC - UNRAVELING THE SIGNALING PATHWAYS OF NF (cont.) .... Culture, 6th Fl.
Chairs: Frank McCormick, University of California, San Francisco
Vijaya Ramesh, Harvard Medical School/Massachusetts General Hospital

4:00 PM 4:20 PM Pathways Regulating Ras Activity
Frank McCormick, University of California, San Francisco

4:20 PM 4:35 PM Loss of NF2 Function in Schwann Cells Induces Dedifferentiation and Activation of Nerve Repair
Mechanisms by Mimicking Impaired Axon-Schwann Cell Interaction
Jan Manent, Inserm U674, France: Abstract Platform

4:35 PM 4:55 PM Junctional Specializations of Schwann Cells
Steven Scherer, University of Pennsylvania

4:55 PM 5:10 PM Function of Merlin in Controlling Membrane Receptor Distribution and Signaling
Andrea McClatchey: on behalf of Zachary Morris, Harvard Medical School/Brigham & Women’s Hospital: Abstract Platform

5:10 PM 5:30 PM Rho- GTPase in Schwann Cells
Ueli Suter, University of Zurich, Switzerland

8:30 PM – 10:30 PM Join us for drinks and presentation of the 2009 Friedrich von Recklinghausen Award
Kells Irish Restaurant and Pub
112 SW Second Avenue
(between SW Ash and SW Pine Streets)
See Map B at back of Program & Abstract Book for directions

Monday • June 15, 2009

8:00 AM 8:30 AM BREAKFAST AVAILABLE

SESSION 6: RECENT ADVANCES IN UNDERSTANDING NF1 COGNITIVE DEFICITS ... Design & Fashion, 6th Fl.
Chair: Kathryn North, Children’s Hospital at Westmead, Australia

8:30 AM 8:40 AM Overview and Issues to Consider in Developing Effective Therapies for NF1 Learning Disabilities
Kathryn North, Children’s Hospital at Westmead, Australia

8:40 AM 9:00 AM Reading and Other Cognitive Concerns in NF-1: And An Ongoing Trial of Intervention
Sheryl Rimrodt, Kennedy Krieger Institute

9:00 AM 9:30 AM Mechanisms of Learning Disabilities Associated with Disruptions of Ras/MAPK Aignaling: From Lab to Clinic
Alcino Silva, University of California, Los Angeles

9:30 AM 9:50 AM Clinical Trials - How Do We Move From Mouse to Humans?
Jonathan Payne, Children’s Hospital at Westmead, Australia

9:50 AM 10:05 AM The Role of NF1 Exon 9a in Neuronal Function
Ype Elgersma, Erasmus University, The Netherlands: Abstract Platform
### SCHEDULE

**Monday • June 15, 2009**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker/Institution</th>
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<tbody>
<tr>
<td>10:05 AM</td>
<td>Pharmacological Effects on Cognition in a Fly Model of NF1</td>
<td>Linnea R. Vose, New York Medical College, <em>2008 Young Investigator Award Recipient</em>: Abstract Platform</td>
</tr>
<tr>
<td>10:20 AM</td>
<td>NF1: A Personal Perspective</td>
<td>Sarah Marugg, <em>Patient Advocate</em></td>
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<tr>
<td>10:30 AM</td>
<td>BREAK</td>
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**SESSION 7: NF2 AND SCHWANNOMATOSIS: WHAT’S NEW?**

*Design & Fashion, 6th Fl.*

**Chair:** Joe Kissil, *The Wistar Institute*

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<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker/Institution</th>
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</thead>
<tbody>
<tr>
<td>11:00 AM</td>
<td>Schwannomatosis Natural History Study Update</td>
<td>Allan Belzberg, <em>Johns Hopkins University</em></td>
</tr>
<tr>
<td>11:20 AM</td>
<td>Pathology of Neurofibromas and Schwannomas</td>
<td>Anat Stemmer-Rachamimov, <em>Harvard Medical School/Massachusetts General Hospital</em></td>
</tr>
<tr>
<td>11:40 AM</td>
<td>NF2 Anti-Angiogenic Trial</td>
<td>Emmanuelle di Tomaso, <em>Harvard Medical School/Massachusetts General Hospital</em></td>
</tr>
<tr>
<td>12:00 PM</td>
<td>Expression Profiling of Schwannomas</td>
<td>D. Gareth Evans, <em>St. Mary’s Hospital, Manchester</em>: Abstract Platform</td>
</tr>
<tr>
<td>12:15 PM</td>
<td>Timing and Impact of Surgery in NF2</td>
<td>Rachael Hornigold, <em>Guy’s and St Thomas’ NHS Trust Hospital</em>: Abstract Platform</td>
</tr>
<tr>
<td>12:30 PM</td>
<td>Drosophila as a Model to Study the Role of <em>SMARCB1</em> in Schwannomatosis</td>
<td>James A. Walker, <em>Harvard Medical School/Massachusetts General Hospital</em>: Abstract Platform</td>
</tr>
<tr>
<td>12:45 PM</td>
<td>Schwannomatosis: A Personal Perspective</td>
<td>Lora Stradley, <em>Patient Advocate</em></td>
</tr>
<tr>
<td>1:00 PM</td>
<td>LUNCH (on your own)</td>
<td></td>
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</table>

**2:30 PM - 6:30 PM**

**Poster Session**

Studio & Gallery, 6th Fl.

- 2:30 - 4:30: Poster Session I - stand by posters (odd numbers)
- 4:30 - 6:30: Poster Session II - stand by posters (even numbers)

Wine and beer will be served after 5:00 PM

**2:00 PM - 4:00 PM**

**Satellite Session**

Culture, 6th Fl.

Shared experiences and challenges in the NF Clinic: An open discussion for health care professionals
**SCHEDULE**

**Tuesday • June 16, 2009**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>7:30 AM</td>
<td>BREAKFAST AVAILABLE</td>
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</tbody>
</table>

*** NOTE: Sessions 8A and 8B run concurrently ***
Session 8B commences at 8:00 AM!

**SESSION 8A: NOVEL CELLULAR AND ANIMAL MODELS OF NF ........................ Design & Fashion, 6th Fl.**

**Chair:** Michel Kalamardies, *Hospital Beaujon, Paris*

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>8:30 AM</td>
<td>NF2 Meningioma Mouse Model&lt;br&gt;Michel Kalamardies, <em>Hospital Beaujon, Paris</em></td>
</tr>
<tr>
<td>8:50 AM</td>
<td>SWI/SNF Factors in Schwann cells: Regulators of Schwannomatosis Pain and Tumor Growth? Larry Sherman, <em>Oregon Health Sciences University</em></td>
</tr>
<tr>
<td>9:10 AM</td>
<td>Novel Mouse Models of MPNSTs&lt;br&gt;Lou Chang, <em>University of Michigan</em>: Abstract Platform</td>
</tr>
<tr>
<td>9:25 AM</td>
<td>NF1 Heterozygous Environment in Initiation and Progression of Plexiform Neurofibromas&lt;br&gt;Yuan Zhu, <em>University of Michigan</em></td>
</tr>
<tr>
<td>9:45 AM</td>
<td>Merlin Regulates Epithelial Cell Polarity and Proliferation via a Junctional Polarity Complex&lt;br&gt;Andrew B. Gladden, <em>Harvard Medical School/Massachusetts General Hospital</em>: Abstract Platform</td>
</tr>
<tr>
<td>10:00 AM</td>
<td>Nerve Gene Expression Patterning in NF1 Mouse Models&lt;br&gt;Walter J. Jessen, <em>Cincinnati Children’s Medical Center</em>: Abstract Platform</td>
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</tbody>
</table>

**SESSION 8B: PROGRESS IN MANAGEMENT OF NF1 BONE ABNORMALITIES .......... Culture, 6th Fl.**

**Chair:** Dave Stevenson, *University of Utah*

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>8:00 AM</td>
<td>Dural Ectasia in NF1&lt;br&gt;David Feldman, <em>NYU Langone Medical Center</em></td>
</tr>
<tr>
<td>8:20 AM</td>
<td>Osteoporosis in NF1-related Bone Abnormalities&lt;br&gt;Janet Hock, <em>Maine Institute for Human Genetics and Health</em></td>
</tr>
<tr>
<td>8:40 AM</td>
<td>Spine Abnormalities in Asymptomatic Children with NF1&lt;br&gt;David Viskochil, <em>University of Utah</em>: Abstract Platform</td>
</tr>
<tr>
<td>8:55 AM</td>
<td>NF1 Focal Bone Defects - Identification of the Cellular Culprits by Conditional Mouse Models&lt;br&gt;Florent Elefteriou, <em>Vanderbilt University</em></td>
</tr>
<tr>
<td>9:15 AM</td>
<td>Molecular Aspects of NF1-related Bone Abnormalities&lt;br&gt;Kevin McHugh, <em>Harvard Medical School</em></td>
</tr>
<tr>
<td>9:35 AM</td>
<td>NF1-deficient Osteoclasts In Vivo and In Vitro&lt;br&gt;Juha Pellonen, <em>University of Turku, Finland</em></td>
</tr>
<tr>
<td>9:55 AM</td>
<td>Local Delivery of Low Dose Lovastatin Improves Fracture Healing in NF1 Loss-Of-Function Osteoblasts&lt;br&gt;Weixi Wang, <em>Vanderbilt University</em>, <em>(2007 Young Investigator Award Recipient)</em>: Abstract Platform</td>
</tr>
</tbody>
</table>
Orthopedic Animal Models of NF1: An Update
Aaron Schindeler, The Children’s Hospital at Westmead, Australia, (2008 Young Investigator Award Recipient):
Abstract Platform

10:15 AM 10:30 AM  BREAK

SESSION 9: NF THERAPEUTIC TARGETS AND PRECLINICAL DRUG SCREENING ....... Design & Fashion, 6th Fl.
Chair: Nancy Ratner, Cincinnati Children’s Hospital

10:30 AM 11:25 AM  KEYNOTE PRESENTATION
The Multiple Faces of RAS: Oncogene, Tumor Suppressor, and Tumor Susceptibility Gene
Allan Balmain, University of California, San Francisco

11:25 AM 11:45 AM  Structure Based Design of Novel Kinase Inhibitors for NF and Other Cancers
Ronen Marmorstein, The Wistar Institute

11:45 AM 12:00 PM  Development of a Non-surgical Treatment for Dermal Neurofibromas in NF1
Ruihong Chen, NexGenix Pharmaceuticals and NYU Medical Center: Abstract Platform

12:00 PM 12:15 PM  Overactivation of Ral in MPNST: Cell Signaling and Therapeutic Ramifications
Faris Farassati, University of Kansas: Abstract Platform

12:15 PM 12:30 PM  Regulation of MAPK Signaling by the Mammalian Ste-20 Like Kinase-2
Geoffrey Kilili, Tufts University, (2007 Young Investigator Award Recipient): Abstract Platform

12:30 PM 12:45 PM  Regulation of Rac by PAK in Human Schwannoma
C. Oliver Hanemann, Peninsula College of Medicine and Dentistry: Abstract Platform

12:45 PM 1:00 PM  Using Gene Expression Analysis to Identify NF1 Clinical Targets
Nancy Ratner, Cincinnati Children’s Hospital: Abstract Platform

Poster Winners Announced, Closing Remarks
Kathryn North, Children’s Hospital at Westmead, Australia and Joe Kissil, The Wistar Institute

2009
Children’s Tumor Foundation
ABSTRACTS

Keynote Presentations

TSC1/TSC2: a signaling hub with roles in tuberous sclerosis, neurodevelopment, and cancer

David Kwiatkowski
Harvard Medical School/Brigham & Women’s Hospital

Tuberous sclerosis (TSC) is an autosomal dominant tumor syndrome, with many similarities but also many differences in comparison to neurofibromatosis type 1. In this presentation, I will review the molecular genetics of TSC, its core clinical features and major clinical morbidity, our current understanding of pathogenesis of the disease, current management, and ongoing clinical trials.

The protein products of the genes which cause TSC, TSC1 and TSC2, are at the central hub of a signaling pathway that extends from surface receptors through both the PI3K and ERK signaling pathways to TSC1/TSC2 to regulate activation of mTORC1, a core kinase that regulates protein synthesis, ribosome biogenesis, and cellular growth. Our current understanding of this signaling circuit, and particularly the mechanisms by which TSC1/TSC2 function is regulated to affect mTORC1 activation, will also be discussed.

The TSC1/TSC2 proteins play a particularly important role in brain development, reflected by the neuropathology and neurological manifestations seen in patients. The role of these proteins in brain progenitor cells, neurons, and astrocytes will also be reviewed. Several published brain models of TSC in the mouse will be reviewed, as well as recent unpublished results on a new model that replicates TSC tubers.

Finally, the role of these proteins in human cancer will be considered. Although in general these genes are not common targets for mutation/inactivation in human cancer, there are a few tumors in which they seem to be an important target. Recent and unpublished mouse models of cancer development will be discussed, and considered in the light of recent advances in TSC signaling pathways.

A decade of modeling NF1 in the mouse

Luis Parada
University of Texas Southwestern Medical Center

2009 Friedrich von Recklinghausen Award Recipient

Neurofibromatosis (NF1) is one of the most prevalent genetic disorders of the nervous system. NF1 patients invariably develop neurofibromas, a complex benign tumor that readily progresses to malignancy. Neurofibromin, the tumor suppressor protein encoded by NF1, has rasGap activity, implicating ras pathway constitutive activation as a major consequence of NF1 loss of function. We have undertaken to model NF1 in mice through conventional and conditional knock out technologies. Mice lacking NF1 in Schwann cell precursors develop neurofibromas.

We have uncovered a genetic contribution of the environment to this tumor formation that may be attributable to mast cell involvement. Dermal neurofibromas arise in adolescents and also during pregnancy. While cellularly identical to plexiform tumors, dermal tumors have limited growth potential and how and from what cell they arise remains a mystery. We are using our mouse modeling strategies to answer these questions.

NF1 patients also have increased incidence of glioblastoma formation. We have sought to model these tumors for which no effective therapies have been developed. According to the WHO classification, four grades of astrocytoma exist. Grade I (pilocytic astrocytoma) is benign. Grade II (low grade astrocytoma) is characterized by infiltrative cells that home on neuronal bodies (perineurial satellosis). Grade III (anaplastic astrocytoma) is cell dense and highly proliferative. Grade IV (glioblastoma multiforme) is characterized by pseudopalisading, necrotic foci, and intense microvascularization. All forms of astrocytoma express primitive cell markers such as nestin. In addition, all forms of astrocytoma appear throughout the brain but do not leave the CNS.

These observations suggest that the CNS provides a niche that is required for tumor growth and that spontaneous tumorgenesis occurs throughout.
Historically, the prevalent model for astrocytoma formation invoked mechanisms of glial cell dedifferentiation, followed by genetic and epigenetic signals that drive neoplastic transformation. However, the recent appreciation of the existence of stem cells in the lateral ventricles and dentate gyrus has raised the question of a potential role for stem cells in tumor formation. We have developed tumor suppressor-based mouse models of glioblastoma that histologically and molecularly resemble human glioblastoma. Through analysis of a variety of criteria, our results provide concrete evidence that stem cells could account for the origin of glioblastoma. Studies of signal transduction pathway alteration in these progressive tumors may provide insights into therapeutic targeting.

Acknowledgements: Funding provided by ACS, CDMRP NFRP, NIH, Simons Foundation

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Luis Parada, Ph.D. will open the NF Conference with a keynote presentation - A decade of modeling NF1 in the mouse. Dr. Parada, professor and Chair of the Department of Developmental Biology is an internationally recognized developmental neurobiologist whose work focuses on the molecular and genetic mechanisms that regulate the development and survival of nerve cells in the embryos of mammals as well as on molecular mechanisms of cancers of the nervous system. He discovered the identity of the Nerve Growth Factor (NGF) receptor critical in signalling embryonic nerve cells to survive or die. Scientific journal articles documenting his research into this nerve-cell survival signaling mechanism were so widely quoted that for a time Dr. Parada was among the most cited biologists in the world. He remains among the ISI’s Highly Cited scientists over the past twenty years. Before coming to Dallas in 1994, Dr. Parada, a native of Bogota, Colombia, directed the Molecular Embryology Section of the National Cancer Institute’s Mammalian Genetics Laboratory. His work there focused on proto-oncogenes—genes with normal biological functions that can cause cancer when they function abnormally. Dr. Parada earned his undergraduate degree from the University of Wisconsin-Madison and his doctoral degree in biology from the Massachusetts Institute of Technology. One of Dr. Parada’s specialties is developing genetically altered mouse models that can be used to help researchers learn how neural pathways develop and to model cancer. Dr. Parada holds the Diana and Richard C. Strauss Distinguished Chair in Developmental Biology and the Southwestern Ball Distinguished Chair in Basic Neuroscience Research. He serves on numerous Academic and Foundation Boards currently including the National Advisory Council of the National Institutes for Neurological Disorders & Stroke, the Pew Scholars Foundation Advisory Board and the Howard Hughes Medical Institute. He is an elected member of the American Academy of Arts & Sciences and of the Institute of Medicine of the National Academy of Sciences.

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The multiple faces of RAS: oncogene, tumor suppressor, and tumor susceptibility gene

Allan Balmain

*University of California, San Francisco*

No abstract submitted

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Alan Balmain, Ph.D., FRSE is the Barbara Bass Bakar Distinguished Professor in Cancer Genetics, Co-Leader of the Cancer Genetics Program, and Director, Genome Analysis Core Facility, Helen Diller Family Comprehensive Cancer Center at the at the University of California, San Francisco. He will talk on his main research interest the elucidation of the molecular mechanisms of multistage carcinogenesis, with particular emphasis on mouse models of chemically induced skin tumor development via the development of transgenic or knock-out mice to investigate the biological consequences of genetic change for cell behaviour during transformation. These have proved invaluable for studies of the causal genetic and biological changes associated with tumor progression. Dr. Balmain has a Ph.D. from the University of Glasgow.
NF1 clinical trials of the CDMRP NFRP Clinical Trials Consortium

Roger Packer  
Children’s National Medical Center

The Department of Defense sponsored Neurofibromatosis Clinical Trials Consortium (DOD/NFCTC) is now entering its third year of operation. The NFCTC was constituted to develop biologically-based, innovative therapies for patients with neurofibromatosis. Areas of initial focus included the treatment of: 1) plexiform neurofibromas; 2) progressive low-grade gliomas; 3) the neurocognitive deficits of children with neurofibromatosis type 1; 4) and malignant peripheral nerve sheath tumors. Progress has been made rapidly in these areas. The first trial opened by the DOD/NFCTC was a Phase II study of treatment of progressive and/or symptomatic plexiform neurofibromas with an mTOR inhibitor, rapamycin (B. Weiss, Cincinnati PI). This trial has utilized innovative centralized neuroimaging assessment and has exceeded its initial accrual goals. Accrual in stratum A evaluating patients with symptomatic plexiform neurofibromas entered 12 patients has been closed for analysis and, to date, 26 patients have been entered on stratum B, evaluating symptomatic, radiographically progressive plexiform neurofibromas. Interim analysis on stratum A should be complete within the next three months. Therapy has been extremely well tolerated, and data submission, to date, has also been excellent. A second study evaluating the efficacy of lovastatin in children between 10 and 17 years of age with selective cognitive deficits has been approved by the DOD and is set to open as early as May of 2009. The Phase 1 portion of this study was completed by Dr. M. Acosta at Children’s National Medical Center. This is a prospective randomized trial being performed in collaboration with the University of Sydney (K. North, PI). It is expected that accrual will take 12 to 18 months. A third study on progressive low-grade gliomas is nearing approval. If approved, it will evaluate a new mTOR inhibitor, Rad 001, in patients with recurrent progressive low-grade gliomas. For malignant peripheral nerve sheath tumors, a study is being proposed in collaboration with a national sarcoma working group to evaluate biologic agents with chemotherapy for children and adults with recurrent MPNSTs. In addition to the above activities, the DOD/CTNFC is evaluating ancillary studies in neurocognitive assessment, the epidemiology of inherited and sporadic NF1, and therapies to prevent refracture in tibial dysplasia.

The DODCTNFC is functioning well and already has two approved studies (one accruing patients) and two other studies set to open within the next year. Accrual has been excellent, as has been compliance.

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Towards a treatment of the cognitive deficits associated with NF1

Ype Elgersma  
Erasmus University, The Netherlands

Last year, we completed a trial to determine the effect of Simvastatin on neuropsychological neurophysiological and neuroradiological outcome measures in children with Neurofibromatosis type 1. 62 children with Neurofibromatosis type 1 participated in a randomized, double-blind, placebo-controlled trial. We did not find significant improvement of Simvastatin treated children as compared to the placebo treated group for the primary outcome measures. Regarding the secondary outcome measures, we found a significant improvement in only one test. In this presentation I will give an overview of this trial, and discuss what we have learned and how we should proceed.

Full Author List: Lianne Krab, Arja De Goede-Bolder, Femke Aarsen, Saskia Pluijim, Marlies Bouman, Jos. van der Geest, Maarten Lequin, Coriene Catsman-Berrevoets, Willem Frans Arts, Steven Kushner, Alcino Silva (UCLA), Chris De Zeeuw, Henriëtte Moll, Ype Elgersma. All authors are from the Erasmus University Medical Center, Rotterdam, The Netherlands, unless otherwise indicated.

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NF1 optic pathway glioma trials

Nicole Ullrich  
Children’s Hospital Boston

One of the most frequently observed tumors in patients with NF1 is the optic pathway glioma. Optic pathway tumors account for 2-5% of all brain tumors in childhood. The incidence of optic pathway glioma in children with NF1 is approximately 15%; however, the incidence of symptomatic optic pathway gliomas is closer to 3-5% with a median age of onset of 4.9 years. While these tumors generally behave in a benign fashion and may not demonstrate progression on imaging, clinical course can be unpredictable and local expansion may result in progressive loss of visual acuity and visual function. Questions remain regarding the criteria that constitute radiographic and clinical progression. In addition, the best methods of visual and imaging monitoring of both asymptomatic and symptomatic OPGs in children with NF1 has not been clearly determined or uniformly accepted.

Therapeutic interventions for optic pathway tumors include surgery, radiation, chemotherapy, or a combination therein. Surgical enervation of a blind eye is an option to prevent further extension of the tumor into the optic chiasm; however, surgery is rarely undertaken for these unresactable tumors. Radiation
therapy has been shown to halt further tumor progression and/or result in durable tumor shrinkage; however, there are increasing concerns regarding the late effects of radiation, such as neuroendocrine and neurocognitive sequelae as well as the development of vasculopathy of the moyamoya type in this population, particularly in children treated at a young age. In addition, there are concerns of secondary tumorigenesis and malignant transformation.

There is increasing data that demonstrates the effectiveness of chemotherapy in the treatment of progressive low grade glioma, and chemotherapy is now the standard initial treatment approach for children with NF1 and signs of progression, based on either radiographic progression or loss of vision. The combination of vincristine and carbotaxplatin has been utilized in children with progressive optic pathway gliomas with a documented decreased in tumor bulk in many patients. However, this regimen rarely results in recovery of vision and many patients continue to exhibit progressive visual deterioration.

In the NF1 population in particular, with concurrent risks of radiation and alkylator-based therapy, no standard salvage chemotherapy regimen has been identified for progressive optic pathway glioma. The NF Clinical Trials Consortium is planning a phase II study to evaluate the response of children with NF1 and chemotherapy-refractory progressive symptomatic low grade gliomas, including OPGs, to everolimus, a suppressor of the mTOR pathway.

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Trials for NF1 plexiform neurofibromas and MPNSTs

Brigitte Widemann
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No abstract submitted

Development of Therapies for Cutaneous T-Cell Lymphoma: Pipeline for Dermal Neurofibromas?

Madeleine Duvic
University of Texas M.D. Anderson Cancer Center

Mycosis fungoides (MF) and Sézary Syndrome (SS), the erythrodermic, leukemic variant, are the most commonly encountered cutaneous T-cell lymphomas (CTCL) with an incidence of only 0.45/100,000 persons. Psoriasis (Ps) is similar to MF - epidermal hyperplasia is induced by a T-cell immune response affecting 2% of the population. Both MF and Ps have been the focus of intense drug discovery with clinical trials leading to FDA approval of multiple new targeted agents in the last decade. Development of clinical assessment tools has facilitated this process. Novel retinoids with receptor selectivity include tazarotene (RAR beta/gamma selective) for psoriasis, beoxretone (RXR selective) for CTCL, and allitretin (pan-retinoid for Kaposi’s). Topical retinoids are safe and effective in blocking epidermal proliferation but they induce collagen synthesis. Anti-retinoids (agonists) block other retinoids and might block collagen synthesis in neurofibromas, and steroids are notorious in their ability to block collagen causing atrophy. Biological response modulators targeting 1) co-stimulatory molecules (Raptiva, Amivive), 2) TNF-alpha/receptor (Embrel, Remicaide, Humara), and key cytokines (IL-23 antibody) have increased response rates in psoriasis. Ontak (denileukin difftox) was approved in 1997 as the first targeted fusion protein. Like a stealth bomber, this fusion toxin binds to the IL-2 receptor killing activated T cells, and is active in both MF and psoriasis. Any ligand can be incorporated into a recombinant fusion toxin to kill cells bearing its receptor on their surface with obvious selectivity issues. Surface proteins CD2, CD4, CD30, CD52, CCR4 have also been targeted by therapeutic antibodies in MF/SS. Small molecule inhibitors designed to inhibit key enzymes, especially tyrosine kinases (i.e., gleevac) or receptors like EGF-R (i.e., Tarceva, Iressa) are expected to inhibit psoriasis as well as carcinomas. Small molecule histone deacetylase inhibitors (HDAC-i) have broad anti-cancer activities inducing apoptosis, blocking cell proliferation and angiogenesis and might be active in NF tumors. Development of agents to block neurofibroma growth or formation could evolve from an increased understanding of mast cell/Schwann cell/fibroblast interactions that lead to TGF-beta induced collagen synthesis. Thus, mast cell inhibitors, TGF-beta anti-sense, TGF-fusion proteins or antibodies and anti-retinoids or high potency steroids would be expected to block collagen synthesis underlying NF. Another target, the Ras-b-abl pathway, is also attractive for development of small molecule inhibitors for NF patients.

NF1 Dermal Neurofibromas – Ongoing Trials

Scott Plotkin
Harvard Medical School/Massachusetts General Hospital

There is wide variation in cutaneous neurofibroma burden among patients with neurofibromatosis 1 (NF1). More than half of subjects older than thirty have greater than 100 cutaneous tumors. Although physical pain or bleeding may occur in some tumors when they are irritated, cosmetic disfigurement is the main source of morbidity related to cutaneous tumors and often leads to significant psychological distress. Immunohistochemical data suggest that blockade of VEGF signaling is likely to be beneficial for treatment of these tumors. Ranibizumab is a high affinity Fab that neutralizes all forms of VEGF-A and is approved for treatment of age-related macular degeneration. To date, we have treated seven NF1 patients with four or more cutaneous neurofibromas ≥ 5 mm in size by injecting ranibizumab 0.5 mg into 3 tumors on day 1 and by injecting saline solution into one additional tumor on day 1. Tumors treated with ranibizumab were removed on days 8, 15, and 29; tumors treated with saline were removed on day 29. Tumor response was monitored by calipers; the primary endpoint was the change in tumor volume in treated tumors. All subjects have tolerated treatment with ranibizumab with only occasional episodes of mild discomfort (grade 1). Efficacy data is not yet available. These initial results show that patients with NF1 are willing to participate in clinical
NF2 clinical trials: an overview

Jaishri Blakeley
Johns Hopkins University

There has been a great deal of progress in clinical trial development for patients with Neurofibromatosis Type II (NF2). Increasing understanding of the pathophysiology NF2-related tumors has led to recognition of the potential use of FDA approved agents for NF2 patients. One promising class of agents is small molecule tyrosine kinase inhibitors (TKI) of epidermal growth factor receptor (EGFR). EGFR is overactive in many malignancies and has been implicated in the growth of Merlin deficient cells (Curto et al, 2007; Cole et al, 2008). There are 5 FDA approved agents that inhibit EGFR and its family of receptors. The challenge is choosing which agent to study, in which patients and against what tumors. One major consideration is determining what agent reaches therapeutic concentrations in the tumor. A recently launched trial assesses delivery of lapatinib to VS in patients with NF2 related and sporadic VS. Patients who are planned for VS resection take the oral EGFR/ErbB2 inhibitor lapatinib for 10 days prior to surgery. Resected tissue is analyzed to assess: 1) the concentration of lapatinib in VS at steady state, 2) the effect of lapatinib exposure in vivo, and 3) the differences in drug concentration and tumor response in sporadic versus NF2-related VS.

Another promising class of agents is antiangiogenesis inhibitors. There are at least 10 agents FDA approved for various cancers that could be applied to NF2 related tumors. Early translational data suggests that vascular endothelial growth factor (VEGF) is expressed on both sporadic and NF2-related VS and clinical experience suggests that VEGF inhibitors may have efficacy in patients with progressive VS (DiTomaso et al, ASCO, 2007; Plotkin et al, SNO, 2008). A clinical trial with PTC-299 (decreases circulating VEGF) is set to open in the summer of 2009. The goals of the trial are to determine if PTC-299 decreases the size of tumor or improves hearing function. Small molecular TKI that target multiple angiogenic growth factors are also entering investigation. Plans are underway for a randomized trial of sorafenib in patients with NF2 in the United Kingdom. Sorafenib is a TKI of several angiogenesis related receptors that has shown activity in tumors that have a high frequency of NF2 mutation.

The pace for developing clinical trials for NF2 has accelerated dramatically. Where a year ago there were no trials specifically designed for NF2 patients, within the next year there will be 3; all with promise of identifying effective therapies for patients with NF2 in the near future.

Jaishri Blakeley is currently a Children’s Tumor Foundation Clinical Trial Award Recipient.

Phase 2 Study of PTC299 in NF2

Harry Miao
PTC Therapeutics

Background: NF2 is a serious, progressive, disabling, and potentially life threatening disorder with few adequate treatment options. Angiogenesis plays an important role in NF2-associated vestibular schwannoma (VS) progression. Preliminary clinical data indicate that anti-angiogenesis may be an effective therapeutic strategy in NF2. PTC Therapeutics has developed the novel oral drug, PTC299, to suppress tumor growth by selective post transcriptional inhibition of vascular endothelial growth factor (VEGF) production. PTC299 potently inhibits angiogenesis and tumor growth in multiple preclinical tumor models; efficiently crosses the blood brain barrier; and has been generally well tolerated in safety pharmacology and toxicology studies as well as Phase 1 clinical studies in healthy volunteers and ongoing Phase 1/2 trials in patients with cancer.

Methods: Participants will include patients with NF2 who are not good surgical candidates and have progressive VS growth or progressive hearing loss related to VS. Participants will receive 100 mg of PTC299 BID for up to 48 weeks. The primary endpoint will be response rate, defined as the proportion of patients with a ≥20% reduction in tumor volume as assessed by magnetic resonance imaging (MRI), and/or an increase in percent word recognition that meets predefined 95% critical difference criteria. Secondary efficacy end points are latency of wave V on brainstem auditory evoked responses, and otoacoustic emissions; tinnitus as assessed by questionnaires; tumor perfusion as assessed by dynamic contrast enhanced MRI (DCE-MRI); and concentrations of circulating VEGF and other angiogenic proteins. Pharmacokinetics, safety, and compliance will also be evaluated. A two-stage design will be used, with 11 participants in Stage 1 and 14 participants in Stage 2. The 25-patient sample size is intended to differentiate a null-hypothesis response rate of ≤5% against an alternative-hypothesis response rate of ≥25%.

Results: Participant enrollment is projected to initiate in Q2009. Available information on the pharmacology of PTC299 and baseline data from the trial will be presented.

Conclusion: Positive PTC299 effects on NF2 tumor size and tumor-related symptoms would support the importance of angiogenesis in NF2 growth and offer the potential for development of PTC299 as a new treatment option for patients with NF2.

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Acknowledgements: Funding provided by CDMRP NFRP
The Children’s Tumor Foundation NF Preclinical Consortium (NFPC): an Update

Mila McCurrach
NFPC Project Manager

Consortium Mission: The Children’s Tumor Foundation Neurofibromatosis Preclinical Consortium (NFPC), part of a $5M investment of the Foundation to accelerate NF research to the clinic, was established in 2007 and will be supported by the Foundation through to 2011. NFPC is a collaborative group of six research centers, each using animal models (xenograft and genetically engineered) and cell lines of different tumors of NF1 and NF2. The Centers of the Consortium have entered into a fully collaborative and sharing relationship with the common goal of identifying and advancing the most promising candidate NF therapies through preclinical testing in an expedited manner so that these drugs might then advance to clinical trials. The interactive structure of the Consortium is intended to permit a drug to be tested in multiple NF tumor models at the same time, so that data to be compared across as many models as possible to fully evaluate the drug’s potential as an NF tumor therapy. Where possible, the 6 Centers of the Consortium will screen each drug. The NFPC’s first commercial collaboration is with Novartis.

Centers of the NF Preclinical Consortium
• University of California, San Francisco - Director: Kevin Shannon; Focus: NF1 myeloid leukemia
• Washington University School of Medicine - Director: David Gutmann; Focus: NF1 optic pathway glioma
• Cincinnati Children’s Hospital Medical Center - Director: Timothy Cripe; Focus: NF1 plexiform neurofibromas
• House Ear Institute - Director: Marco Giovannini; Focus: NF2 schwannoma, meningioma
• Harvard Medical School/Massachusetts General Hospital - Director: Andrea McClatchey; Focus: NF2 schwannoma
• Harvard Medical School/Brigham & Women’s Hospital: Director: Karen Cichowski, Ph.D. Focus: NF1 malignant peripheral nerve sheath tumors

Oversight Committee of the NF Preclinical Consortium: To oversee NFPC the Children’s Tumor Foundation has appointed a team that includes seasoned industry professionals, NF researchers and physicians with the collective experience of advancing therapeutics through preclinical drug screening and into the clinic and marketplace.
• Jay Gibbs (Chair), AstraZeneca Pharmaceuticals
• Gideon E. Bollag, Plexxikon Inc.
• Sara A. Courtneidge, The Burnham Inst. for Medical Research
• Larry Gelbert, Eli Lilly and Company
• Filippo G. Giancotti, Memorial Sloan-Kettering Cancer Center New York
• Janet M Hock, Maine Institute for Human Genetics and Health
• Christopher L. Moertel, Children’s Hospitals and Clinics of Minnesota
• Michael J. Morin, Oncovia, Inc.
• Roger J. Packer, Children’s National Medical Center
• Edward Stern, Esq., Legal Counsel to the CTF Medical Advisory Committee

Initiation of a Neurofibromatosis BioBank and Patient Registry

Mindell Seidlin
Consultant, Children’s Tumor Foundation

It has been recognized for some time that the availability of well annotated tissue specimens from patients with NF would be an important resource for furthering fundamental understanding of these disorders and advancing towards novel therapeutic interventions. In recent years, tissue bank initiatives have been undertaken with foundation support for a variety of other genetic and rare disorders. The experiences of these other foundations can serve to accelerate and guide development of an NF BioBank.

Many of the challenges associated with recruiting patients through individual investigators can be overcome by giving patients or parents the option of directly registering for participation in a registry and bank. Later, when tissue becomes available, mechanisms for obtaining, transporting, storing and distributing the tissues can be implemented. Such a resource would not only improve access to clinical samples for NF researchers, but also empower NF patients with the ability to participate directly in research and be guaranteed that their donated tissue be effectively utilized. To maximize value, it is envisioned that a BioBank will be partnered with a Patient Registry.

In May 2009, the Children’s Tumor Foundation convened a small working group to share ideas on how an NF BioBank might be structured and utilized. Attendees included experts from NF clinical care and research, information technology and data management, bioethics, established biobanking services and patient representatives.

Dr. Seidlin will share some initial ideas drawn from this workshop on how an NF BioBank might be structured. Following the NF Conference, meeting attendees will be invited to provide feedback on BioBank planning via an online survey.

Link to survey: https://www.surveymonkey.com/s.aspx?sm=hIMzk6Ya17yd4wgt1DqYtg_3d_3d

Co-author: Ian Atkinson
Defining the NF1 phenotype

Bruce Korf
University of Alabama at Birmingham

Neurofibromatosis 1 is well known for its wide range of phenotypic variability. This variability poses significant challenges, both in defining endpoints for clinical trials and in establishing genotype-phenotype correlations. Phenotypic variability has been attributed to many factors, including allelic heterogeneity, modifying genes, environmental exposures, age, parity, and stochastic factors. To the extent possible, use of quantitative measures can provide a rigorous and reproducible approach to NF1 phenotyping, and has been useful both in clinical trials and genetic studies. We have applied a quantitative approach to definition of the rate of growth of neurofibromas, as well as to café-au-lait macules. Plexiform neurofibromas take many morphological forms, and are best quantified using volumetric MRI. Volumetric approaches now permit accurate measurement of the rate of tumor growth and are being used in clinical trials. Neurofibromas on the skin are easier to visualize, yet, ironically, more difficult to quantify. A simple classification scheme divides these into dermal, intradermal, and subdermal discrete neurofibromas. We are now using a combination of photography and laser scanning to quantify the volume of dermal tumors to follow their rate of growth. Café-au-lait spots differ in size, morphology, and degree of pigmentation; the latter can be quantified using reflectance photometry, and reveals pigmentary heterogeneity among café-au-lait spots within an individual that is independent of sun exposure. A limited number of genotype-phenotype correlations are beginning to emerge. This includes well-recognized phenotypes associated with large NF1 gene deletions, mosaicism, and a three base deletion in exon 17 associated with lack of dermal tumors. Another distinct phenotype characterized by a paucity of dermal tumors but an extreme burden of internal tumors is also emerging. Further definition of these subphenotypes and dissection of molecular correlates will benefit from standardized and quantitative approaches to phenotyping and development of a database for collection and annotation of phenotypic and genotypic heterogeneity.

NF1 in old age: International Interdisciplinary Analysis of the Issues

John Mulvihill
University of Oklahoma

The clinical picture of NF1 in childhood and adolescence is well described. The issues of NF1 in old age are less clear. There are case reports suggesting the medical problems, but there is no specific information to guide clinicians in the care of these patients. Mortality studies of NF1 indicate that the more severe complications of neurofibromatosis such as malignant peripheral nerve sheath tumors and pheochromocytomas tend to occur before the age of 50. The CDC death certificate study (Rasmussen SA, Yang Q, Friedman JM, Am J Hum Genet 2001; 68:1110) showed that about 1/3 of people with NF1 die before age 45.

The objective of this study is to define the issues that individuals with NF1 have in old age. The expected outcome is that people with NF1 who survive to age 50 have a milder disease on average than young NF1 patients, but there may be exceptions. There are three specific aims: (1) Quantify and compare the mortalities and co-morbidities of NF1 in persons over and under age 50 years in the US, Canada, and Denmark; (2) Develop a clinical profile of NF1 after age 50 years, through analyses of special clinical cohorts; (3) Conduct focus groups and pilot quality of life studies to identify additional issues for formal investigation and make preliminary management recommendations

The strategy is to analyze existing databases and conduct focus groups and preliminary clinical studies in three centers in three countries with experienced NF1 investigators to obtain a multifaceted view of issues of aging in NF1. In a summary this project could set a model for geriatric genetics.

Results: Interim results are in hand for a new analysis of US death certificates with NF1 as a cause or a comorbidity in death; for focus groups, for mortality in the Danish cohort from 1944, and from the International NF Registry.

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Genetic modification of NF1 phenotypes

Andre Bernards
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Drosophila NF1 null mutants exhibit a learning defect and a 20% reduction in post-embryonic growth. Both defects are suppressed by increasing signaling through the cAMP-dependent protein kinase A (PKA) pathway. The size defect is also – less readily – suppressed by decreasing Ras signaling strength. However, multiple lines of evidence have led us to conclude that excess Ras signaling in larval neurons is directly responsible for the size defect, and that any role for cAMP/PKA is secondary. Others have disagreed with this conclusion and argued that NF1 has distinct Ras and cAMP-related functions. To further define mechanisms responsible for NF1 defects, we have conducted genetic modifier screens. The most extensively analyzed confirmed suppressor
of the NF1 size and learning defects encodes a neuronal receptor tyrosine kinase. Importantly, overexpression of this kinase phenocopies both size and learning defects, arguing that a common signaling defect is responsible. Other yet to be confirmed size defect suppressors include another neuronal receptor tyrosine kinase, as well as an adenylyl cyclase coupled neuropeptide receptor.

Among the few recognized phenotype-genotype correlations in human NF1 is that microdeletion patients often suffer from severe defects, including high numbers of early onset neurofibromas. We have been interested in modifiers of NF1 tumor burden and have performed preliminary analysis of two conserved microRNA genes located within the microdeletion region, approximately 200 kb downstream of NF1. We have identified targets for both miRNAs, and analyzed effects of manipulating their expression in human glioma cells. Results of both projects will be presented.

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Whole body MRI evaluation in NF1, NF2, and Schwannomatosis

Scott Plotkin
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Introduction: Neurofibromatosis 1 (NF1), NF2, and schwannomatosis are a group of related genetic disorders in which affected individuals share the predisposition to develop multiple neurofibromas and schwannomas. The prevalence of internal tumors is not known because current estimates are based on regional MRI scans that may not detect occult tumors. A rapid and sensitive method to detect internal tumors is highly desirable since they can cause neurologic dysfunction, compress vital structures, or transform into malignant tumors. Whole-body MRI (WBMRI) is an imaging technique by which the entire body can be imaged in a relatively short time without the use of ionizing radiation. Methods: We performed WBMRI in subjects with NF1, NF2, or schwannomatosis as part of an IRB-approved research study. Each subject was imaged from head to ankles in the supine position using a 1.5 Tesla magnet, integrated body coil, and no intravenous contrast. Using five acquisitions, the entire body was imaged using a fat suppressed fluid sensitive STIR sequence. The images were then fused into a single whole body DICOM image. The number and type of tumors (discrete vs plexiform) were identified by a board-certified radiologist and tumor volume was calculated using semi-automated analysis. Results: A total of 143 subjects were imaged (NF1 spectrum—77; NF2 spectrum—30, schwannomatosis—28; schwannomatosis at risk—8). The median age was 40 years (range, 18 to 97 years). A total of 920 tumors were identified in 83 subjects (60%) who had at least one internal tumor. The median number of tumors in affected individuals was 5 (range, 1 to 63 tumors). Overall, the legs harbored the greatest number of tumors (30%), followed by the thorax (19%), pelvis (17%), abdomen (12%), arms (8%), head/neck (8%), and other locations (4%). Forty-seven percent of internal tumors were classified as plexiform yet these tumors contributed 83% of the tumor burden by volume. Conclusions: WBMRI scan is a powerful tool to evaluate the number, size, and distribution of internal tumors in patients with neurofibromatosis. This technique provides unique phenotypic information for genetic studies on NF1, NF2, and schwannomatosis. A total of 250 subjects will be enrolled in this international prospective observational study.

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Clinical, pathological and molecular variables predictive of MPNST outcome

Ian McCutcheon
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Objective: Improved staging systems for malignant peripheral nerve sheath tumor (MPNST) prognostication and management are needed. Consequently, we sought to identify clinical, pathologic and molecular predictors of outcome in patients with/without neurofibromatosis type 1 (NF-1)-associated MPNST treated at UTMDACC

Methods: MPNST patients treated from 1986 to 2006 (n=140) were identified; 72 had NF-1 syndrome and 68 did not. A comprehensive database was constructed. Formalin-fixed paraffin-embedded neurofibroma or MPNST blocks were assembled in a tissue microarray; expression of molecular markers was evaluated immunohistochemically. Univariable and multivariable analyses identified independent factors prognostic of local recurrence, distant metastasis and disease specific survival (DSS).

Results: DSS at 10 years was 31.6% for 87 primary disease patients, 25.9% for 26 recurrent patients, and 7.5% for 27 metastatic patients after median follow up of 91 months. The five-year DSS for localized tumor patients was 35% for NF-1 syndrome patients and 50% for sporadic patients. MPNST ≥10 cm at diagnosis, partial resection, and metastasis development were significant negative predictors of DSS; localized tumors that lacked S-100 immunoreactivity had a five-fold increased risk of developing distant metastasis. Ki67, VEGF, p53, EGFR, and pMEK were all over-expressed in MPNST compared to benign neurofibromas. Expression of EGFR and nuclear p53 were associated with significantly worse disease-specific MPNST survival.

Conclusions: MPNST is a markedly metastatic and aggressive poor prognosis tumor. Multiple clinical, pathological, and molecular markers identified in this study, coupled with findings from previous series, should be considered for an improved MPNST staging system useful for prognostic assessment and management decisions.

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Photodynamic therapy in children with NF1 and plexiform neurofibromas

Michael Fisher
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Plexiform neurofibromas (PN) are common tumors that cause serious morbidity in patients with neurofibromatosis type 1 by severe disfigurement, compression of vital structures, or malignant transformation. Complete surgical excision is the only known effective therapy; however, this is rarely possible due to local infiltration of normal tissue. Radiotherapy and chemotherapy have not shown efficacy in PN. Newer approaches, including anti-angiogenesis agents, farnesyl transferase inhibitors, and inhibitors of growth factor pathways are in early development. These predominantly non-cytotoxic strategies may slow or inhibit PN growth; however, they are not likely to result in significant volume reduction of large PN. Photodynamic Therapy (PDT) is a locoregional tumor treatment in which a systemically administered photosensitizer (e.g. LS11) is activated locally by illuminating the diseased tissue with light of a specific wavelength. Light activation of LS11 leads to the formation of reactive oxygen species that cause damage primarily to the vascular endothelial cells leading to vascular thrombosis, occlusion, and death of tumor cells. A recently, developed novel approach to PDT uses disposable, implantable (by interventional radiology) LED light sources (Litx™). Preclinical and clinical studies in adults with refractory solid tumors indicate that this strategy results in a high rate of tumor volume reduction and is well tolerated by patients. However, the safety and tolerability of PDT using the LitxTM system in children has not been established.

We recently opened a phase I study to determine the maximal tolerated dose (MTD) of light combined with LS11 using the Litx™ system for PDT treatment of PN in children. The treatment is completed within one day and patients are monitored for toxicity and treatment response during the 12 week study period. Secondary objectives include response evaluation using anatomic MRI, dynamic contrast enhanced MRI, and quality of life questionnaires.

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Velopharyngeal insufficiency in NF 1- A clinical sign of underestimated clinical significance?

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Speech abnormalities including difficulties with articulation are reported in at least 25% of children with NF1. Velopharyngeal insufficiency (VPI) has previously been described in a small number of patients with NF1, but is not a well known complication of this condition. We have noted VPI in a series of patients with NF1. Amongst these patients, VPI has caused significant impairment in speech intelligibility and, in some cases, required surgical intervention.
Vascular endothelial cell dysfunction in NF1

Kimberly Jett
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Vasculopathy is one of the most serious, non-neurocutaneous manifestations of NF1. NF1 vasculopathy is usually asymptomatic, and the first clinical manifestation may be a life-threatening or fatal event. The pathogenesis of NF1 vasculopathy is not clearly understood but is thought to result from haploinsufficiency of neurofibromin in vascular smooth muscle cells, endothelial cells and bone marrow derived inflammatory cells. Recent preliminary data in genetically engineered mice indicated that Nf1+/− bone marrow derived macrophages directly contribute to vasocclusive disease and that Nf1+/− mice have evidence of vascular inflammation (Lasater abstract). Based on these observations, we used flow-mediated vasodilation (FMD) and glyceryl-trinitrate-mediated dilation (NMD) to assess endothelial and smooth muscle cell function. Further, poly-chromatic flow cytometry (PFC) analysis was used to test for evidence of vascular inflammation from the peripheral blood of NF1 patients and age/sex matched controls. In this study, we analyzed eight NF1 patients. Three individuals had known NF1 vasculopathy, one had hypertension, and four had no history of cardiovascular disease. All eight patients had low FMD, indicative of dysfunction of the vascular endothelium. In contrast, NMD, a test of vascular smooth muscle function, was normal in all of the patients studied except one with known atherosclerotic vascular disease as well as NF1 vasculopathy.

Novel application of PFC has been used to identify distinct populations of monocytes in peripheral blood that have been linked to vascular disease in other patient populations, with lesions similar to those observed in NF1 patients1. Pro-inflammatory monocytes have been linked to inflammatory cytokine production and increased endothelial transmigration of macrophages in other studies of vascular disease2. Interestingly, PFC analysis of peripheral blood from NF1 patients demonstrated a dramatically increased population of pro-inflammatory CD16++CD14+ monocytes that is not observed in healthy, aged matched controls. In this study, FMD and NMD data demonstrate that vascular endothelial function is altered in NF1 patients. Further, we show for the first time that NF1 patients have evidence of chronic inflammation that could lead to vascular inflammation and subsequent vascular disease.

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Digital image archiving and analysis for patients with NF1 and NF2

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Magnetic resonance imaging (MRI) is commonly used to evaluate vestibular schwannoma (VS) in patients with NF2, and plexiform neurofibroma (PN) in patients with NF1. In making treatment decisions for clinical care or clinical trials, it is important to accurately determine whether a lesion is growing, and if so, at what rate. Unfortunately, the current state of clinical practice does not typically include longitudinal volumetric measurements for accurate lesion growth tracking. In addition, many NF patients receive care at multiple different specialty centers, complicating comparisons because of differing protocols and the unavailability of prior comparison images from remote institutions. To address the clinical and research need for longitudinal tumor imaging analyses, we developed a centralized service to receive MRI scans electronically, perform volumetric analyses, and report resulting images, measurements, and graphs. We integrated a secure image transfer system for electronic transmission of scans to our analysis center, either from images stored on CD, loaded and transferred from a desktop PC, or transferred from the local PACS system through our portal. We developed a secure, Web-based reporting system for longitudinal tracking of these NF tumor metrics, viewable remotely by referring physicians and study staff. We implemented this digital image archiving, analysis, and reporting system locally, and are piloting it with select outside hospitals. To date, we have entered imaging data from 17 NF1 and 75 NF2 patients, as well as 13 patients with sporadic VS. We have received and processed volumetric analyses for scans from clinics at 10 US hospitals and one foreign institute. We are interested in expanding this service to provide access for NF patients and their care providers at many clinics to quantitative, reproducible volumetric data for scans from remote hospitals and imaging centers. The ultimate goal of this service is to improve care for NF patients by providing reliable longitudinal measurements of tumor growth.

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Cell of Origin and Molecular Mechanisms of Tumor Initiation in a Mouse Model of Malignant Astrocytoma
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Malignant astrocytomas are infiltrative and incurable brain tumors. NF1 patients, in particular, have greatly increased risk of developing these tumors compared to the general population. Despite profound therapeutic implications, the identity of the cell (or cells) of origin has not been rigorously determined. We previously reported mouse models based on conditional inactivation of the human astrocytoma-relevant tumor suppressors Nf1, p53, and Pten, wherein through somatic loss of heterozygosity, mutant mice develop tumors with 100% penetrance. In the present study, we show that tumor suppressor inactivation in neural stem and progenitor cells is both necessary and sufficient to induce astrocytoma formation. We demonstrate in vivo that transformed cells and their progeny undergo infiltration and multlineage differentiation during tumorigenesis. We also show evidence that early on during astrocytoma development, tumor suppressor heterozygous neural stem/progenitor cultures from pre-symptomatic mice show aberrant growth advantage and altered differentiation, thus identifying a pretumorigenic cell population. Gene expression profiling and analysis of cell cycle regulation during early stages of tumorigenesis provide insight into the molecular events involved during brain tumor initiation.

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Sheila Llaguno Alcantara is currently a Children’s Tumor Foundation Young Investigator Award Recipient

Tumor initiating cells in malignant peripheral nerve sheath tumors
Johanna Buchstaller
University of Michigan

About 10% of patients with neurofibromatosis develop malignant peripheral nerve sheath tumors (MPNSTs), very aggressive and invasive soft tissue sarcomas, which are often refractory to chemo-and radiotherapy. We have shown that plexiform neurofibromas and MPNSTs appear to arise from differentiated glia (Cancer Cell 13:129). This project’s objective is to determine if the progression of MPNSTs follows a cancer stem cell model, in which only a minority of cancer cells are capable of extensive proliferation or the formation of new tumors. To test this we are using mouse models for MPNSTs bearing mutations in Nf1, Ink4Arf and p53, genes that are also affected in human patients. By performing limit dilution transplantation assays, we find that a high number of single primary cells from these tumors form tumorigenic spheres. These results suggest that growth of MPNSTs in this mouse model does not follow a cancer stem cell model and that most tumors cells are capable of extensive proliferation. Thus, all cancer cells need to be targeted during tumor therapy in MPNSTs bearing these mutations.

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The natural product Schweinfurthin inhibits growth of multiple NF1-associated tumors and acts through an NF1-dependent pathway affecting Rho signaling and cytoskeletal alteration
Karlyne M. Reilly
National Cancer Institute-Frederick

Although most therapeutic approaches to NF1 have focused on the Ras signaling pathway and the MAPK and AKT downstream signaling pathways, there is preliminary evidence for involvement of neurofibromin in Rho signaling. We are investigating a candidate therapeutic, Schweinfurthin, extracted from a tree native to Cameroon, Africa, that we have shown has activity against brain tumor lines in the NCI60 cell panel. We find that treatment of cells with Schweinfurthin blocks the Rho signaling pathway and alters the actin cytoskeleton. Wild-type and Nf1-/-;p53-/-;cis primary astrocytes are resistant to the
effects of Schweinfurthin and reintroduction of the rasGAP domain of neurofibromin into astrocytoma tumor cells renders them resistant to Schweinfurthin. This effect is not seen upon reintroduction of p53, suggesting that activity of Schweinfurthin may depend on homozygous loss of Nf1. Interestingly, the lung tumor cell line A549 is resistant to Schweinfurthin and carries an activating mutation in K-ras, suggesting that the role of Nf1 loss in Schweinfurthin sensitivity is not merely through activation of ras signaling. To determine whether the activity of Schweinfurthin is specific to brain tumors, we tested the sensitivity of mouse malignant peripheral nerve sheath tumor to Schweinfurthin. We find similar results in peripheral nerve sheath tumors as seen in astrocytoma cells, further supporting the importance of neurofibromin in the pathway targeted by Schweinfurthin. This data further raises the possibility that inhibitors of Rho signaling may show promise in the treatment of Nf1.

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**Differential impact of neurofibromatosis-1 (Nf1) loss on neural stem cells from different brain regions**

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Cancer stem cells (CSCs) make up a small population (<5%) in solid tumors including glioma and ependymomas in the brain. Recent studies have suggested that CSCs are the cells in these brain tumors with the greatest capacities to growth and regenerate the original tumor. In addition, CSCs share some biological and biochemical properties with normal neural stem cells (NSCs) in the brain. In this regard, several studies have indicated that CSCs may arise from genetically-transformed normal NSCs. Previous work from our laboratory demonstrated that pilocytic astrocytomas from different regions of the brain have different patterns of gene expression patterns. This observation suggests that progenitor cells from different brain regions might have unique properties relative to glioma formation. In this study, we sought to determine whether loss of neurofibromin expression affects NSC renewal, proliferation, and multi-lineage differentiation in region-specific manner. To generate Nf1-deficient NSC cultures, we isolated NSCs from the neocortex and brainstem of postnatal day (PN) 1 Nf1floxed mice, and inactivated Nf1 gene expression by adenoviral Cre-mediated excision. We then performed proliferation and self-renewal assays as well as examined the ability of these NSCs to give rise to astrocytes, neurons, and oligodendrocytes. To examine the region-specific effect of Nf1 gene inactivation in brain lipid binding protein neural precursor cells, we took advantage of a recently-generated mouse strain (Nf1floxed; BLBP-Cre conditional knockout mouse) in which neurofibromin expression is abrogated at E9.5 in neural stem/progenitor cells. The number of BLBP- and Olig2-positive precursor cells and GFAP-positive astrocytes were quantitated in the neocortex and brainstem of PN8 and PN18 mice. We found that NSC proliferation is differently affected by Nf1 gene deficiency in a region-specific fashion in vivo and in vitro.

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*Da Yong Lee is currently a Children’s Tumor Foundation Young Investigator Award Recipient*

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**Tumor-derived Merlin mutations abrogate binding to a novel Cul4 E3 ubiquitin ligase in the nucleus**

Jonathan Cooper  
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Contact-mediated inhibition of cell proliferation is critical for proper tissue morphogenesis, wound healing, and other repair processes. Current models suggest that the FERM domain protein Merlin, encoded by the tumor suppressor NF2, inhibits mitogenic signaling at or near the plasma membrane. However, the biochemical function of Merlin is poorly understood and further insight into Merlin’s function would be beneficial in understanding its role in contact-mediated inhibition. We have discovered that the closed, growth inhibitory form of Merlin accumulates in the nucleus and binds to the receptor component of a novel Cul4 E3 ubiquitin ligase. Biochemical and functional studies have indicated that Merlin’s binding suppresses the ubiquitin conjugating activity of the ligase, placing Merlin upstream of the ligase. Several tumor-derived mutants of Merlin, including missense mutants mapping to the surface of the FERM domain, display reduced binding to the ligase. Several tumor-derived mutations are predicted to disrupt folding of two subdomains of the FERM domain. Moreover, these tumor-derived mutants of Merlin fail to interact in vitro with the ligase. Combined with data suggesting an inability of several tumor-derived mutations to be defective in nuclear translocation, it has become evident that pathogenic Merlin mutations inhibit the ability of Merlin to translocate into the nucleus, interact with the ligase, or a combination of the two. These findings support the idea that Merlin suppresses tumorigenesis by translocating to the nucleus to inhibit the Cul4 E3 ubiquitin ligase-dependent gene expression.

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Functional relevance of the merlin/RasGAP interaction

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Merlin/NF2 is the responsible tumour suppressor in both familial and sporadic Neurofibromatosis type 2 (NF2) disease. Merlin belongs to the ERM (ezrin, radixin and moesin) family of proteins, which link membrane proteins to the cortical actin cytoskeleton. Our lab has previously shown that merlin mediates contact inhibition of growth by inhibiting the activation of both Ras and Rac. In addition, ongoing work in our lab identified that ezrin (or the ezrin-related proteins moesin and radixin) in association with F-actin recruits and activates the GEF (guanine nucleotide exchange factor) SOS to activate Ras. Merlin cannot bind SOS but counteracts ezrin by competing for the same binding sites at the plasma membrane. This led us to hypothesize that merlin inhibits Ras activation by antagonizing the SOS-ezrin complex from relevant sites of activity. If replacing ezrin were the only mechanism, N-terminal half molecules should suffice to replace ezrin from its binding site and exert full tumor suppressor activity. However, from co-transfection experiments with half molecules, N-terminal or C-terminal alone or in combination, we found that the C-terminus enhances tumor suppression. We have searched for this enhancing activity by co-immunoprecipitation. So far we have found that merlin can complex with RasGAP and RhoGAP, GTPase activating protein important for the down regulation of Ras activity. We are currently dissecting the functional relevance of these interactions providing further insight into merlin function and highlighting the importance of GAPs activity during contact inhibition of growth.

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**Clinical and mutational spectrum of the NF1-like syndrome (Legius syndrome)**

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Autosomal dominant inactivating SPRED1 mutations have recently been described in 5 families with a neurofibromatosis type 1-like syndrome (NFLS). The phenotype consists of café-au-lait-macules (CALM), axillary freckling and macrocephaly. The full clinical spectrum of this new disorder has not yet been investigated.

We performed SPRED1 mutation analysis in 1318 unrelated patients presenting with a broad range of signs typically found in neurofibromatosis type 1 (NF1) and in whom no NF1 mutation was present in peripheral blood lymphocytes. A comparison between clinical findings in patients with a SPRED1 mutation versus those with a NF1 mutation and those without known mutation was made. Functional assays were used to evaluate the pathogenicity of identified missense mutations.

We identified 34 different SPRED1 mutations in 43 probands: twenty seven were pathogenic (including 2 missense mutations) and 7 missense mutations were classified as probably benign. Forty eight percent of SPRED1-positive patients fulfilled NIH NF1 diagnostic criteria based on the presence of >5 CALM +/- freckling or a NF1-compatible family history. We estimate that 1.2-2.9% of individuals with the clinical diagnosis of NF1 have NFLS.

A high SPRED1 mutation detection rate was found in NF1 mutation-negative families with an autosomal dominant phenotype with CALM +/- freckling and no other NF1 features. The NF1 diagnostic criteria are not specific, since 48% of patients with NFLS fulfilled these criteria. NFLS is not associated with the high incidence of peripheral and central nervous system tumors seen in NF1. We suggest a less intensive medical follow-up program for patients with NFLS.

**SPRED1 mutations in Legius syndrome: another clinically useful genotype for dissecting the NF1 phenotype**

Meena Upadhyaya  
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Mutations of the SPRED1 gene, part of the family of Sprouty (Spry)/Spred proteins that ‘down-regulate’ mitogen-activated protein kinase (MAPK) signaling,
Cognition in mice and man with SPRED1 mutations

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SPRED1 is a negative regulator of RAF activation by RAS-GTP. Inactivating heterozygous mutations in SPRED1 are the cause of an autosomal dominant NF1-like syndrome (Legius syndrome). Some of the children with SPRED1 mutations reportedly have learning and/or speech difficulties. We investigated a group of 9 children with a SPRED1 mutation and compared IQ test scores with 7 siblings. Due to the small number of children no differences were significant. The mean performal IQ score in the SPRED1 group was 15 IQ points lower compared to the sibling group. The lowest scores were seen on the performal subtests. Twelve of the 13 subtests of the WISC showed lower scores in the SPRED1 group compared to the sibling group and this is significant (p = .001).

Analysis of cognition in Spred1 knockout mice showed a significantly slower learning in hippocampus dependent tests such as the hidden Morris Water Maze and the T-maze. These findings correlated with a decreased long term potentiation (LTP) in hippocampal slices and an increased long term depression in Spred1 knockout mice. Biochemical analysis of hippocampal slices after theta burst stimulation for LTP showed an increase in MEK and ERK phosphorylation and the T-maze. These findings correlated with a decreased long term potentiation (LTP) in hippocampal slices and an increased long term depression in Spred1 knockout mice. Biochemical analysis of hippocampal slices after theta burst stimulation for LTP showed an increase in MEK and ERK phosphorylation.

Cognitive defects are present in humans and mice with a SPRED1 mutation but are generally milder than in the NF1 group. The mechanism of the cognitive defects seems to be similar in SPRED1 and NF1.

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RASA1 (a RasGap) and CM-AVM (capillary malformation - arteriovenous malformation)

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Vascular malformations are localized errors of vascular development. They are often identified on the skin as “birthmarks” of various sizes and shapes. They usually grow slowly with the growth of the child. They may also be encountered in other organs, such as the liver, intestine and the brain. The lesions are consisted of tortuous vascular channels of various types, with continuous endothelium surrounded by various numbers of support cells. Most of these lesions occur sporadically, but also as part of a syndrome or as an inherited disorder.

During the past years, we have identified several genes mutated in inherited vascular malformations. In one study, using a genome wide linkage approach, we identified that several families with autosomal dominant capacity malformations were linked to 5q13. Detailed clinical phenotyping unravelled a newly recognised clinical entity, which we named CM-AVM for capillary malformation – arteriovenous malformation, as several patients, but not all, had fast-flow vascular anomalies in addition to the cutaneous capillary malformations. Subsequently, we discovered mutations in the RASA1 gene, encoding a RASGTPase called p120-RasGAP, a NF1 homologue. All mutations likely result in loss-of-function. More recent studies have unravelled that the clinical spectrum includes Parkes Weber syndrome and vein of Galen aneurysmal malformation. Moreover, some patients have specific neurologic tumors.

In 1994, when we mapped the 9p21 locus for inherited cutaneomucosal venous malformation, VMCM, we hypothesized that the variation in size, number and localization of the multifocal lesions may follow Knudson’s double-hit hypothesis for retinoblastoma. Proof for paradigmatic inheritance, the need for a combination of an inherited change with a somatic second-hit in the same gene, has started to pile up for some inherited vascular anomalies, supporting the idea that the inherited mutations have only recessive effects at tissular level. This may also be true for CM-AVM, and highlights the importance of assessing for tissue-based genetic changes, especially acquired genetic changes, as possible pathophysiological causes, which have been largely overlooked in developmental disorders.

http://www.deDuveInstitute.be/vikkula

Noonan syndrome

Amy Roberts  
Children’s Hospital Boston

Noonan syndrome (NS) is an autosomal dominant disorder with an estimated incidence of 1/1000-2500. Those with the diagnosis often have distinctive facial features, short stature, congenital heart disease (pulmonary valve stenosis, hypertrophic cardiomyopathy, atrial septal defect), learning disabilities, and less commonly renal malformation, bleeding disorder, Arnold Chiari malformation, and/or juvenile myelomonocytic leukemia. To date four genes have been found to cause NS, all members of the Ras-MAPK pathway: PTPN11, KRAS, SOS1, and RAF1. There are several disorders long noted to be phenotypically related that are now known to also be caused by perturbations of this pathway including LEOPARD syndrome, Cardio-facio-cutaneous syndrome, Costello syndrome, and NF-1. The phenotypic features, medical and developmental problems, diagnosis, and molecular genetics of Noonan syndrome will be discussed.

A Pathway to Comprehensive Care: The UCSF NF/Ras Pathway Genetics Clinic

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Comprehensive care for rare syndromes can be challenging. Many providers are not well-versed in the appropriate medical care for such patients, and rare syndrome are typically diagnosed in childhood, meaning that transition to adult care can be problematic. UCSF has developed a new clinical model for a class of genetic syndromes defined by germline mutations in genes which encode components of the Ras/mitogen activated protein kinase (MAPK) pathway. These syndromes include neurofibromatosis type 1 (NF1), Noonan syndrome, Costello syndrome, cardio-facio-cutaneous syndrome, LEOPARD syndrome, Legius syndrome, capillary malformation-arteriovenous malformation syndrome, and gingival fibromatosis. The clinic includes care for individuals with schwannomatosis and neurofibromatosis type 2 (NF2), although they are not specifically part of the Ras/MAPK pathway. UCSF’s clinic is unique because it is pathway-based and encompasses a group of syndromes with similar underlying pathogenic mechanisms, and thus, overlapping phenotypes. Although individually, each of these syndromes is considered “rare”, together, this group of genetic syndromes may in fact represent one of the most common classes of Mendelian genetic disorders. UCSF’s clinic model is adapted from the Children’s Tumor Foundation’s structure for their national satellite clinics, which provide multidisciplinary sub-specialty care, and transition of care for patients with NF1, NF2 and schwannomatosis. We expanded upon this basic concept by specializing in these and all other known Ras/MAPK pathway syndromes. We also expanded our subspecialty care to include women’s health, obstetrics, and prenatal services. Our specialty clinic is particularly unique in that it 1) is based on a pathway in which a common molecular etiology results in overlapping phenotypes and thus, similar multispecialty care needs; 2) provides comprehensive care for individuals of all ages with rare syndromes of the Ras/MAPK pathway; 3) includes a provider network of over 50 specialists at a single institution; including prenatal, pediatric and adult care; 4) seamlessly transitions pediatric patients to adult care; 5) has an external advocacy advisory board and 7) has an internal scientific advisory board. We feel that this unprecedented clinic will be a model for other genetic clinics with the capacity to provide comprehensive care to individuals with rare genetics syndromes, and serve as a clinical core for translational medicine and clinical trials.

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Molecular consequences of mTOR misregulation in tumor syndromes

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Studies over the past 8 years from diverse fields of biology have demonstrated that the aberrant activation of mTOR complex 1 (mTORC1) is a shared molecular event in the development and progression of many human tumor syndromes. It is now clear that the major pathways regulated by the tumor suppressors mutated in these diseases (e.g., NF1, PTEN, LKB1, TSC1, TSC2) converge on the control of mTORC1 and much progress has been made in understanding the molecular details of these pathways. However, the downstream consequences of aberrant mTORC1 activation toward the pathology and therapeutic response of these tumor syndromes remain poorly defined.

We are using cell and tumor models affecting the tuberous sclerosis complex (TSC) tumor suppressor genes, TSC1 and TSC2, to understand the cellular and molecular effects of uncontrolled mTORC1 activity. The TSC1-TSC2 complex acts as a monitor of intracellular and extracellular growth parameters (e.g., nutrients, energy, stress, and growth factors) and is a critical negative regulator of mTORC1. Therefore, loss of function of this complex results in elevated mTORC1 signaling that is no longer sensitive to perturbations in cellular growth conditions. Using a combination of TSC gene disruption and rapamycin to, respectively, activate and inhibit mTORC1, we have identified a set of transcripts that are strictly regulated by mTORC1 signaling. Through this approach, we have characterized a metabolic gene regulatory network stimulated by mTORC1 that alters the bioenergetic properties of cells to promote anabolic processes underlying cell growth and proliferation. In addition, we have found that aberrant mTORC1 signaling triggers adaptive stress response pathways that dampen the deleterious effects of these metabolic changes and uncontrolled protein synthesis. These alterations in basic cellular metabolism and basal activation of stress responses offer novel points of therapeutic intervention. We are actively exploring these areas as potential targets of cytotoxic agents to selectively kill tumor cells exhibiting elevated mTORC1 activity, a molecular defect common to neurofibromatosis, TSC, Cowden’s disease, Peutz Jegher’s syndrome, and the majority of human cancers.

Title of Abstract: Merlin modulates Rac-Pak and downstream MAPK signaling through tight junction-associated proteins.

Chunling Yi
The Wistar Institute

Neurofibromatosis Type 2 (NF2) is caused by mutations at the Nf2 gene, which codes for a protein termed merlin. Available evidence suggests that merlin suppresses cell proliferation and tumorigenesis by regulating multiple signaling pathways. To search for novel merlin targets/regulators, we have undertaken an unbiased biochemical approach that has led to the purification of a novel protein complex composed of merlin and a number of tight junction associated proteins. We show that merlin directly interacts with one of these tight junction proteins through their mutual coiled-coiled domains. Through confocal imaging, we have established that merlin, similar to its binding partner, is localized to both tight junctions and adherens junctions. Although merlin does not appear to require this novel binding partner for its initial recruitment to cell junctions during junctional assembly, the localization of merlin to mature junctions is regulated by this protein. We will present data that place this novel merlin-interacting protein downstream of merlin as a positive regulator of Rac-Pak and downstream MAPK signaling. Taken together, our study delineates a novel merlin-mediated signaling route at the site of cell:cell junctions, which may contribute to the tumor suppressive function of merlin.

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How basic research promotes insight into NF1 disease pathogenesis and therapeutic design

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Treatment options for patients with NF1 are limited, in particular for those that develop unresectable malignant peripheral nerve sheath tumors (MPNSTs). However, efforts in identifying and testing appropriate targeted therapies are currently underway. One strategy involves the use of mTOR inhibitors, which have been shown to promote stable disease in mouse tumor models and are currently being assessed in human clinical trials. However, as with many tumor types, it is likely that sequential or combination therapies may ultimately be more effective. In order to develop such therapies we have been engaged in a biology-driven effort toward therapeutic discovery. Specifically, we have been delineating both the mechanisms of NF1 pathogenesis and investigating the biology of therapeutic response in murine models in order to rationally develop new therapies. As such we have been focusing our efforts on using mTOR inhibitors as a platform for developing novel drug combinations. Notably, while we have identified several promising combinations, in each case in vitro studies were not predictive of the therapeutic response in vivo. These findings highlight the importance of performing basic biological studies and utilizing appropriate mouse models in drug discovery efforts.

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ErbB2 and c-Jun N-terminal kinase (JNK) signaling contribute to vestibular schwannoma growth and radiosensitivity

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Neurofibromatosis type II results from mutation of the tumor suppressor gene, Merlin, and leads to bilateral vestibular schwannomas (VSs) and other central nervous system tumors. Understanding the mechanisms that contribute to schwannoma formation and growth underlies the rational development of novel therapies. We find that ErbB2, a receptor tyrosine kinase essential for Schwann cell (SC) development and proliferation, constitutively resides in lipid rafts and is active in VS cells. Similarly, denervation of SCs by axotomy leads to inactivation of Merlin by protein kinase A-mediated serine 518 phosphorylation and movement of ErbB2 into lipid rafts, correlated with increased proliferation. Inhibition of ErbB2 decreases VS cell proliferation and radiosensitivity in vitro and growth of human VS xenografts in nude mice. Using human vestibular schwannoma specimens and primary cultures, we have begun to examine the contribution of merlin-sensitive intracellular kinases to VS growth. Mitogen activated protein kinase kinase/extracellular signal-regulated protein kinase (MEK/ERK), phosphatidylinositol-3 kinase (PI3-K)/Akt, and c-Jun N-terminal kinase (JNK) are persistently active in VS cells independent of ErbB2 signaling. Inhibition of MEK, PI3-K, or JNK reduces VS proliferation while inhibition of JNK, but not MEK or PI3-K, also results in VS cell apoptosis. By contrast, JNK inhibitors fail to induce apoptosis in normal SCs, suggesting that JNK may represent a specific therapeutic target for schwannoma cells. Inhibition of persistent JNK activity increases superoxide levels in VS cell mitochondria and overexpression of manganese superoxide dismutase prevents VS cell death due to JNK inhibition. Finally, inhibition of JNK increases the radiosensitivity of cultured VS cells. These data suggest that persistent JNK activity protects VS cells from apoptosis by limiting oxidative stress in the mitochondria.

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Analysis of p21-activated kinase function in NF2 signaling in vivo

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Neurofibromatosis type 2 (NF2) is a genetic disorder in which affected individuals are predisposed to the development of intracranial and spinal neoplasms including bilateral vestibular Schwannomas, meningiomas, and epedemymomas. It is not known how loss of the NF2 gene product, Merlin, causes Schwann cells to overproliferate, but recent data from our laboratory and others suggests that Merlin may signal through the p21-activated kinases (Paks). In this work, we show that expression of a peptide inhibitor of Pak1, Pak2, and Pak3 restores normal morphology and slows the growth of cells transformed with a dominant mutant of Merlin. Furthermore, these effects are also maintained in vivo, as xenografts of these cells in mice show markedly less growth when the Pak inhibitor is expressed, but not when an inactive mutant of this inhibitor is expressed. Interestingly, the few tumors that do develop in animals bearing the Pak inhibitor display elevated expression levels of the dominant NF2 mutant. The growth of these xenograft tumors does not correlate with the level of extracellular regulated kinase (ERK) activity. These results suggest that Paks indeed represent key downstream signaling components for Merlin, but that the effects of Pak on cell proliferation and tumor growth are not mediated by ERK.

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An E3 Ubiquitin Ligase That Controls Ras Signaling Through the Degradation of the NF1 Tumor Suppressor

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The NF1 gene encodes a tumor suppressor protein, neurofibromin, which acts as a negative regulator of Ras signaling. Ras critically regulates cell growth and proliferation in most tissues, and moreover, mutations in Ras proteins and many upstream and downstream effectors have been found in the majority of all human cancers. Notably, loss-of-function mutations in the NF1 gene are responsible for the familial cancer syndrome neurofibromatosis type I (NF1). NF1 patients are predisposed to developing a variety of tumor-types, including benign and malignant tumors in the central and peripheral nervous systems, attributed in part to aberrant Ras signaling in these tissues. However, little is known of the mechanisms that regulate neurofibromin expression and function. Previously, we have shown that neurofibromin is dynamically regulated by the ubiquitin-proteasome system in a variety of mammalian cell types, concurrent with Ras activation. However, the specific effectors that induce the degradation of neurofibromin have not yet been described. Presently, we have elucidated a novel mechanism that may control Ras signaling through the directed degradation of neurofibromin. We have identified an E3 ubiquitin ligase that is specifically required for neurofibromin degradation, and thus may serve as a regulator of neurofibromin stability. Furthermore, depletion of this ubiquitin ligase specifically attenuates Ras activation and significantly decreases the activation of the extracellular signal-regulated kinase (ERK), a critical effector of Ras signaling, resulting in impaired cellular proliferation. In this context, these defects appear to be dependent on the regulation of the Ras pathway by neurofibromin, since its genetic ablation restores the activation of ERK downstream of growth factor receptor activation.

Understanding the mechanisms that regulate neurofibromin degradation may provide the opportunity for designing or applying effective therapies aimed at increasing neurofibromin protein stability. This may be of particular significance to NF1 patients, since even haploinsufficiency at the NF1 locus has been shown to contribute to some symptoms of the disease in both model systems and human patients, including tumor development. Moreover, a mechanism of enhanced neurofibromin stability may be amenable to manipulation in the treatment of other cancer types fueled by mutations that result in the unscheduled activation of Ras.

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Pathways Regulating Ras Activity

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Levels of Ras.GTP, the active form of the Ras protein, are regulated by the balance between Guanine Nucleotides Exchange Factors and GTPase Activating Proteins such as neurofibromin. This balance changes dramatically during growth factor exposure, so that Ras proteins switch briefly from GDP- to GTP-bound states. This dynamic process is facilitated by rapid translocation of regulatory proteins to the plasma membrane during signaling, and by post-translational modifications to the regulatory proteins themselves. Downstream signaling to effectors, such as the Raf kinases and PI 3’ kinases is dependent on levels of Ras.GTP but modulated by other proteins, whose functions and modes of action are less well known. For example, ephrin receptors, and proteins of the Sprouty/SPRED family, negatively regulate Ras signaling downstream through distinct but poorly understood mechanisms. Likewise, wild-type Ras proteins interfere with downstream signaling from oncogenic Ras mutants, and have a profound effect on Ras’ ability to form tumors, at least in mouse models of cancer. The mechanism by which normal Ras proteins could interfere with mutant forms is unclear.

Downstream effector pathways also have major effects on engagement of receptors with the Ras through feedback loops that remain poorly characterized. For example, pharmacologic inhibition of ERK phosphorylation leads to up-regulation of signaling from EGF-R to the PI 3’ kinase pathway: as a result, tumor cells become insensitive to these inhibitors.

Each of these poorly characterized pathways can play an important role in diseases caused by hyper-activation of the Ras pathway. Some advanced stage cancers appear to be “addicted” to B-Raf, for example, and die when exposed to levels of MEK inhibitors that are not toxic to normal cells, or to other cancer cells that do not contain mutant B-Raf. On the other hand, inhibition of MEK activity reduces addiction and allows some cells to escape therapy. These considerations are relevant to treatment strategies for NF1-related diseases. It is critical to determine the degree to which such cells are “addicted” to hyper-activated Ras pathways and to what extent is their response to therapy likely to be affected by feedback loops and de-sensitization.

Loss of NF2 function in Schwann cells induces dedifferentiation and activation of nerve repair mechanisms by mimicking impaired axon-Schwann cell interaction

Jan Manent
INSERM U674 / Functional Genomics of Solid Tumors Laboratory, France

The hallmark of the dominant inherited peripheral nerve tumor disorder neurofibromatosis type 2 (NF2) is the development of bilateral vestibular schwannomas in which both copies of the NF2 gene are inactivated. To understand schwannoma pathophysiology and NF2 gene function in Schwann cells (SCs), we evaluated the transcriptome of Nf2-deficient primary mouse SCs and tumors from NF2 mice and patients. We show that in mouse SCs, loss of Nf2 leads to the activation of a molecular program reminiscent of myelination via the PI3K/Akt pathway. We further show that primary mouse Nf2 SC cultures are enriched for genes activated in normal developing immature SCs, supporting tumor dedifferentiation. Additionally, mouse schwannoma transcription profiles share striking similarity to developing immature SCs. Comparison of mouse and human schwannoma gene expression lead us to the identification of a common transcriptional signature. Genetic network analysis again revealed significant associations with nerve development and nerve repair wound healing that are normally induced when the axon-SC interaction is disrupted. Altogether, these data suggest that loss of NF2 mimics loss of axonal contact, thereby inducing activation of gene programs involved in nerve repair in a cell-autonomous manner. We therefore propose that NF2 plays a role in axon-SC cross-talk and that loss of this specific function is relevant to schwannoma formation. This provides a novel view on schwannoma development whereby NF2 tumors arise as a consequence of abnormal activation of nerve repair processes in SC that are usually triggered following nerve injury. Our results suggest that inhibition of the molecular program of nerve repair may represent a novel therapeutic approach to control schwannoma growth.

Junctional specializations of Schwann cells

Steven S. Scherer
University of Pennsylvania

Schwann cells have diverse phenotypes, in which gap junctions, tight junctions, and adherens junctions are deployed in different ways. Myelinating...
Schwann cells have gap, tight, and adherens junctions that are comprised of connexin32, claudin-19, and E-cadherin, respectively. These are found between the layers of the myelin sheath itself, in areas of non-compact myelin known as the paranodal region and Schmidt-Lanterman incisures. Myelinating Schwann cells also have specialized contacts with the axons at nodes of Ranvier and the flanking paranodal regions; these specializations are mediated by a different set of cell adhesion molecules. Merlin and ERM proteins are also found in these areas of non-compact myelin, and the conditional deletion of merlin results in shorter myelin internodes, increased numbers of Schmidt-Lanterman incisures, and disorganized paranodes. Non-myelinating Schwann cells are less complicated, having gap junctions and adherens junctions between individual cells. N-cadherin forms the adherens junctions; the connexin that forms the gap junctions has not been determined. Cultured Schwann cells are also joined by adherens junctions comprised of N-cadherin. Beta-catenin is associated with the adherens junctions of myelinating, non-myelinating, and cultured Schwann cells. Cultured NF2-deficient Schwannoma cells, in contrast, have poorly formed adherens junctions and an irregular distribution of N-cadherin and beta-catenin on apposed membranes; this is thought to contribute to tumorigenesis.

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Function of Merlin in controlling membrane receptor distribution and signaling

Zachary Morris (Presented by Andrea McClatchey)
Harvard Medical School/Massachusetts General Hospital

Cells organize their membranes by creating architecturally specialized domains that define local signaling environments. The neurofibromatosis type 2 (NF2) tumor suppressor, Merlin, is closely related to Ezrin, Radixin and Moesin (the ERM proteins), which are thought to organize specialized membrane domains by providing regulated linkage between membrane proteins and the underlying cortical cytoskeleton. Recent studies in Drosophila and our own studies in mammalian cells indicate that Merlin can control the surface availability of certain membrane receptors, such as the Epidermal Growth Factor Receptor (EGFR). We found that Merlin coordinates the establishment of stable cell junctions with inhibition of EGFR internalization and signaling. In the absence of Merlin, EGFR signaling persists at confluence and cells fail to undergo contact-dependent inhibition of proliferation. In part through the use of single particle tracking of individual EGFR molecules, our most recent studies reveal that at confluence, Merlin immobilizes EGFR at the cell surface through its localization to the cortical actin cytoskeleton. Importantly, however, we also found that Merlin governs the mobility and distribution of EGFR within the plasma membrane in a contact-independent manner. In fact, Merlin-mediated distribution of EGFR dictates whether the receptor is poised to undergo clathrin-dependent or clathrin-independent endocytosis, which, in turn, dictates whether it can be silenced at confluence. This work yields important insights into how Merlin controls the output of EGFR and other receptors and more broadly suggests novel mechanisms of both receptor control and tumor suppressor activity. These studies also highlight the potential importance of pharmacologic EGFR inhibitors for reversing the phenotypic consequences of NF2-deficiency. With that in mind, we have examined the efficacy of several EGFR inhibitors in preclinical in vitro studies, including a novel class of protein silencing covalent pan-ErbB inhibitors developed by Avila Therapeutics. Pilot studies indicate that these compounds work very effectively to achieve a prolonged block in proliferation and signaling in NF2-/- cells, and are superior in both efficacy and duration of action to Tarceva.

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Rho-GTPases in Schwann cells

Ueli Suter
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During development Schwann cells have to interpret different extracellular cues to regulate their migration, proliferation, and the remarkable morphological changes associated with the sorting, ensheathment and myelination of axons. I will present the crucial role in these processes of Integrin-linked-kinase (ILK), a focal-adhesion-protein that associates with multiple binding partners to link integrins to the actin cytoskeleton and that is thought to participate in integrin and growth factor mediated signaling. In this context, I will also discuss the putative roles of RhoGTPase and AKT signaling in signal transduction in Schwann cells during development and in regeneration.

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Reading and other cognitive concerns in NF1, and an Ongoing Trial of Intervention

Sheryl Rimrodt
Kennedy Krieger Institute

For parents of children with NF1, cognitive impairments are the most frequently reported concern...[occurring in] approximately 35% to 65% of children with NF1 have learning disabilities" (Levine et al, 2006). For this reason, our lab has engaged in studies to better characterize the cognitive profile of children with NF1 as well as the corresponding neural correlates. In this session, we will present data from a recent investigation including preliminary data that describes the broader features of visual spatial deficits commonly associated with NF1. Additionally, we have encouraging preliminary data from a clinical trial of intensive instructional intervention that we are currently conducting.

Molecular and cellular mechanisms underlying the learning disabilities associated with disruptions of Ras/MAPK signaling: from the lab to the clinic

Alcino J. Silva
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Ras-dependent activation of MAPK has a key role in synaptic plasticity and learning and memory, and disruptions in Ras/MAPK signaling underlie a number of genetic disorders that affect cognitive function. For example, mutations in the Neurofibromatosis Type 1 (NF1) gene, encoding Neurofibromin, a p21Ras GTPase Activating Protein (GAP), cause learning disabilities and attention deficits. Our studies have shown that the learning and memory deficits of a mouse model of NF1 (nf1 +/-) are caused by excessive Ras/MAPK signaling leading to hyperphosphorylation of synapsin I, and subsequent enhanced GABA release, which in turn result in impairments in the induction of long-term potentiation (LTP), a cellular mechanism of learning and memory. Consistent with increased GABA-mediated inhibition, we (collaboration with the Cannon lab at UCLA) found evidence for brain hypoactivation in fMRI studies of NF1 patients. Recently, we discovered that statins, at concentrations ineffective in controls, can reverse the enhanced p21Ras activity in the brain of nf1 +/- mice, rescue their LTP deficits, and reverse their spatial learning and attention impairments. Strikingly, recently completed pilot clinical trials (collaboration with the Elgersma laboratory in Rotterdam) uncovered suggestive evidence that statins may also be able to reverse cognitive deficits in children with NF1. Additionally, our laboratory is studying the molecular, cellular, systems and behavioral mechanisms of other genetic conditions that involve Ras/MAPK signaling and learning disabilities. Results from these studies will be reported.

From mice to humans: extrapolating from the mouse behavioral phenotype to human clinical trials

Jonathan M. Payne
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Mouse studies investigating the functional correlates of the NF1 gene mutation have shown that abnormal Ras signalling plays a role in cognitive deficits in memory and learning. In NF1 +/- mice, treatment with lovastatin, a non-selective HMG-CoA reductase inhibitor, can reverse these deficits, both at a functional level and by rescuing long-term potentiation (LTP), a cellular mechanism critical for effective learning and memory.

When considering the applicability of treatment effects in animals to humans, it is important to understand the nature of the impairment across both species. NF1 +/- mice display reversible impairments on the Morris water maze, a measure of visuospatial learning that has been shown to rely on the integrity of the hippocampal formation. As LTP deficits are also observed in the hippocampus of NF1 +/- mice, it is possible that hippocampal-based cognitive impairments will also be reversible in humans with NF1.

The efficacy of lovastatin as a treatment of cognitive deficits in children with NF1 is currently being investigated in a double-blind, placebo-controlled, Phase II study. We are administering the Paired Associate Learning (PAL) subtest from the Cambridge Neuropsychological Test Automated Battery (CANTAB) as a primary outcome measure for this study based on (a) its sensitivity to changes in human hippocampal function (b) its evolution from animal behaviour paradigms to facilitate cross-species studies of cognition (c) its validation in clinical populations and (d) multiple versions of the test to minimize practice effects. We will present findings that indicate children with NF1 make significantly more errors on the PAL task when compared to a control group and approximately 40% of children with NF1 displayed an impairment (>1SD below normative mean).

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The role of NF1 Exon 9a in neuronal function

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Neurofibromatosis type I (NF1) is an autosomal dominant disorder caused by a heterozygous loss of function mutations within the NF1 gene that encodes a Ras GTPase-activating protein neurofibromin. Mutations on this gene often cause learning, behavioral and attention problems.

Although NF1 is expressed in all cells of the central nervous system, expression of NF1 containing alternatively spliced exon 9a is restricted to the CNS neurons with high expression in the forebrain. The existence of a neuron-specific isoform raises the possibility that this isoform has unique properties by virtue of the presence of exon 9a and that this exon may have modulatory effect on Ras function of neurofibromin in neurons. However, the role of this alternatively spliced isoform is unknown.

To study the role of the NF1-Ex9a isoform in function of neurofibromin in neurons, we created a mouse lacking the NF1-Ex9a isoform. Although this mutation does not affect total levels of NF1, marked deficits are observed in synaptic plasticity and learning. In addition we observed that the phenotype is caused by increased inhibition. These deficits strongly resemble the phenotype of heterozygous NF1 knock-out mice, suggesting that the Exon 9a isoform plays an essential role in neuronal function, through a yet unidentified mechanism.

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Pharmacological effects on cognition in a fly model of NF1

Linnea R. Vose
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Neurofibromatosis Type 1 is characterized by disfiguring peripheral nervous system tumors and a high incidence of cognitive defects, including learning disabilities. Clinically relevant mutations in the protein neurofibromin (NF1) have been implicated in poor learning and long term memory (LTM) in mice, flies, and humans. There are two main intracellular signaling pathways influenced by NF1 that regulate learning and LTM. NF1 increases activity of an AC/PKA pathway implicated in learning in flies. It also decreases Ras activity in a MEK/MAPK pathway affecting LTM in flies and spatial memory in mice. Many inhibitory compounds exist for molecules in these pathways, including some drugs currently approved for use in humans, such as lovastatin, rolipram, and rapamycin. Previously, Silva and colleagues showed that treating NF1 deficient mice with lovastatin can improve spatial memory and reverse deficits in long term potentiation (LTP).

We hypothesize that compounds targeting the AC/PKA or MEK/MAPK pathways will have modulating effects on learning and/or LTM in flies. We further hypothesize that drugs targeting the downstream Rheb/mTOR pathway may also affect NF1 associated learning or memory deficits.

Compounds were fed to adult or larval Drosophila and uptake was monitored using food dye. Effects on survival, locomotion, and sensory responses were examined. Additionally, effects of the drugs were monitored at the molecular level using Western blot analysis and protein activity assays. Adult flies were tested for learning using an established associative learning protocol that measures the ability of the flies to distinguish between two odors, one of which was paired with an electric shock. Larvae were tested using a similar shock-odor protocol recently developed in our laboratory. Treatment with lovastatin and rolipram rescues learning defects in both adult and larval NF1 mutants. Tests with rapamycin are in process. Interestingly, learning of wild type larvae is negatively affected by lovastatin. Such a developmental side effect may be clinically relevant when treating young NF1 patients.

Our larval learning system pairing shock and odor provides a rapid (one minute) and robust protocol that will be useful for screening drugs and novel compounds that can advance to be tested in mouse models and eventual clinical trials in humans. Understanding the basis of cognitive dysfunction in flies will also guide further basic research in fly and mammalian NF1 models.

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Schwannomatosis Natural History Study Update

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Background: To date, there is very little known about schwannomatosis. It is thought to be a rare disorder with the incidence still to be determined. It is currently characterized by the presence of multiple schwannomas throughout the body and the absence of features of neurofibromatosis type 2. Diagnostic criteria have been established based on clinical presentation, however genetic testing that would be of utility to a large percentage of affected patients and their families cannot be performed, as a comprehensive understanding of the underlying genetic mechanism(s) remains undefined. The natural history of schwannomatosis has yet to be elucidated. As such, the advancement of novel treatment modalities is hindered by the inability to clearly demonstrate an alteration in natural history of the disorder.

Objectives: This study will allow for the development of a robust database designed to answer critical, hypothesis-driven research questions concerning the epidemiology and natural history of Schwannomatosis. The three specific aims begin with establishing a core group of internationally recognized experts in the clinical treatment of schwannomatosis. They will be charged with formulating key questions that a natural history study should answer and specifically those which can be realistically answered in a preliminary study. The participation of the various experts we have brought together in formulating the initial hypothesis driven questions will help to ensure participation and compliance in gathering data from their centers. The second specific aim requires the translation of hypotheses generated questions into database based research. Key data points for a web based database will be established and methodology for entry and maintenance of the database developed. In the final specific aim the database will be created including standardized case report forms and a web based computer program. A trial period of patient data entry will occur to assess for accuracy, ease of data entry and usability. Data analysis of the preliminary entries should provide some information concerning the natural history of schwannomatosis. This will also serve as the foundation for a subsequent, and more ambitious, proposal to have world-wide participation in a web based robust Schwannomatosis database.

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Molecular Pathology of Schwannomas and Neurofibromas

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Schwannomas and neurofibromas may occur in two clinical settings: as sporadic tumors in the general population or as multiple tumors as part of a syndrome in genetically predisposed individuals. Although the clinical and histological distinction is often straightforward, there are cases in which neurofibromas and schwannomas are very difficult to differentiate.

Recent studies have implicated INI1/SMARCB1 protein product (INI1), a component of a transcription complex, in the pathogenesis of schwannomas. INI1/ SMARCB1 germline mutations were first described in two family members of with familial schwannomatosis in which tumors were found to have both constitutional and somatic mutations of the SMARCB1 gene and showed a mosaic pattern of loss of INI1 expression by immunohistochemistry. The involvement of the INI1/SMARCB1 was confirmed multiple, independent series of schwannomatosis studies. We performed an immunohistochemistry analysis on 45 schwannomas from patients with multiple schwanna syndrome and on 38 solitary, sporadic schwannomas from non-syndromic patients to investigate the role of INI1 in multiple schwanna syndromes as well as in sporadic, solitary schwannomas. A mosaic pattern of INI1 expression was seen in 93% of tumors from familial schwannomatosis patients, 55% of tumors from sporadic schwannomatosis, 83% of NF2-associated tumors and only 5% of solitary, sporadic schwannomas. These results confirm a role for INI1/SMARCB1 in multiple schwanna syndromes and suggest that a different pathway of tumorigenesis occurs in solitary, sporadic tumors.

INI1/ SMARCB1 involvement in schwannomatosis (both familial and sporadic) tumors is significantly higher in our series than that reported in studies of INI1/ SMARCB1 mutation analysis. This discrepancy may be caused by difference of sensitivity and specificity of techniques of analysis or alternatively, because of an epigenetic mechanism of INI1/ SMARCB1 inactivation in a subset of tumors. It is unclear if INI1/ SMARCB1 plays any role in the pathogenesis of Schwann cell tumors in NF1 (neurofibromas or MPNST).

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Preliminary success with anti-angiogenic therapy of NF2-related tumors: one year later

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Neurofibromatosis 2 (NF2) is characterized by the presence of bilateral VS and multiple meningiomas for which alternative treatments are desperately needed. Profound hearing loss is a devastating complication of this disease. Using formalin-fixed, paraffin-embedded archival specimens, we studied vascular patterning, quantified parameters of vessel morphology (number, size and distribution), and analyzed angiogenic molecules, such as vascular endothelial growth factor (VEGF) and its receptors VEGFR2, neuropilin 1 (NRP1), and neuropilin 2 (NRP2) in 43 archival vestibular schwannoma specimens. Our findings (higher MVD, larger vessel diameter, expression of VEGF and its receptors) suggested a role for VEGF in schwannoma pathophysiology.

We treated 10 NF2 patients (six men and four women with a median age of 25 years; range, 16 to 53 years) with progressive vestibular schwannomas and chronic, progressive hearing loss in their only hearing ear using bevacizumab, an anti-VEGF monoclonal antibody. The mean annual tumor volume growth rate prior to treatment was 70% (range, 26% to 121%). The median duration of treatment was 11+ months (range, 3 to 19 months); the first six patients were each followed for at least one year. Eight of the patients continue to be treated with bevacizumab. Patient 1 required resection of his vestibular schwannoma 19 months after initiating treatment due to progressive tumor growth and patient 7 died of complications related to spinal cord surgery five months after discontinuing bevacizumab treatment. Nine of ten tumors shrank after treatment with bevacizumab, with 6 of 10 tumors (60%) achieving a radiographic response. A hearing response was seen in 4 of 6 patients (66%) who were capable of hearing improvement. In the remaining 4 patients, 3 had normal hearing at baseline (and thus could not improve) and 1 had surgical resection of both auditory nerves. The results suggest that the pathophysiology of hearing loss from neurofibromatosis-associated vestibular schwannomas is related, in part, to the action of VEGF and provide the first evidence that a medical treatment can restore functional hearing in a subset of NF2 patients.

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Expression profiling of schwannomas

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Background: Histologically it is not possible to differentiate between sporadic schwannomas, those occurring as part of schwannomatosis or those in patients with NF2. Germline mutation testing for NF2 or SMARCB1 may be informative in a subset of patients. However, for many individuals the initial presentation with a schwannoma provides a significant diagnostic and prognostic dilemma. We set out to establish if schwannomas could be differentiated on the basis of transcript expression profile.

Methods: Expression array profiling was performed on RNA extracted from 15 fresh schwannoma tissue samples (2 schwannomatosis, 6 NF2, 7 unilateral vestibular schwannoma (UVS)) and compared to RNA profiles from 3 normal nerve and 2 fibroma controls) using the Affymetrix Human U133 Plus 2.0 array. An empirical Bayes T-test was used to select significant differential expression (p<0.05) between the schwannoma subtypes and between controls and all schwannomas collectively. Hierarchical clustering analysis indicated that the gene expression in NF2 and UVS schwannomas is very similar as reflected by the small number of genes that are differentially expressed between these two groups. In addition, there was no apparent clustering relating to the size of tumour, location (right/left) or gender of the patient. SNAP29 was the only differentially expressed gene on chromosome 22 and was up-regulated 2.3-fold in NF2 schwannomas relative to schwannomatosis. STXBP1 on chromosome 9q was up-regulated 2.6-fold in both NF2 and UVS schwannomas relative to schwannomatosis. We will present detailed results following verification experiments and data from further downstream analysis for enrichment of specific pathways and enrichment based on chromosomal location, which is currently ongoing.

Conclusion: The results from this study provide an initial molecular phenotype to aid differentiation of schwannomas and predict clinical outcome and may provide targets for therapeutic intervention.


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Timing and impact of surgery in NF2

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Introduction: The appropriateness and timing of surgical intervention is a key factor in the management of NF2 interventions, and decisions should be made within a multi-disciplinary setting. The majority of patients will undergo multiple surgical procedures within their lifetime, many resulting in significant post-operative deficits. To our knowledge, there has been no previous published systematic review of all surgical interventions and post-operative deficits within a cohort of NF2 patients.

Aim: To identify the number of surgical procedures, timing and post-operative deficits in patients managed within the specialist NF2 clinic at Guy’s and St Thomas’ Hospital. To determine whether other features apart from symptoms, neurological deficit and rate of growth had any impact on surgical intervention. To use this information to produce a protocol.

Methods: A retrospective case note analysis of patients with a confirmed diagnosis of NF2, managed in the specialist clinic between 1992 and 2008. Number and timing of surgical procedures and post-operative complications were reviewed and correlated to a number of parameters including tumour type and burden, associated clinical features (e.g. neurofibromatous neuropathy), age at presentation and genotype.

Results: A total of 61 patients with a diagnosis of somatic or mosaic NF2 were identified. 32 were male, 29 were female. Mean age at the time of database analysis was 40 years, with a range of 6-81 years. Preliminary data revealed that 79.3% of our cohort had had some form of surgical intervention. Time between onset of symptom and surgery had a range of 0 – 20 years, with a mean of 5.3 years. Number of procedures ranged from 1 – 8, with a mean of 3.4. 24% of procedures were for excision of vestibular schwannoma. 19% were for complications of surgery (facial palsy) and 15% were for shunt insertions and revisions. 9% were for spinal procedures.

Discussion: All surgical interventions have their own risks, and correct timing is essential to avoid unnecessary complications and also to avoid the need for emergency procedures. Patients who required shunt insertion and those who required surgery for facial palsy were most likely to require multiple procedures. Indications for surgery have greatly changed over time, with a trend to be more conservative in latter years, for example, not all patients with brain stem compression will undergo surgery in the absence of other markers, such as papilloedema. We will present a protocol for surgical intervention.

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Drosophila as a model to study the role of SMARCB1 in Schwannomatosis

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Recent genetic studies have revealed germline mutations in the tumor-suppressor gene SMARCB1 in familial schwannomatosis. SMARCB1 is a core component of the SWI/SNF chromatin-remodeling complex, which regulates transcription and plays crucial roles in the control of proliferation and differentiation. We are developing a Drosophila model of Schwannomatosis, to help elucidate the specific molecular events that give rise to this disease. Transgenic fly lines have been generated that express human SMARCB1 or its Drosophila ortholog, Snr1, under the control of the UAS/Gal4 system. Expression of Snr1 or either of the two alternatively spliced forms of SMARCB1 is able to compensate for loss of Snr1 in flies. We have also made transgenes encoding SMARCB1 bearing mutations found in patients with familial cases of schwannomatosis. These are being tested for their ability to rescue the lethality associated with the Snr1 mutant. In this way we hope to be able to determine the molecular consequences of these patient mutations.

SMARCB1 is the only human SWI/SNF subunit thus far implicated as a potent tumor suppressor. We hypothesize that SMARCB1 may have an important role, independent from that of being a core constituent of the SWI/SNF complex. We hope to uncover new functions of SMARCB1 and Snr1 using our genetically tractable Drosophila model system. RNA interference (RNAi) is being used to knockdown the expression of Snr1 in whole animals or in specific tissues with the UAS/Gal4 system. The resulting RNAi phenotypes are ideal for conducting genetic modifier screens to look for novel Snr1 interactions. In the first instance, we are examining whether Snr1 genetically interacts with NF2, which is also mutated in schwannomas. Novel interactors from our genetic screens will also be tested for modification of phenotypes caused by RNAi of other components of the Brahma complex, the Drosophila equivalent of the SWI/SNF complex, to identify genetic interactions unique to Snr1. Together these approaches using a Drosophila model system should improve our understanding of SMARCB1 and its orthologs, providing a clearer path to tackling schwannomatosis.


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**Session 8A: Novel cellular and animal models of NF**

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**Single lineage derivation of meningothelial and fibroblastic meningiomas induced by NF2 loss in PGDS-expressing primordial meningeal cells**

**Michel Kalamarides**  
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Meningiomas account for approximately one-third of all primary central nervous system tumors. It has long been assumed that the arachnoid cap cells capping arachnoid villi are the cell of origin of meningiomas but no reference has been made to specific arachnoid subsets or parts of the meninges. An association between NF2 gene status, tumor site and histologic subtype has been demonstrated, but it remains uncertain which cell type(s) in the arachnoid cell lineage during development and/or adulthood is the cellular target for NF2 mutation and how loss of NF2 in these cells leads to development of different meningioma subtypes. The frequent NF2 gene inactivation event in human meningiomas has been exploited in mice to generate an animal model that phenocopies human meningioma tumorigenesis and thus allow for in vivo investigation of tumor development and use in translational studies. Inactivation of the NF2 gene in mouse leptomeningeal cells using adeno virus-mediated Cre recombinase intrathecal delivery in conditional NF2 knockout newborn mice (NF2flox/flox) resulted in meningioma development, confirming the critical role of the NF2 gene as growth regulator for leptomeningeal cells (Kalamarides et al., 2002). While this model has represented a significant advance in mouse meningioma modeling, there are persistent questions as the meningioma cell of origin. To address this question, we have identified a specific marker of arachnoid cells. The prostaglandin D2 synthase (PGDS) gene is specifically expressed in arachnoid cells of mice and humans. Human and mouse meningiomas also show intense PGDS immunoreactivity.

In this study, we have used this arachnoid-specific promoter to generate conditional NF2 knockout mice with restricted biallelic NF2 inactivation in the primordial meninges, before formation of the meningeal layers. Using genetic and injection methods (adCre) we found that the timing of NF2 bialleic inactivation in a PGDS+ meningeal precursor cell is responsible for the developmental heterogeneity of meningioma subtypes. Pre-natal and early post-natal biallelic NF2 inactivation in PGDS+ progenitor cells can give rise to meningiomas, whereas more mature cells cannot.

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**Role of SWI/SNF factors in Schwann cells: Potential regulators of pain and tumor growth in Schwannomatosis**

**Larry S. Sherman**  
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Patients with Schwannomatosis suffer from both schwannomas, resulting from abnormal Schwann cell proliferation, and chronic pain. Mutations in two genes, NF2 and SNF5 (also called SMARCB1 and INI1) have been observed in schwannomas from schwannomatosis patients. SNF5 is a subunit of SWI/SNF chromatin remodeling complexes that can regulate both cell cycle progression and the transcriptional activation of a wide range of genes. The role of SWI/SNF complexes in Schwann cells was not previously known. We observed Schwann cell hyperproliferation along peripheral nerves of mice with conditional mutations in brahma-related gene-1 (Brg1), an ATPase subunit of SWI/SNF complexes. This effect was cell autonomous as loss of Brg1 in primary cultures of Schwann cells was sufficient to induce a 2-3 fold increase in bromodeoxyuridine (BrdU) uptake in vitro. In contrast, mice with conditional mutations in the Snf5 gene and Snf5-null Schwann cells grown in vitro did not have significant changes in Schwann cell proliferation.

Given the role of SWI/SNF chromatin remodeling factors in regulating gene expression, we tested the possibility that loss of Snf5 could influence the expression of factors that contribute to pain. One such factor known to be expressed by Schwann cells is brain-derived-neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors. Growing evidence has implicated BDNF in neuropathic pain and increased pain sensitivity. We found that rhabdoid tumors with SNF5 mutations have elevated levels of BDNF transcripts, and that Schwann cells lacking Snf5 express twice the amount of BDNF as wild type Schwann cells. Furthermore, wild type capsaicin-sensitive dorsal root ganglion neurons exposed to supernatants from Snf5-mutant Schwann cells demonstrated increased Ca2+ fluctuations, consistent with the hypothesis that BDNF and/or other factors released by Snf5-mutant Schwann cells may promote pain. We are currently testing if these effects on sensory neurons can be reversed by BDNF-neutralizing antibodies, and whether mice with conditional Snf5 mutations have increased pain sensitivity.

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*Larry Sherman is currently a Children’s Tumor Foundation Schwannomatosis Award Recipient*
Novel Murine Models of Malignant Peripheral Nerve Sheath Tumors

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Malignant peripheral nerve sheath tumors (MPNSTs) are one of the most lethal consequences for Neurofibromatosis type I (NF1) patients. Although NF1 patients have approximately a 10% lifetime risk of acquiring MPNSTs, these tumors are highly aggressive, with a 5 year survival rate of approximately 50%. Currently, surgical resection is the primary treatment option, with complete excision of small and early stage tumors correlated with higher survival rates. Unfortunately, no diagnostic markers consistently differentiate MPNSTs from neurofibromas, and few chemotherapies have been demonstrated to be efficacious in the treatment of MPNSTs, largely due to the relative rarity of these tumors. In this study, we present two novel murine models of NF1 that generate MPNSTs with high penetrance. These two models differ in their timing of Nf1 inactivation, where one model loses Nf1 in conjunction with p53, while our other model inactivates Nf1 first with subsequent loss of p53. While both models produce MPNSTs, if Nf1 is inactivated prior to p53 loss, neurofibromas develop, mimicking NF1 patients. As both these models utilize Schwann cell lineage specific ablation of a single p53 allele, they generate predominately MPNSTs. Comparison of these two mouse models enables elucidation of the role of neurofibromas in MPNST development. Finally, p53 ablation is only of the DNA transcription activation domain, allowing identification of malignant tumors though staining of stabilized p53 protein. These novel murine models enable us to generate large numbers of MPNSTs, allowing testing for diagnostic markers to differentiate between benign and malignant peripheral nerve sheath tumors, and offer improved NF1 models for pre-clinical therapeutic trials.

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Role of the Nf1 heterozygous microenvironment in the development of benign and malignant peripheral nerve sheath tumor

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Neurofibromatosis type 1 (NF1) is a common inherited neurological disease, affecting 1 in 3500 newborns worldwide. Individuals afflicted with NF1 carry germline loss-of-function mutations in the NF1 gene and are predisposed to a wide spectrum of neoplasms, including tumors in the peripheral and central nervous system (PNS and CNS). The hallmark feature of NF1 is the development of benign peripheral nerve sheath tumor, also known as neurofibroma. There are two subtypes of neurofibroma: discrete neurofibroma is typically small, localized and often arise in the dermis or epidermis of the skin whereas plexiform neurofibroma extends along the length of peripheral nerves and may involve multiple fascicles of a nerve or multiple branches of a large nerve. Genetic studies from human tumors and mouse models have established that bi-allelic inactivation of NF1 in the Schwann cell lineage is required for neurofibroma development. However, the contribution of the NF1 heterozygous microenvironment to neurofibroma formation has been controversial. In this study, by using a conditional Nf1 allele, we establish a mouse model that develops plexiform neurofibromas throughout the PNS as well as discrete neurofibromas in the skin. In the p53 heterozygous background, some of benign lesions progress to malignant peripheral nerve sheath tumors (MPNST). Furthermore, we demonstrate that the Nf1 heterozygous microenvironment is critical for neurofibroma progression, but not for initiation or malignant transformation. Together, our study not only establishes a novel mouse model for neurofibroma and MPNST, but also provides important insights into the role of the Nf1 heterozygous microenvironment in the development of benign and malignant peripheral nerve sheath tumor.

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Merlin Regulates Epithelial Cell Polarity and Proliferation by Establishing a Junctional Polarity Complex

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The neurofibromatosis type 2 (NF2) protein, Merlin, is a unique cytoskeleton-associated tumor suppressor whose loss is associated with defects in morphogenesis and metastatic tumor development in mouse models. We have shown that Merlin function is critical for the establishment of adherens junctions (AJ) and contact-dependent inhibition of proliferation in cells. During epidermal development, the basal cells of the skin rely upon coordinated regulation of cell junction and polarity components to trigger subsequent differentiation and barrier formation in the adult epidermis. Given the requirement for Merlin in AJ formation in vitro, and the importance of cell junction components in the basal cells of the skin we investigated the function of Merlin during skin development in vivo. We found that K14-Cre;N122lox/lox mice display a dramatic epidermal phenotype, with little initial hair growth and severe dehydration leading to death during early postnatal development. The altered barrier function of the Nf2-deficient skin is due in part to altered cell:cell junctions, in particular the incomplete establishment of both AJ and tight junctions. Histological analysis of K14-Cre;N122lox/lox skin reveals a pronounced expansion of the epidermal progenitor cell population in the basal layer. This expansion can be ascribed to altered cell:cell communication and defective basal cell polarity during skin development. In fact, molecular studies suggest that Merlin is a vital bridge between AJ and polarity components. These studies introduce new evidence for a role for Merlin in epithelial progenitor cell adhesion, proliferation and polarity and provide a platform for further studies of Merlin function in these processes.

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Nerve gene expression patterning in NF1 mouse models

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Many mouse models of neurofibromatosis type 1 (NF1) display abnormalities in the organization of unmyelinated peripheral nerve fibers. These irregularities may model the initial structural changes that occur with neurofibroma development or growth and thus provide mechanistic insight into the roles of relevant candidate genes and biological pathways in NF1 tumorigenesis. To elucidate the molecular changes that occur, we compared the transcriptome of adult nerves from five mouse models – CNPase-hEGFR/+ (n = 4), CNPase-hEGFR/EGFR (n = 4), CNP-HRas12V/+ (n = 6), Nf1 flox/flox; DhhCre (n = 3) and NPCis (n = 4) – and evaluated changes in gene expression relative to Nf1 flox/- (n = 3) and Nf1 flox/flox (n = 4) controls. We identified 1,783 transcripts statistically different (p = 0.01) and performed unsupervised two-way hierarchical cluster analysis to distinguish specific subtypes. Consistent with previously observed pathophysiological studies, CNPase-hEGFR/+ and DhhCre; Nf1 flox/flox mice displayed similar patterns of gene expression. Interestingly, many genes changed in nerves overexpressing a single copy of EGFR were found to be unchanged in nerves overexpressing two copies. We identified six principle patterns of gene expression and assessed each cluster to explore the potential biological significance. Three subtypes emerged from this analysis. Transcripts up- or down-regulated across all mouse nerve samples were enriched for genes associated with apoptotic regulation, unfolded protein response, nervous system development and angiogenesis and cell cycle progression. Transcripts up- or down-regulated across NPCis, Nf1 flox/flox; DhhCre, CNPase-hEGFR/+ and a subset of CNP-HRas12V/+ nerve samples were enriched for genes associated with complement and coagulation cascades, wound healing, cell adhesion and migration, and the intermediate filament cytoskeleton. Lastly, transcripts up- or down-regulated across CNPase-hEGFR/+ and CNPase-hEGFR/EGFR and Nf1 flox/flox; DhhCre nerve samples were enriched for genes associated with intermediate filament bundle assembly, nervous system development, angiogenesis, Wnt signaling, cell adhesion, response to wounding, cholesterol biosynthesis and the endoplasmic reticulum. These results demonstrate that transcriptional changes in NF1 mouse models are diverse and represent different molecular aspects of the disease. Further analysis and comparison to human tumors will be useful for the identification of early biological events that may be amenable to non-surgical treatment(s) to either prevent tumor formation or inhibit neurofibroma growth.

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Dural ectasia in NF1

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Dural ectasia, an uncommon spinal manifestation of neurofibromatosis type 1, is a circumferential expansion of the thecal sac that erodes surrounding osseous and ligamentous structures. Subsequent vertebral destabilization and dislocation, angular deformities, and spontaneous fractures can develop. Nevertheless, canal expansion protects the spinal cord often preventing neurological damage.

Multiple theories as to the exact cause of dural ectasia have been proposed, yet a single, unifying hypothesis remains unclear. One explanation is a primary neurofibroma or mass effect causes a pressure-induced osteolysis within and/or around the site leading to a secondary dural expansion. Abnormally elevated pressure within the dural sac would lead to the expansion of the dural sac and eventually may erode osseous structures nearby. This theory fails to account for cases of dural ectasia in the absence of any such mass. As well, intracranial pressure and intraspinal pressure are not increased in NF1. Therefore this theory is unlikely.

A more likely theory incorporates the association between the dural ectasia found in Marfan’s syndrome and NF1. A primary mesodermal dysplasia affecting the development of bone, vascular, reticular, and other collagenous and elastic connective tissues is likely to create an environment that leads to Dural Ectasia. Skeletal and reticular abnormalities are both common to NF1. Neurofibromatosis has features consistent with a congenital mesodermal defect. Such congenital defects may cause secondary dural dilation as well as osseous erosions.

Posterior spinal fusion has been utilized to manage the unstable spine in the presence of dural ectasia. More severe cases may require further intervention including circumferential fusion. In either case, special attention must be paid to the potential for weakened or eroded pedicles as pedicle screws may be inadequate. A vascularized fibular graft should accompany the fusion as all bone added is often resorbed. To avoid recurrence and erosion of the fibula graft, some have wrapped the fibula in titanium mesh for protection. The literature is sparse in terms of the long term results of treating dural ectasia.

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References:

Osteoporosis in NF1-related bone abnormalities

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Osteoporosis is a metabolic skeletal disease in which low bone density and poor bone quality increase fracture risk. Osteoporosis is most common in post-menopausal women and aged men. Diagnosis of osteoporosis in humans depends mostly on densitometry, as a surrogate marker for fragility fractures. One risk factor for osteoporosis is failure to accrue sufficient bone mass by the time skeletal growth completes. Therapies for older osteoporotics are targeted to either inhibit bone resorption to preserve remaining bone, or to stimulate bone turnover and increase bone formation. This presentation will review current concepts of osteoporosis and therapies, and their application in NF1.

Markedly decreased bone mineral density has been reported in at least 50% of NF1 humans. The clinical implications of low BMD in NF1 are unclear. Preclinical studies of Nf1 mouse models report impaired osteoblasts and over-active osteoclasts. A few studies suggest NF1 humans may be at increased risk for bone fractures, the hallmark of osteoporosis, at an unusually early age. Elevated markers of bone resorption and low serum 25(OH)D in some NF1 humans may contribute to failure to accrue optimal skeletal mass, and sub-optimal bone quality. Premature osteoporosis in NF1 humans presents challenges in accurate diagnosis and in age-appropriate therapies. Clinical research is needed to better determine bone quality and fracture risk in NF1. Osteoporotic drugs developed for an aging osteoporotic population are not optimized for younger NF1 humans, and may be contra-indicated in children. The implications of NF1 should be considered in diagnosis and treatment of osteoporosis.
Spine Abnormalities in Asymptomatic Children with Neurofibromatosis Type 1 (NF1)

Dave Viskochil  
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Children with NF1 are at risk to develop progressive scoliosis. The etiology of this manifestation is unknown; however, associated findings include vertebral scalloping, rib penciling, dural ectasia, and paraspinal neurofibromas. To identify potential spine features in NF1 patients who develop dystrophic scoliosis, a multi-center cohort of asymptomatic prepubertal children with NF1 were screened for spine abnormalities upon entry to the study. The primary outcome of this 3-year natural history study is to determine the incidence of dystrophic scoliosis in a cohort of 110 subjects. Additional bone health indices are determined as secondary outcome measures.

Children with NF1 who did not have scoliosis by physical examination were enrolled through 1 of 4 NF Clinics. Each subject was evaluated by thoracic MRI, scoliosis series, DXA, and pQCT. The MRIs and scoliosis series were examined and scored by a single radiologist (KM) for the following: scoliosis >9 degrees, scalloping ratio at T5 and L1, vertebral wedging in the sagittal or coronal planes, spindling of the transverse process, rib penciling, dural ectasia, paraspinal neurofibroma, and meningocele.

Fifty-nine prepubertal enrollees have been evaluated. The age range of this cohort is 6 to 9 years, 60% are male, and 52% are sporadic cases. Thirty subjects have normal imaging on both spine MRI and PA/lateral spine radiography, and 29 had at least 1 abnormality. These abnormalities include 16 with paraspinal neurofibroma, 5 with dural ectasia, 1 with meningocele, 9 with an abnormal vertebral body, 7 with measurable scoliosis between 10 and 16 degrees, and 1 with rib penciling.

In summary, half of the asymptomatic prepubertal NF1 enrollees without scoliosis on physical examination had at least 1 spine abnormality. Of those with abnormal findings, 55% have at least 1 paraspinal neurofibroma, 31% have vertebral abnormalities, and 17% have dural ectasia.

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NF1 focal bone defects - Identification of the cellular culprits by conditional mouse models

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Neurofibromatosis type I (NF1) is characterized by skeletal defects that can be categorized as general and focal. General defects include low BMD, increased resorption, low vitamin D levels and should be amenable to treatment. However, NF1 vertebral dystrophic lesions, as well as bowing of the tibia and subsequent non-union, are two focal lesions associated with high morbidity and whose etiology is unknown. Consequently, no treatment other than invasive surgical interventions exists. Clinical investigations aimed at clarifying the molecular mechanisms underlying these bony abnormalities are obviously difficult and limited in nature. Some laboratories, including ours, thus turned to mouse models with two goals: to determine the role of NF1 in bone cells and to generate preclinical models to assess the efficacy of rationale treatments. Following a half-decade of efforts, supported by CTF and NIH, we now have in hand a number of different mouse models and potential pharmacological strategies have emerged from these models. Each of these models, due to the nature of the genetic manipulation used to create them, does not recapitulate the totality of the skeletal lesions characteristic of NF1. However, their comparison allowed a much better understanding of the role of NF1 during bone development and regeneration, identified defects not yet well defined in human NF1 lesions, enabled us to generate new hypotheses related to the etiology of the NF1 bony defects and led to the assessment of various pharmacological strategies to improve bone healing in a NF1 context. Although the gap between these pre-clinical data and the first clinical trials is still wide, we now have a much better view of the mechanisms to tackle and stronger hope for improved NF1 orthopedic care.

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Molecular Aspects of NF1-Related Bone Abnormalities

Kevin McHugh  
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No abstract submitted
NF1-deficient osteoclasts in vivo and in vitro

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Aim: Our aim was to address the role of osteoclasts in the pathogenesis of NF1-related bone lesions.

Material and methods (I): In order to elucidate the catabolic component of bone dynamics in NF1, osteoclast precursors were isolated from peripheral blood of 16 patients with NF1, and allowed to differentiate into osteoclast-like cells (OLCs) on bone slices for 10 days in alpha-MEM (Gibco) with 10% iFCS (Gibco) supplemented with penicillin-streptomycin, and the growth factors RANK ligand 20ng/ml and M-CSF 10ng/ml.

Results (II): Mice harboring tartrate resistant acidic phosphatase (TRAP) promoter-driven Cre recombinase were mated with the NF1flox/flox mice. The resulting transgenic mice harbored Nf1 +/- or Nf1-/- osteoclasts in otherwise Nf1+/+ background.

Conclusion: Even when isolated from the factors mediated by other cell types of the NF1 microenvironment, NF1-deficient osteoclasts of men and mice display altered phenotype.

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Local delivery of low dose lovastatin improves the fracture healing defect caused by Nf1 loss-of-function in osteoblasts

Weixi Wang
Vanderbilt University

NF1 tibia pseudarthrosis leads to lifelong disability and amputation in the most severe cases. The role of Nf1 in bone fracture healing remains unknown and consequently no therapeutic strategies other than surgical management are available. In this study, we used the Nf1ob-/- conditional mouse model and a distal tibia fracture protocol to determine whether lack of Nf1 in osteoblasts impairs bone healing. Longitudinal 3D-microtomography and histomorphometric measurements indicated that at 21 and 28 days post-fracture, the callus of Nf1ob-/- mice is bigger and less calcified than the one of WT littermates, and still contains cartilaginous remnants and extended osteoid surfaces which have already disappeared in WT animals. Most importantly, biomechanical tests at 28 days post-fracture revealed a significant reduction of mechanical properties in Nf1ob-/- compared to WT calluses. Therefore, loss of Nf1 function in mature osteoblasts delays bone healing and callus mechanical properties in the Nf1ob-/- mouse model, which can thus be used as a pre-clinical model to test rational strategies aimed at preventing or correcting the bone healing abnormalities associated with loss of Nf1 function. In vitro, Nf1-/- osteoblasts are characterized by constitutive Ras and ERK activation, which can be corrected by the inhibitory effect of lovastatin on Ras prenylation. Based on this proof-of-concept result, we treated WT and Nf1ob-/- mice, immediately following fracture, with a single injection of lovastatin nanoparticles that progressively release low doses of lovastatin. Callus bone volume and the number and surface of cartilaginous remnants were decreased in lovastatin-treated Nf1ob-/- mice compared to non-treated controls, and the difference in both trabecular and cortical bone volume between WT and Nf1ob-/- calluses became no longer significantly different upon lovastatin treatment. Most importantly, from a clinical point of view, lovastatin treatment in Nf1ob-/- mice improved their biomechanical properties to an extent that made them no longer different from WT calluses. All together, these results suggest that local delivery of low dose lovastatin may have a good potential to improve bone healing in NF1 patients. (This work is supported by the Young Investigator Award and Drug Discovery Award from the Children’s Tumor Foundation.)

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Weixi Wang is currently a Children’s Tumor Foundation Young Investigator Award Recipient
Orthopedic animal models of NF1: an update

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Congenital tibial dysplasia associated with NF1 is an anteriolateral bowing of the tibia that is prone to fracture and subsequent non-union or pseudarthrosis. There is emerging range of genetic mouse models of NF1 deficiency that display bone healing defects. We have previously used the Nf1 +/- mouse to show impaired bone healing in the distal tibia as well as a reduced response to bone morphogenetic proteins (BMPs). These experiments indicate that the bone healing deficiency in NF1 is complex and likely to involve defects in multiple cell types including osteoprogenitors, osteoclasts, chondrocytes, and fibroblasts. We now have two major aims. First, we seek to develop a more advanced model of congenital pseudarthrosis that adequately represents the double inactivation of NF1 that is believed to occur in local bone lesions. Second, we plan to use our orthopaedic animal models to test existing and emerging therapies for bone repair in an NF1 setting.

To model double inactivation of the NF1 gene in mice, we have sourced the conditional Nf1floxed strain. We have crossed this mouse with the Nf1 +/- strain to generate Nf1floxed/- mice, which possess one functional null allele, and one cre-sensitive allele. Local delivery of cre-recombinase to a fracture site should enable regionalized double inactivation of NF1, analogous to a pseudarthrosis. To accomplish this, we have produced cre-expressing lentivirus and adenovirus constructs. These viruses have been tested in vitro, where they induce cre-dependent gene expression in primary cells from reporter mice, and excise the floxed allele from Nf1floxed/+ mice. In vivo tests are underway. We have continued to explore new methods for improving fracture repair in Nf1+- mice. We have hypothesized that improved muscle coverage would lead to an improved prognosis. In mice, we have found that muscle flaps are prone to swelling and infection, and yield suboptimal results when combined with BMPs. However, we have identified a minimal-carrier BMP delivery system that may yield improved results, particularly when combined with anti-resorptive drugs.

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Aaron Schindeler is currently a Children’s Tumor Foundation Young Investigator Award Recipient
Development of a Non-surgical Treatment for Dermal Neurofibromas in Neurofibromatosis Type 1 (NF1)

Ruihong Chen
NexGenix Pharmaceuticals

Background and Aims: Multiple dermal neurofibromas (DNFs) develop in 94% of adults with neurofibromatosis type 1 (NF1), and range in number from a few to several thousand in an affected individual. DNFs cause disfigurement, pain and pruritus and are a major cause of psychological distress and isolation in the NF1 population. The only treatments available, surgical removal by scalpel, electro-dessication or laser, typically require multiple injections of anesthetic, and the time required averages 10-15 minutes per lesion. Removal of multiple lesions under general anesthesia substantially increases the risk and cost. We screened a variety of non-surgical approaches to DNF removal using parental formulations of generic drugs, with the intent of developing a safe, economically feasible non-surgical treatment.

Methods: Using cell-based assays, DNF explants, DNF xenografts, and small proof of principle clinical trials conducted in Mexico City and Rio de Janeiro, we evaluated drugs with known abilities for vascular disruption, anti-angiogenesis or biological response modification.

Results: Specially formulated doxycycline (NX101), an antibiotic in wide usage with known vascular disruption and possible anti-angiogenic capabilities, was demonstrated in a proof-of-principle open-label single dose clinical trial to be quite effective in clearing DNFs. Diclofenac, a biological response modifier when applied topically, was effective in a minority of lesions. Combining the 2 drugs did not produce a synergistic effect.

NX101 caused 17 of 18 lesions in 6 patients to become necrotic and fall off after 1-2 injections, leaving a scab which healed normally within several weeks. A subsequent dose-ranging trial has identified an optimal dose for further development. Side effects were minimal and included minor pruritus and discomfort at the injection site. No complications were observed in follow-up visits that extended to 12 months. There was no evidence of re-growth of lesions in the follow-up period. NX101 is not carcinogenic or immunosuppressive.

Conclusions: NX101 is an intraleisional treatment for DNFs which cause vascular disruption, necrosis and clearing of lesions within days after 1-2 injections. It can be applied quickly with no anesthetic, and has an excellent safety profile. Further clinical trials with a unique burst/delayed release formulation are planned for DNFs and plexiform neurofibromas.

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Overactivation of Ral in MPNST: Cell signaling and therapeutic ramifications

Faris Farassati
University of Kansas Medical School

The Ras family of guanine-nucleotide bound proteins exerts a fundamental role in cell biology and constitutes an important area of cancer research due to its significant involvement in the development and progression of malignancies. Ras-like (Ral) proteins are crucial members of this family, shown to play a pivotal role in human tumors. Because Ral guanine nucleotide exchange factors (Ral-GEFs) are direct effectors of Ras, the Ral signaling pathway has been traditionally considered a Ras-effector pathway. Although highly similar to Ras, Ral proteins (RalA and RalB) involve a series of distinctly different effectors which influence gene expression and translation through interaction with ZO-1 associated nucleic acid binding protein (ZONAB) and RalA binding protein 1 (RalBP1).

The molecular events involved in the malignant transformation of benign neurofibromas to MPNST are poorly defined. Inactivation of both copies of the NF1-gene has been demonstrated in benign human neurofibromas and shown to cause tumors in murine models. Loss of heterozygosity (LOH) of NF1 and p53 has frequently been observed in human MPNST. In this study, we show that while Ras activation as well as activation of a series of its down-stream effector pathways are observed in a fraction of MPNST cells, RalA is activated globally in all studied mouse and human MPNST cells and tumor samples as compared to non-transformed Schwann cells. Silencing Ral or inhibiting it with a dominant negative Ral (Ral S28N) caused a significant reduction in proliferation, invasiveness, and in-vivo tumorigenicity of MPNST cells. Silencing Ral also reduced the expression of epithelial-mesenchymal transition (EMT) markers such as b-catenin, N-cadherin and Slug. Expression of NF1-GTPase-related domain (NF1-GRD) diminished the levels of Ral activation, proliferation, invasiveness, and cell cycle progression, but cell death increased. Interestingly, exposure of MPNST cells to geranyl-geranyl trasferase inhibitors (GGTIs) decreased cell proliferation in concentrations which reduced Ral activation but had no meaningful effects on Ras activation. On such basis we propose Ral pathway as a worthy target for gene and drug therapy of MPNST which might lead to development of novel agents against this deadly disease.


Acknowledgements: Supported by a CDMRP NFRP grant to Faris Farassati.
Regulation of MAPK signaling by the Mammalian Ste-20 like Kinase-2

Geoffrey Kilili
Tufts Medical Center

In Drosophila, the orthologue of the Mammalian sterile-20-like kinases 1&2 (Mst1, 2) Hippo (hpo) functions as a suppressor of cell proliferation. Epistatic studies in Drosophila also indicated that, the Drosophila orthologues of Merlin (dMer and expanded-EX) act upstream of, and positively regulate hpo. Loss of function mutations in the mammalian orthologue of dMerlin, hMerlin, underlie Neurofibromatosis type 2 (NF2), a familial autosomal dominant cancer syndrome characterized by the development of bilateral vestibular schwannomas and meningiomas. We used RNAi knock down and adenoviral over expression systems to determine if Merlin acted upstream of Mst2 in human cells.

Over expression of Merlin in Hei-193 cells, a schwannoma cell line, led to apoptotic cell death, which was not affected by Mst2 knockdown in any way. Co-transfection of Merlin and Mst2 in HEK 293 cells did not promote or potentiate Mst2 phosphorylation or its kinase activity. These data indicate that, in human cells, Merlin may not act upstream of or positively regulate Mst2 as established in Drosophila.

Merlin is known to regulate mitogen mediated MAPK signaling. The MAP3K kinase, Raf-1, in a kinase independent manner has been shown to interact and mediate inhibition of the pro-apoptotic activation of Mst2. However, a kinase independent role for Mst2 in regulation of mitogen mediated MAPK signaling was not tested. To further elucidate the functional nature of the hippo pathway in mammalian cells, we asked if knockdown of Mst2 in human derived cell lines affected MAPK signaling.

Knockdown of Mst2 in the schwannoma cell line Hei-193, and SKOV3, an ovarian cancer cell line, led to slowed cell proliferation and decreased phosphorylation of the downstream target of the Rafs, MEK1/2 in response to epidermal growth factor (EGF) stimulation. We traced this decrease in MAPK signaling to an increase in phosphorylation of Raf-1 on Serine 259 a known inhibitory site. Indeed when assayed in vitro with purified MEK-1 as substrate, Raf-1 immunoprecipitated from Mst2 knockdown cells exhibited less kinase activity compared to control samples. Raf-1 Serine 259 is a known AKT target. Surprisingly, we observed increased phosphorylation of AKT in Mst2 knockdown cells, an observation already reported at least once before by others.

These data indicate that, Mst2 is required for optimal mitogen mediated MAPK signaling. Mst2 is a negative regulator of AKT, thus knockdown of Mst2 leads to up regulated AKT activity and thus, increased phosphorylation of Raf-1 on serine 259, an inhibitory site. This negatively affects Raf-1 kinase activity and thus MAPK signaling, which would perhaps explain the slowed cell proliferation upon Mst2 knockdown. Interestingly, Mst2 seems to be a positive regulator of MAPK signaling, a growth/proliferative pathway and a negative regulator of the PI3K/AKT, a survival pathway. Merlin is known to negatively regulate both of these pathways. This raises the idea that either Merlin and Mst2 function separately in mammalian cells to regulate MAPKs or their relationship is more complex than the straightforward epistatic relationship observed in Drosophila studies.

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Geoffrey Kilili currently a Children’s Tumor Foundation Young Investigator Award Recipient

Regulation of Rac by PAK in human schwannoma

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Peninsula College for Medicine and Dentistry, United Kingdom

Merlin loss causes benign tumors of the nervous system, mainly schwannomas and meningiomas. Schwannomas show enhanced Rac1 and Cdc42 activity, p21-activated kinase 2 (PAK2) activation and increased ruffling and cell adhesion. PAK regulates activation of merlin. PAK has been proposed as a potential therapeutic target in schwannomas. However where PAK stands in the Rac pathway is insufficiently characterised. We used a peptidergic inhibitor of the GEF Pix interaction with Rac and APK and a novel small molecule PAK inhibitor, IPA-3, to investigate the role of PAK activation on Rac1/Cdc42 activity, cell spreading and adhesion in human primary schwannoma and Schwann cells. We show that IPA-3 blocks activation of PAK2 at Ser192/197 that antagonises PAKs interaction with Pix. Accordingly, Pix-mediated Rac1 activation is decreased in IPA-3 treated schwannoma cells, indicating that PAK acts upstream of Rac. We show that this Rac activation at the level of focal adhesions in schwannoma cells is essential for cell spreading and adhesion in Schwann and schwannoma cells.

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Using Gene Expression Analysis to Identify NF1 Clinical Targets

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In humans, neurofibromatosis type 1 (NF1) mutation predisposes individuals to development of benign neurofibromas, incurable peripheral nerve tumors, and to malignant peripheral nerve sheath tumors (MPNST). These NF1 peripheral nerve tumors lack effective predictors and therapies. We used transcriptional profiling to distinguish primary normal Schwann cells (n = 10) from NF1-derived primary Schwann cells, malignant peripheral nerve sheath tumor (MPNST) cell lines, and benign and malignant tumors (n = 67). The integrative analyses identify specific proteins as biomarkers and possibly therapeutic targets to fight NF1, and provide numerous additional candidates for further analysis. We validated differential expression of 82 genes including the neural crest transcription factor SOX9 and SOX9 predicted transcriptional targets. For example, SOX9 immunoreactivity was robust in neurofibroma and MPNST tissue sections. Using shRNAs targeting genes up-regulated in neurofibromas and MPNST we are testing the role of SOX9 and its targets in cell growth and tumorigenesis. Targeting SOX9 caused MPNST cell death. Sox9 regulates EYA4 which is highly expressed in MPNST. EYA proteins interact with Dach and/or SIX proteins to bind DNA and regulate gene transcription. Strikingly, using shRNAs to target EYA4 blocks MPNST tumor formation. Thus SOX9 is a biomarker of neurofibroma and MPNST, and SOX9 and its downstream targets may represent therapeutic targets in NF1.


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Parent Reported Executive Function Profiles in Children with NF-1 with or without Comorbid Attention Deficits

Maria T. Acosta  
Children’s National Medical Center, George Washington University Medical Center

Objective: To determine if there are differential executive functions profiles in children with NF-1 (NF1-AD) and children with NF-1 and comorbid attention deficits (NF1+AD). Participants/Methods: This is a retrospective study of 36 NF-1 patients characterized clinically as having general regulatory control difficulties. Clinical neuropsychological evaluations were obtained from chart review and 25 patients had complete data. Patients were categorized into two groups - significant attention deficits (NF1+AD) or no attention deficits (NF1-AD) using a cutoff score of seven on the TEA-Ch Sky Search DT. Fifteen of the 25 patients were categorized as NF1+AD (60%) based on these criteria. Results: Significant (p < .05) differences were identified between the two groups on the parent BRIEF for the Behavioral Regulation Index (NF1+AD, M = 66.5, SD = 8.78; NF1-AD, M = 54.0 SD = 8.01) and the Global Executive Composite (NF1+AD, M = 71.0, SD = 8.85; NF1-AD, M = 53.9, SD = 18.79). There was a trend toward higher levels of anxiety in the NF1+AD group by parent report (NF1+AD, M = 59.0, SD = 15.39; NF1-AD, M = 48.8, SD = 9.39). Conclusions: These data reveal significant differences in reported executive functioning between NF1 children with and without significant comorbid attention deficits with regard to global executive functions, and particularly within the behavioral regulation domain, which includes skills such as inhibitory control, cognitive flexibility and emotional control. Higher levels of anxiety appear to be more prevalent in the children with comorbid attention deficits as well.

Lovastatin as a Pharmacological Treatment for Learning Disabilities in Patients with NF1: Results of a Phase 1 Safety Study

Presenter: Maria Acosta  
Children's National Medical Center, George Washington University Medical Center

Objectives: We will present results of a Phase 1 study assessing the safety and tolerability of Lovastatin in neurofibromatosis type 1 (NF1). Eight patients have been added to the original cohort, in order to increase safety information and evaluation of pharmacokinetics and metabolism of lovastatin in NF1 children. Additional objectives included identifying patterns of brain activation using fMRI during a working memory task. A multicenter, international phase 2 efficacy study is currently in progress. Methods: Twenty three subjects have been enrolled to date. Assessments were completed pre-treatment and 12 weeks post-treatment. Baseline evaluations included physical and neurological examinations, neuropsychological testing, blood samples. Post-treatment included a repeat of baseline assessments as well as a 12 hour PK collection. Results: To date, 21 of 23 patients have finished the 12 week period. No side effects have been reported related to Lovastatin. No differences in safety data have been observed as our numbers have increased. Laboratory results were within normal limits for all parameters for all study participants. Total cholesterol levels decreased an average of 18% (range 4 - 28%) across all groups and stayed within normal limits. The decrease of cholesterol levels was not related to the total final dose. No alterations in muscle enzymes, liver function, pancreatic enzyme were observed. Neither myoglobinuria nor CBC alterations were observed in any of our patients and all liver enzymes were within the normal range. Conclusions: Lovastatin has been well tolerated within our sample. Changes in cholesterol are unrelated to Lovastatin dose. Our results support the ongoing Phase 2 study. Broad variability in the Lovastatin metabolism is expected as per the medication properties. PK data analysis is pending.

Leucine-rich pentatricopeptide repeat cassette (LRPPRC): a novel interacting protein of the tubulin binding domain (TBD) of Neurofibromin

Vedant Arun  
University of Toronto, Canada, Hospital for Sick Children’s Research Institute and The Arthur and Sonia Labatt Brain Tumor Research Centre

Neurofibromatosis 1 (NF1) is the most common tumor predisposing syndrome in humans, with an incidence of 1:3500 live births. Neurofibromin acts as a p21-Ras-GAP to directly interact with and inactive p21-Ras, through its’ GAP Related Domain (GRD). Evidence suggests that non-Ras-GAP functions mediated through interactions with domains outside of the GRD are of importance. The large size and thereby lack of full-length Nf1 cDNA led us to use GST-fusion proteins of neurofibromin domains, coupled with gel-based mass-spectrometric (MS) proteomic analysis. We identified LRPPRC and Dyneme as previously unreported NF1-TBD interacting proteins. LRPPRC was of interest as it is mutated in the Leigh Syndrome French Canadian (LSFC) variant, a cytochrome-oxidase deficiency syndrome, characterized by neurodegeneration, developmental- mental- and growth-retardation, thereby having some similarities with non-tumor manifestations of NF1. Studies including sub-cellular fractionation and immunofluorescence (IF) using spinning disc confocal microscopy have confirmed that NF1 and LRPPRC colocalize; and by means of LC/MS analysis, immunoprecipitation, reverse bait immunoprecipitation and in situ proximity ligation assay (PLA) we have established that this is a true interaction that occurs in the cytoplasm, mitochondria and nucleus of human schwann cells. Current studies are focused on elucidating the functional relevance of this interaction by NF1 and LRPPRC siRNA knock-down, to better understand the biological relevance of this novel interaction towards some of the overlap of neurological and developmental manifestations between the LSFC and NF1.

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Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder characterized by the development of benign tumors of the peripheral and central nervous system (CNS) including schwannomas, meningiomas, and ependymomas. The gene responsible for the development of NF2 (schwannomin or merlin) acts as a tumor suppressor gene. Disease expression is quite variable resulting in a spectrum of different phenotypes. While there is an association between NF1 and malignant neurofibroma, the development of malignant tumors is not considered a feature of NF2. The hallmark of NF2 is development of bilateral vestibular schwannomas with a consequent hearing loss. Patients often have a large burden of intracranial tumors, particularly meningiomas, and
require frequent interventions. Surgery has been a standard therapy, but stereotactic radiotherapy (SRT) has been increasingly used for the management of these tumors. It offers some enticing advantages over surgery particularly in regards to short-term risk. Because of the high frequency of multiple intracranial tumors in NF2 patients, SRT is an attractive option in this patient group. Common long-term complications of radiosurgery include cranial neuropathies, and less commonly vasculopathy, while the risk for malignancy after SRT is not well delineated. While there are no known instances of spontaneous malignant degeneration of tumors in NF2 patients, there are a few reports of malignant schwannoma, meningioma and epedymoma after the SRT. We present the first documented case of rhabdomyosarcoma following SRT for a NF2-associated vestibular schwannoma. Patients with NF2 have a tumor suppressor gene defect and may be more vulnerable for development of secondary malignancy after treatment involving radiation, when compared to patients with isolated tumors. Decision about selection of therapy in NF2 patients, particularly ones in young age, must include consideration of long term complications, particularly radiation-induced malignancies.

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NF1 and RKIP (Raf kinase inhibitor protein) are partially co-localized in the nucleus of astrocytes

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In the yeast S. cerevisiae, the homologs of neurofibromin (NF1) are Ira1p and Ira2p proteins. Like NF1, these proteins are Ras-GAP (GTPase activating protein). The GAP activity is carried out by a homologous central domain of the proteins called GRD (GAP related domain). The homology between NF1 and Ira proteins extends on both sides of their GRD, encompassing a large region of 1480 residues. In 2004, our group showed that Ira2p was able to interact with the Tfs1 protein and that this interaction resulted in the inhibition of Ira2p activity on Ras (1). We could identify the Ira2p domain responsible for this interaction and called it TBD for Tfs1-binding domain. TBD is conserved in NF1 and corresponds almost exactly to its SecPH domain which was discovered by K. Scheffzek’s group (2). Tfs1p belongs to the family of PEBP (phosphatidylethanolamine binding protein). In human, three members of the PEBP family can be found: RKIP (Raf kinase inhibitor protein), MRPL36 (a mitochondrial ribosomal protein), and PEBP4. RKIP is the best characterized protein. It is very abundant in the brain and has been shown to inhibit different kinases (Raf-1, Tks-1, NIK-1, Gsk-2). It is furthermore involved in metastasis suppression in different cancers. In order to know if the Ira2p/Tfs1p interaction was conserved in mammalian cells, two-hybrid and co-immunoprecipitation experiments were performed between NF1 and the human PEBPs. No interaction could be shown by these techniques. Immunofluorescence experiments were then undertaken on cell lines of human and rat astrocytes using anti-NF1 and anti-RKIP antibodies. They demonstrate the existence of a partial co-localisation of the two proteins in the nucleus. This co-localisation more specifically involves the PKC-phosphorylated RKIP. RET experiments are in progress in order to know if this co-localisation corresponds to an interaction. The nuclear localization of NF1 has already been described in the literature however nothing is known about the role NF1 might play in the nucleus. Since phosphorylated RKIP is associated to centrosomes and kinetochores and regulates the spindle checkpoint in different mammalian cell lines, the partial co-localisation of NF1 and RKIP might give insights on the role played by NF1 in the nucleus. References: 1- Chautard et al., Eukaryot. Cell 2004, 3: 459. 2- D’Angelo et al., EMBO Rep., 2006, 7: 174.

Additional Authors: Fabienne Godin, Sandrine Villette, Béatrice Vallée, Michel Doudeau, Chantal Pichon, Jean-Vianney Barnier, Tobias Hevor, Aurélie Gombault - a: Centre de Biophysique moléculaire, CNRS, Orleans, France; b: NBCM, CNRS, Gif/Yvette, France; c: Laboratoire de Neurobiologie, Université d’Orleans, France

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Shedding Light on the Cafe-au-lait Macule in NF1

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Introduction & Purpose: The cafe-au-lait macule (CALM) in NF1 exhibits phenotypic variance although this has not been fully characterized. Recent investigations have found that NF1 CALM melanocytes have a mutation in both copies of the NF1 gene and that melanocytes of non-CALM NF1 skin show the germline mutation only; the inherited mutation is identical from melanocyte-to-melanocyte but the “second-hit” mutation varies. Our objective was to quantify the pigmentation of the CALM in NF1 and determine if there are significant intra- and interpersonal differences. Methods: Lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer. To account for sun exposure, the difference between lesions of a suitable size and their adjacent, non-CALM skin were analyzed with a narrow-band reflectance photospectrometer.

Results: The variation in pigmentation after adjusting for age, sex, and sun exposure status was primarily explained by cafe au lait spots (48.42%, p<0.0001) and secondly by individuals (33.01%, p = 0.0094). Utilizing the linear mixed model to check the consistency of pigmentation within cafe au lait spots, we found that 3.85% (p<0.0001) of the variance was random, likely attributable to meter variability. Conclusions: Multiple insights were gained, most significant of which was the finding that CALMs between patients and within an individual vary in a statistically-significant manner, providing evidence of interfamilial as well as intrapersonal phenotypic variability. Given our increased understanding of the melanocyte in NF1, we propose that the differences in pigmentation of the CALM are related to the underlying genetic mutations. It is uncertain, however, how the mutation directly affects coloration; one hypothesis is that color intensity has an inverse relationship to the loss of function of neurofibromin.

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Elevation of Protein Content in Perilymph but not Endolymph in NF2 Associated Vestibular Schwannomas

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Purpose: First, to ascertain whether the elevation of protein content in the inner ear associated with vestibular schwannomas (VS) was confined to the elevation of the perilymph or whether this abnormality extended to the endolymph. Second, to determine whether endolymphic hydrops was associated with VS. Methods: Normally, signal from endolymph and perilymph is completely suppressed on FLAIR MRI. Changes of the T1 relaxation time, as occurs with increasing protein or contrast agent concentration, restores high signal. Intravenous contrast accumulates slowly in the perilymph but is restricted from the endolymph. Thus, delayed FLAIR imaging performed 5-10 h after injection demonstrates the endolymph as a “filling defect” within enhanced perilymph. We used high resolution precontrast FLAIR to assess protein concentration, and delayed postcontrast FLAIR to assess the for endolymphic hydrops.Twelve NF2 patients with 21 vestibular schwannomas enrolled in a natural history study (ClinicalTrials.gov identifier: NCT00598351) were imaged at 3T with a delayed high resolution FLAIR protocol involving two scanning sessions. In the first session, pre contrast T1, FSE T2 and FLAIR, and post contrast T1 were obtained using a single dose of contrast. A second dose of contrast (for spinal MRI) was administered within 30 m of the first. Delayed imaging was performed 5-10 h following the initial injection. All sequences had matching geometries (0.47×0.62×1.8 mm). Results: Of the 21 ears with VS, 4 had prior surgery, 1 total labyrinthectomy, 2 partial labyrinthectomies, and 1 intracranial resection. Intralabyrinthine extension was present in 9 ears. Of the 17 non-surgically treated ears, 13 showed hyperintense signal in the perilymph on precontrast FLAIR in the perilymph in 13 ears. Of these13, 2 showed hyperintensity in the endolymph. On delayed postcontrast FLAIR, enhancement of the perilymph was seen in all cases. Endolymph enhancement occurred in only 2 cases. Of the 17 non-surgically treated ears, none had evidence of endolymphic hydrops. Hydropic enlargement of the scala media was present in 1 of the 2 ears in which a partial labyrinthectomy had been performed. In the other ear, tumor invasion of the labyrinth precluded assessment. Conclusion: Elevated protein concentration in the endolymph was a frequent feature of VS. Elevated protein content in the endolymph was unusual. Furthermore, endolymphic hydrops is not a consequence of VS. Cochlear endolymphic hydrops may develop when the labyrinth is entered surgically.

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Study of the Prevalence of Pain in Children, Adolescents and Adults with NF1

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Background: Neurofibromatosis 1 (NF-1) is an autosomal-dominant disorder with neurological, osseous, and systemic signs/symptoms. Pain is a significant cause of disability in adults with NF-1,[1, 2] but pain has not been thoroughly investigated in the pediatric NF-1 population. Objective: To examine pain prevalence, levels, and distribution in pediatric NF-1 patients (ages 6-17) in relation to NF-1 signs/symptoms; to assess associations of pain parameters with quality of life and disability. Study Design: This was a descriptive, correlational, cross-sectional pilot study with IRB approval. Participants completed questionnaires: Pain Levels – Faces and Box Scales; Pediatric Pain Disability Index; Pain Drawing; Pediatric Quality of Life Inventory. Medical chart data were extracted regarding 26 NF-1 signs/symptoms. Recruitment was from the NF-1 clinic of a St. Louis, Missouri, children’s hospital. Results: Participants (N=18 to date) were 83% male with mean age of 11.7 years (SD=3.0). Mean age at NF-1 diagnosis was 3.8 (SD=3.3). Fourteen (78%) had chronic/recurrent pain. Mean age at pain onset was 6.5 (SD=3.9). Primary pain sites were head/neck (39%), back (44%), and upper extremities (50%). Pain history, pain levels, and number of pain sites were associated primarily with neurofibromas and/or plexiform neurofibromas (78% reported one or both). Pain level was categorized as no-mild, moderate, or severe (n=6/group). Significant linear trends were found, with higher pain levels associated with lower quality of life (0-100 scale: 81.9 vs. 72.1 vs. 59.5; SD=16.9), particularly activity- and school-related life quality, and higher disability (0-70 scale: 0.7 vs. 3.8 vs. 11.2; SD=7.8). More pain sites and years in pain were also associated with lower life quality and higher disability, while years since NF-1 diagnosis was not. With on-going recruitment, sample size is expected to be 3 times higher at abstract presentation. Conclusions: Pain is common in pediatric NF-1 and is associated with neurofibromas and plexiform neurofibromas. Pain level and distribution have significant, negative associations with self-reported quality of life and disability. Attention to pain assessment and treatment in this population is warranted. References: 1) Wolkenstein P et al. (2001) Quality-of-life impairment in neurofibromatosis type 1: a cross-sectional study of 128 cases. Arch Dermatol 137:1421-5; 2) Creange A et al. (1999) Neurological complications of neurofibromatosis type 1 in adulthood. Brain 122:473-81.

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Academic impairment in Brazilian children and adolescent with NF1: a preliminary study

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Studies with English, Dutch, and French-speaking children have shown that NF1 is associated with a variety of medical complications, academic impairments, and behavioral problems. The aim of the present study was to investigate the presence and specificity of academic impairments in a small sample of Portuguese-speaking children and adolescents. Eleven children and adolescents (7 boys and 4 girls) with NF1 and a full scale IQ (FSIQ) above 70 participated in the study. They ranged in age from 8 to 15 years (M = 11.27, SD = 2.28), and none of them presented medical complications. In addition
to the Wechsler Intelligence Scale for Children-III (WISC-III), participants were individually administered tests of reading, spelling and mathematic skills. Similar to the results of previous studies, our participants scored significantly higher on the verbal subtests than on the performance subtests of the WISC-III (Mean Verbal IQ = 103.18, SD = 16.64; Mean Performance IQ = 90.18, SD = 10.78). As a matter of fact, this discrepancy was found in seven of the 11 participants. However, the proportion of participants with learning disabilities was somewhat lower than what has been reported in the literature (30–65%). Only three of the children who participated in the present study (27%) manifested academic difficulties, as defined by a score of at least one standard deviation below the group mean in one or more of the academic achievement tests. However, one of these participants also scored below the normal range on the WISC-III. The remaining two (18%) had IQs within the normal range (FSIQ = 97 and 89), and manifested difficulties in all three academic areas. Interestingly, they both scored relatively worse on the WISC-III subtests associated with the “freedom from distractibility” factor. Concerning the type of learning disability, no isolated literacy impairments were found in our sample. Indeed, reading, spelling and mathematic skills were equally affected among the two children who showed academic impairments despite normal IQs. Finally, our results suggest that attention deficits probably contribute to the academic impairments frequently manifested by individuals with NF1. Reference: Hyman, S.; Shores, A.; North, K. (2006) - Learning disabilities in children with NF 1: subtypes, cognitive profile, and attention-deficit-hyperactivity disorder. Developmental Medicine & Child Neurology, 48, 973-977.

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**Heat shock factor 1 (HSF1) is a potent modifier of the tumorigenesis associated with NF1**

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HSF1 is the master transcriptional regulator of the heat-shock or stress response, an evolutionarily conserved adaptive mechanism that coordinates an extensive array of cellular pathways to fight stress and enhance cellular and organismal survival. In this report, we demonstrate that HSF1 is a potent modifier of the NF1-associated tumorigenesis. In vivo, either Hsf1 haploinsufficiency or complete deficiency significantly prolongs the tumor-free survival of Nf1-cis mice that harbor compound heterozygous deletion of both Nf1 and Trp53 tumor suppressor genes. Furthermore, Hsf1 inhibition alters tumor spectrum of Nf1-cis mice - reducing lymphoma and malignant peripheral nerve sheath tumor (MPNST) incidence but increasing glioma incidence. In cell culture, knockdown HSF1 by small hairpin RNAs (shRNAs) markedly impairs the growth and survival of human MPNST cells. Interestingly, increased HSF1 protein levels are correlated with NF1 deficiency in these MPNST cells. Moreover, immunostaining of human tumor tissues associated with NF1 demonstrates that MPNSTs show an increased nuclear HSF1 staining, an indicator of its activation, compared to benign neurofibromas, suggesting a positive correlation between HSF1 activation and tumor malignant state. Taken together, our in vivo and in vitro data indicate that HSF1 promotes the tumorigenesis initiated by NF1 deficiency and NF1 tumor cells become dependent on HSF1 for their continuous growth and survival. These studies strongly suggest that targeting HSF1 and its mediated stress response may be a novel therapeutic strategy for human NF1-associated malignancies. Current effort focuses on elucidating the molecular mechanisms underlying the elevated HSF1 expression in NF1-deficient cells.

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**Ras signaling enhances permissiveness of malignant peripheral nerve sheath tumor cells to oncolytic Herpes**

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Lack of expression of neurofibromin in neurofibromatosis 1 (NF1) and its lethal derivative, malignant peripheral nerve sheath tumors (MPNSTs), is thought to result in the overactivation of the Ras signaling pathway. Our previous studies have proven that cells with overactivation in the Ras pathway are more permissive to infection with herpes simplex virus 1 and its mutant version R3616. In this study, we show that among five different mouse MPNST cell lines, only the ones with elevated levels of Ras signaling are highly permissive to infection with oncolytic herpes G207. Specific inhibitors of the Ras, ERK and JNK pathway all reduced the synthesis of viral proteins in MPNST cells. The cell lines which contained lower Ras and down-stream signaling activation underwent an enhancement in apoptosis upon exposure to G207. Additionally, mouse SW10 Schwann cells were infectible by parental herpes but resistant to G207. The immortalization of these cells with expression of SV40 large T antigen increased the levels of Ras activation and permissiveness to oncolytic herpes. A Ras/Raf kinase inhibitor reduced the synthesis of HSV-1 and G207 proteins in SW10 cells. The results of this study, therefore, introduce Ras signaling as a divergence turn-point for the response of MPNST cells to an assault by oncolytic herpes.


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The Types, Distribution, and Evolution of Hyperintensities in Children with NF1

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PURPOSE: Many reports have described T2-weighted hyperintensities (hyperintensities) in MRIs performed on children with NF1 but the reported prevalence and significance of these hyperintensities remains controversial. In this study we used T2-weighted and FLAIR images to better characterize hyperintensities in a prospective study of children with NF1. Purposes: (1) to draw a qualitative distinction between two types of hyperintensities, focal and diffuse, (2) to characterize the appearance, prevalence, and anatomical distribution of different types of hyperintensities,(3) to determine how hyperintensities change over time. MATERIALS AND METHODS: Cranial MRIs were performed on 82 children, 43 with NF1 and 39 near relatives (6-13 years old). A second MRI was obtained three years later in 27 of the NF1 subjects and 22 controls. Neuroradiologists blinded to the diagnosis of NF1 analyzed all T2-weighted images and flair images using predetermined criteria to distinguish focal and diffuse hyperintensities. RESULTS: The neuroradiologists reliably identify the two types of hyperintensities. 90% of NF1 subjects had focal hyperintensities and 79% had diffuse hyperintensities. Hyperintensities were rarely found in control subjects. Each type of hyperintensity had a typical anatomical distribution pattern. Focal hyperintensities were most common in the globus pallidus, cerebellar white matter, midbrain and thalamus and diffuse hyperintensities were most common in the hippocampus, around the fourth ventricle, in the thalamus and in the cerebellum. Over a three-year interval, the number of focal hyperintensities in the NF1 subjects significantly decreased and the number of diffuse hyperintensities significantly increased. CONCLUSIONS: NF1 children have focal and diffuse hyperintensities that have different anatomical distributions and that change differently over time. We conclude that hyperintensities in NF1 children are more common than previously reported and the disappearance of hyperintensities may only be the temporal profile for the focal hyperintensities.

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AZD6244 Inhibits ERK1/2 Activation and Proliferation in Human Schwannoma Cells

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Deficiency of the tumor suppressor merlin leads to the development of multiple tumors of the nervous system, such as schwannomas, meningiomas and ependymomas, occurring spontaneously or as part of the hereditary disorder neurofibromatosis type 2 (NF2). NF2 tumors are numerous, develop in adolescence, resulting in increased morbidity and mortality. As these tumors are benign, classical chemotherapy is not effective. As current therapies, surgery and radiosurgery, are local therapies, new systemic treatments are required. To find potential therapeutic targets, we previously deciphered growth factor signaling and the Raf/mitogen-activated kinase (MEK)/extracellular-signal-regulated kinase (ERK) pathway activation and its role in schwannoma growth. Here, we targeted MEK1/2 as it acts as a convergence point for multiple cascades towards ERK1/2 activation and cell proliferation. We used a MEK1/2 inhibitor, AZD6244 (ARRY-142886; Astra Zeneca), for which Phase I data are available. Tissue sections and primary human Schwann and schwannoma cells were used. Immunoblotting and immunohistochemistry were used to detect ERK1/2. Cell growth was monitored by proliferation assays. AZD6244 completely abolished platelet-derived growth factor-DD-mediated ERK1/2 activation and cell proliferation in primary human schwannoma cells. AZD6244 exhibited its maximum effect at the concentration of 458 ng/ml, almost 2-3 fold lower than the plasma concentration observed in patients in clinical trials on other tumors, suggesting that AZD6244 is a potent inhibitor of ERK1/2-mediated cell proliferation in schwannoma cells. Moreover, this drug was not toxic for either schwannoma or Schwann cells, and has been reported to be safe with tolerable side-effects. Thus, AZD6244 can be considered a drug candidate for schwannoma treatment.

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A novel Bmi1 signaling pathway important for tumor growth in NF1 Astrocytoma

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The control of stem/progenitor cell self-renewal and regulatory elements responsible for epigenetic changes could represent new windows of opportunity for therapeutic intervention in NF tumors. Previous evidence suggests that NF brain tumors can arise from stem/progenitor cells. The polycistrome gene Bmi1 is an epigenetic regulator that is a key element required for stem cell self-renewal and epigenetic memory. We provide evidence that Bmi1 expression is highly upregulated in astrocytoma models of NF1 and that overexpression of Bmi1 leads to an increase in tumor growth. We show that phosphorylation of Bmi1 is specifically regulated downstream of EGFR activation by casein kinase 2 and the tumor suppressor PP2A. EGFR-induced phosphorylation of Bmi1 causes it to dissociate from chromatin and leads to alterations in the transcriptional regulation of target genes. Furthermore, mutation of the EGFR-induced phosphorylation site on Bmi1 abolishes its ability to increase proliferation in NF1 tumor cell lines. This data is consistent with previous data showing that EGFR plays an important role in NF tumors. We propose a novel signal transduction pathway linking the EGFR pathway to the epigenetic regulator Bmi1 that is likely to play an important role in tumor proliferation and growth factor mediated epigenetic changes in NF tumor development.

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Merlin is a novel negative regulator of TSC/mTORC1 signaling: Aberrant mTORC1 signaling contributes to benign tumor growth in NF2

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Neurofibromatosis 2 (NF2) is a severe, autosomal dominant neurogenetic disorder characterized by the development of multiple benign tumors of the nervous system, particularly vestibular schwannomas and meningiomas. Inactivating mutations of the NF2 gene result in loss of the encoded protein, merlin, a tumor suppressor whose growth inhibitory mechanisms are not fully understood. We recently identified merlin as a novel negative regulator of the mammalian target of rapamycin complex 1 (mTORC1), a growth factor-, nutrient- and rapamycin-sensitive multiprotein complex that controls cell growth (cell mass) and proliferation. NF2 patient tumors exhibit high levels of S6 phosphorylation, a biomarker of mTORC1 signaling, and in vitro models of human NF2 meningiomas, as well as Nf2-deficient mouse embryonic fibroblasts (MEFs), demonstrate constitutive mTORC1 activation in a growth-factor independent manner. Furthermore, the mTORC1 specific inhibitor, rapamycin, blocks enhanced growth of cultured human merlin-deficient meningioma and merlin-suppressed arachnoidal cells, the normal cell counterpart that meningiomas arise. Our data places merlin upstream of TSC proteins and suggest that intact TSC1/2 complex is required for merlin to inhibit mTORC1. Additionally, we show that hyperactivation of mTORC1 in merlin-deficient arachnoidal meningioma cells results in inhibition of PI3K/Akt signaling in both an insulin-dependent and -independent manner, suggesting mechanisms other than insulin receptor substrate-(IRS) mediated attenuation of Akt signaling may also contribute to Akt inactivation in merlin-deficient cells. Our results show key signaling differences that exist between merlin-deficient human cells and MEFs. We postulate that inhibition of PI3K/Akt signaling resulting from aberrant mTORC1 signaling contributes to the benign nature of NF2 tumors, and plays a protective role in progression to higher grade tumors. Our studies suggest that mTORC1 inhibitors merits consideration for clinical studies as a therapeutic agent against NF2-associated tumors alone, or in combination with PI3K/Akt inhibitors.

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Excess PKA activity affects Nf2 and PAK signaling.

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Signaling events leading to Schwann cell tumor initiation have been extensively characterized, owing to their association with the well-studied inherited tumor syndromes Neurofibromatosis Types 1 and 2. Schwann cell tumors are also observed in patients with the endocrine neoplasia syndrome, Carney Complex (CNC), which results from inactivating mutations in PRKAR1A, the gene encoding the Type 1A regulatory subunit of the cyclic-AMP dependent protein kinase (PKA). We recently described a tissue-specific knockout mouse for Prkar1a in a facial subset of neural crest cells (TEC3KO) that develops benign schwannomas with high penetrance (Jones, et al.; Neoplasia, 2008). Molecular analysis of these tumors demonstrated a profound decrease in the expression of both Nf1 and Nf2 proteins, despite the fact that the mRNA levels were elevated for both genes. Furthermore, small G-protein signaling assays revealed significant activation of Rac1 in TEC3KO schwannomas. In light of these data, we sought to better understand both the molecular basis for loss of the NF proteins, as well as the status of signaling molecules downstream from Rac, namely the p21 activated kinases (PAKs). Genetic interaction studies in vivo showed that mutation of Nf2 enhanced tumorigenesis in the TEC3KO model, whereas mutation of Nf1 had no effect. In light of this, we focused further studies on the interaction of PKA and Nf2. We hypothesized that PKA activation leads to an enhanced degradation of the Nf2 tumor suppressor, thereby causing Schwann cell hyperproliferation. Preliminary experiments confirmed that treatment of rat Schwann cells with a cyclic-AMP analog decreased Nf2 protein levels. Additionally, primary cultures of TEC3KO schwannomas that were treated with MG132 showed a modest increase in Nf2 expression, although these experiments are ongoing. As PAKs may be mediators of Rac signaling, we tested the expression of the six PAK isoforms in TEC3KO tumors. We observed that all six isoforms were upregulated compared to normal mouse Schwann cells and mouse embryonic fibroblasts (MEFs). These results were paralleled by corresponding increases in PAK protein levels in TEC3KO tumors compared to MEFs and rat Schwann cells. Although PAK activity assays are currently ongoing, these results suggest that Rac-mediated PKA activation may play an important role during TEC3KO tumorigenesis. Collectively, these results suggest that Nf2 stability may be compromised during PKA activation, thereby indirectly stimulating the PAK proteins to promote Schwann cell proliferation.

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EGF Receptor Family Activation and Lapatinib in Vestibular Schwannoma

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PURPOSE: Vestibular schwannomas (VS) arising sporadically or in patients with neurofibromatosis-2 (NF2) lack expression of merlin, a putative tumor suppressor gene. Surgery is the mainstay of therapy and no effective medical treatment is known. Recent evidence suggests that merlin deficiency may result in abnormal activation of receptor tyrosine kinases and downstream signaling, promoting tumor growth. Although small-molecule RTK inhibitors are widely available for clinical use, no such therapy has been validated in patients with VS. EXPERIMENTAL DESIGN: To screen for RTK activation, surgical VS specimens from five NF2 patients and four non-NF2 patients were analyzed by phospho-RTK arrays. Downstream signaling pathway activation was analyzed by phospho-MAPK arrays. Activated RTKs and downstream kinases were validated immunohistochemically in five NF2 and four non-NF2 VS samples.
Based on the findings, the small-molecule EGFR/ErbB2 inhibitor lapatinib was selected for evaluation in a preclinical VS model. RESULTS: Phospho-RTK arrays and immunohistochemistry showed consistent activation of EGFR family receptors and Erk1/2 downstream signaling in all samples analyzed. In the preclinical VS model, EGFR/ErbB2 targeted therapy with lapatinib inhibited ErbB2 phosphorylation and downstream Erk1/2 and Akt activation, resulting in decreased proliferation. In addition, the inhibitor of apoptosis (IAP) family protein survivin was consistently expressed in VS and downregulated by lapatinib. CONCLUSION: EGFR family receptor activation is a consistent feature of both sporadic and NF2-related VS. Molecular targeted therapy with lapatinib, a small molecule EGFR/ErbB2 inhibitor, has antiproliferative activity in a preclinical VS model. Based on these findings, a clinical trial with lapatinib for the treatment of VS is currently underway. Survivin expression has been linked to ErbB2 pathway activation in other tumors, and may represent a novel target for treatment of VS.

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**Adaptive and Psychosocial Functioning of Young Children with NF-1: Preliminary Findings**

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Problem and adaptive behavior difficulties were examined in a small preliminary sample of young children with Neurofibromatosis-1 (NF-1). While research has shown that older individuals are at risk for psychosocial and adaptive difficulties, there has been little research in this area about young children. Participants were 16 children with NF-1 (11 boys, 5 girls) between the ages of 3 and 8. Parents completed the Scales of Independent Behavior – Revised (to measure adaptive functioning) and the Behavior Assessment Scale for Children – Second Edition (to measure problem behavior). Children were also administered the Differential Ability Scales – Second Edition to assess intellectual functioning. Mean scores on broad measures of problem behavior and adaptive functioning fell in the average range. When specific areas of function were examined, increased difficulties with hyperactivity [t (15) = 2.53, \( p < .05 \)] and attention [t (15) = 2.40, \( p < .05 \)] were observed at the group level. The proportion of children in this sample with at-risk or clinical levels of problem behavior was compared to that that would be expected based on the normative distribution. Differences were not indicated based on broad areas of problem behavior (e.g., internalizing difficulties, externalizing difficulties). However, when performance at the scale level was examined, participants with NF-1 had increased rates of difficulties with hyperactivity and with anxiety. The proportion of children in this sample with lower adaptive functioning (low average range, borderline range, or delay range) was also examined. Participants with NF-1 had increased rates of difficulties in the areas of motor skills and social and communication skills. Both intellectual functioning and adaptive functioning were negatively associated with problem behavior; children with stronger intellectual functioning tended to have fewer problem behaviors (r = -.40, p = .13) and children with stronger adaptive functioning had significantly fewer problem behaviors (r = -.70, p < .001). In sum, the attention problems, hyperactivity, and anxiety reported for some older children with NF-1 are also observed in some young children with NF-1. These early vulnerabilities may signal a need for proactive supports for some children with NF-1 as they develop. Further implications of these findings will be discussed.

**Early Indicators of Cognitive and Learning Difficulties in Children with NF-1: Preliminary Findings**

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Research indicates that individuals with Neurofibromatosis-1 (NF-1) are at elevated risk for numerous cognitive and behavioral difficulties including intellectual and learning disabilities, language difficulties, and executive function deficits. There is sparse research about the cognitive functioning of young children with NF-1. We report results of a preliminary study of the cognitive functioning of young children with NF-1. Participants were 15 children with NF-1 (10 boys, 5 girls), ages 3 to 8 years. Participants were administered the Differential Ability Scales – Second Edition (DAS-II) to assess intellectual functioning and pre-academic skills and subtests examining visuospatial and fine-motor abilities from the NEPSY – Second Edition (NEPSY-II). Mean DAS-II verbal, nonverbal, and spatial cluster scores fell in the average range. One-sample t-tests compared the participants’ performance to standardized means. Performance was significantly lower than would be expected based on the normative distribution. Differences were not indicated based on broad areas of problem behavior (e.g., internalizing difficulties, externalizing difficulties). However, when performance at the scale level was examined, participants with NF-1 had increased rates of difficulties with hyperactivity and with anxiety. The proportion of children in this sample with lower adaptive functioning (low average range, borderline range, or delay range) was also examined. Participants with NF-1 had increased rates of difficulties in the areas of motor skills and social and communication skills. Both intellectual functioning and adaptive functioning were negatively associated with problem behavior; children with stronger intellectual functioning tended to have fewer problem behaviors (r = -.40, p = .13) and children with stronger adaptive functioning had significantly fewer problem behaviors (r = -.70, p < .001). In sum, the attention problems, hyperactivity, and anxiety reported for some older children with NF-1 are also observed in some young children with NF-1. These early vulnerabilities may signal a need for proactive supports for some children with NF-1 as they develop. Further implications of these findings will be discussed.

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Contribution of Executive Functioning to Academic Achievement in Adolescents with NF-1

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Neurofibromatosis (NF-1) is an inherited neurocutaneous disorder, with an estimated incidence of 1 in 3,000 persons. Many children and adolescents with NF-1 not only suffer significant medical complications, but also deficits in cognitive and academic functioning (Hyman et al., 2005; North, 1998). Executive functioning (EF) deficits have also been described for both children and adults with NF-1 (Ferner et al., 1996; Zoller et al., 1997). Examination of risk factors for poorer academic performance, such as cognitive impairments and EF deficits, is important so steps can be taken to reduce the impact of these difficulties. The purpose of the current study is to examine the relationship between intellectual, academic, and executive functioning. Participants were 27 adolescents ages 12-18 (M age = 13.96, SD = 2.03). They were predominantly Caucasian (88%) and about half (56%) were females. Each participant was administered a measure of cognitive functioning (Kaufman Brief Intelligence Test – Second Edition; KBIT-II), select subtests of the Wechsler Individual Achievement Test – Second Edition (WIAT-II) to assess academic achievement, and tasks examining response inhibition and cognitive flexibility (Dels-Kaplan Executive Function System; D-KEFS). Overall scores on the WIAT-II and D-KEFS fell in the average range and the mean IQ was 94.80 (SD = 17.17). Performance on the measures of academic achievement and EF were significantly and positively correlated with IQ. Older children scored significantly lower on math and spelling tasks and measures of cognitive flexibility. Performance on all WIAT-II subtests was significantly related to measures of inhibition and cognitive flexibility. Regression analyses were used to determine if EF abilities account for variability in academic achievement above and beyond age and IQ. A measure of response inhibition accounted for a significant amount of variance in performance on a word reading task (t = 3.02, p = .008). The relationship between response inhibition and a spelling task approached significance (t = 1.94, p = .07). The decrease in performance with age seen on the WIAT and DKEFS may be related to findings that the prevalence and severity of medical complications tends to increase with age (Riccardi, 1981). Response inhibition may play a role in academic success. Additional implications and directions for future research will be discussed.

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Acceleration of Nf1 deficient bone regeneration by local application of MEK1 inhibitor

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Neurofibromatosis type 1 is an autosomal dominant genetic disease resulting from inactivating mutations in the gene encoding protein called neurofibromin. Neurofibromatosis manifests as a heritable condition with benign tumors of neural tissue located in the skin (neurofibromas) and pigmented skin lesions. Besides these more common clinical problems, many NF1 patients (50 %) have abnormalities of the skeleton. We have previously shown neurofibromin to function in bone development and metabolism, as its biallelic inactivation in early limb bud and cranial mesenchyme results in multiple skeletal phenotypes resembling changes observed in NF1 tibial dysplasia. However, since in the mouse model the affected extremities are not subjected to excessive mechanical load, bone fracturing does not occur spontaneously. Therefore, in order to study Nf1 role in bone repair we established a cortical bone injury model in Nf1Prx1 mice. Our investigation of this model revealed that the bone repair is severely delayed in the Nf1Prx1 mice. Here we present data, indicating local acceleration of the cortical injury healing process in Nf1Prx1 mice by the implantation of the MEK1 inhibitor soaked polymer beads. The mechanism of the bone anabolic effect is clearly associated with the normalization of the osteoblast differentiation, which is confirmed by the results of the in-vitro calvarial osteoblast progenitor differentiation assays. Herby for the first time we report MEK1 inhibition is an effective way to accelerate bone healing in Nf1 deficiency.

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Vascular Inflammation Contributes to Neointima Formation in Nf1 +/- Mice.

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Disorders of the cardiovascular system are one of the least studied complications of NF1. Vascular lesion formation results in increased morbidity and mortality particularly among younger patients. Despite these observations, the mechanism of NF1 vasculopathy remains unknown. Previously, we have shown that Nf1 +/- mice have increased neointima formation in response to mechanical arterial injury compared to wildtype (WT) mice. Further, we demonstrated that the increased neointima formation was driven by an Imatinib Mesylate sensitive molecular pathway. Here, we dissect the role of vascular cells and bone marrow derived cells (BMDCs) in NF1-related vasculopathy. Heterozygous inactivation of Nf1 in vascular smooth muscle cells (VSMCs) or endothelial cells (ECs) alone is insufficient to recapitulate the phenotype of Nf1 +/- mice in response to arterial injury. Utilizing hematopoietic stem cell transfer, we generated WT mice with Nf1 +/- bone marrow (BM) and Nf1 +/- mice with WT BM. In response to arterial injury, WT mice with Nf1 +/- BM have increased neointima formation compared to WT mice. Similarly, Nf1 +/- mice with WT BM have reduced neointima formation compared to Nf1 +/- mice. Mice with Nf1 +/- BM have increased accumulation of BMDCs in the neointima 28 days post-injury. Utilizing immunohistochemistry, we
identified that approximately 50 percent of the BMDCs within the neointima were macrophages, indicating an inflammatory role in vascular lesion formation in Nf1 +/- mice. To further dissect the interaction of vascular cells and BMDCs in neointima formation we utilized cre/lox technology and hematopoietic stem cell transfer to generate mice that were heterozygous for Nf1 in VSMCs and BMDCs (Nf1flox/+SM22cre:Nf1 +/- BM) and heterozygous for Nf1 in ECs and BMDCs (Nf1flox/+Tie2cre:Nf1 +/- BM). In response to arterial injury, Nf1flox/+SM22cre:Nf1 +/- BM mice have a 10-fold increase in neointima formation compared to Nf1flox/+SM22cre mice and are reminiscent of Nf1 +/- mice. Again, these mice have increased accumulation of BMDCs within the neointima. These results suggest that heterozygous inactivation of Nf1 in VSMCs and BMDCs is sufficient for NF1-related vascular lesion formation and that vascular inflammation plays a significant role in neointima formation. The role of inflammation in vascular lesion formation is especially intriguing given that recent studies in our lab indicate that NF1 patients have increased populations of inflammatory and pro-inflammatory monocyte populations in circulation compared to healthy controls.


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Carboxy-terminal regions of merlin regulate both cell growth and cytoskeletal organization

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The NF2 gene product merlin is an atypical tumor suppressor. It is a cytoskeletal component, which in addition to the growth inhibitory properties has morphogenic effects. The morphogenic properties of merlin may play a role in its tumor suppressor mechanism since cytoskeletal defects have been observed in patient derived tumor cells. However, it is unclear how these different functions are linked. Phosphorylation of the carboxy (C)-terminal serine 516 is predicted to unfold merlin and lead to reduced cell growth inhibition and simultaneous merlin-erbin heterodimerization and membrane association. The aim of this study was to characterize the role of merlin’s C-terminal residues in cell growth regulation, cytoskeletal organization, phosphorylation and merlin-erbin binding, and to study whether these functions are linked. In addition to truncating mutations we focused on evolutionarily conserved C-terminal residues 545-547 known to harbour disease causing mutations. To identify the region involved in merlin’s activities, several C-terminally truncated merlin constructs and a construct containing a E545K+E547K missense mutation, disrupting the conserved domain, were expressed in different cell types including N2/-/- MEFs and Schwann cells. The truncated forms of merlin induced a significant increase in membrane-associated cellular projections compared to full-length isoform 1, the effect being dependent on the site of truncation. Residues 538-568 were found to be particularly important for the cell-extension activity of merlin, and merlin 1-547 induced the most drastic phenotype with long, thin cellular processes. Also expression of E545K+E547K resulted in formation of long protrusions. The results indicate that an internal sequence determinant for the cell-extension activity exists in the C-terminal region of merlin. The interplay between merlin and ezrin was also dependent on the length of the merlin C-terminus. In contrast to wild-type isoform 1, the full-length protein with E545K+E547K mutations and some of the C-terminal truncations were able to interact with ezrin. Our combined results suggest that merlin is able to form more complex conformations than just the “open” and “closed” and these conformations require C-terminal residues. Finally, we characterized the growth suppressive properties of merlin in cells expressing active Ras in a soft agar model. Our result show that both merlin isoform 1 and isoform 2 are able to suppress Ras induced cell growth in soft agar, whereas C-terminally mutated merlin constructs showed a decrease in growth suppression. In conclusion, these results indicate that overlapping C-terminal regions of merlin mediate functions related to growth suppression, cellular morphology and control of molecular interactions.

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Merlin suppresses tumorigenesis by inhibiting a novel Cul4 E3 ubiquitin ligase in the nucleus

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Current models suggest that the FERM domain protein Merlin, encoded by the tumor suppressor Neurofibromatosis type 2 (NF2), inhibits mitogenic signaling at or near the plasma membrane. We have discovered that the closed, growth inhibitory form of Merlin accumulates in the nucleus and binds to the receptor component of a novel Cullin 4 (Cul4) E3 ubiquitin ligase. Genetic and biochemical evidence indicates that Merlin functions as a negative regulator of the E3 ubiquitin ligase. Depletion of the receptor component of this ligase blocks exit from contact inhibition and progression through the cell cycle in Merlin-deficient cells. Expression of Merlin and silencing of the E3 ubiquitin ligase receptor induce a largely overlapping program of gene expression, which includes the upregulation of growth arrest and proapoptotic genes and the downregulation of mitogenic and survival genes. Tumor-derived mutants of Merlin fail to accumulate into the nucleus, to bind to the receptor component of the E3 ubiquitin ligase, or to inhibit the ligase. Finally, depletion of the receptor component of the E3 ubiquitin ligase suppresses the ability of Merlin-deficient tumor cells to grow in soft agar and to form tumors in nude mice. These findings indicate that Merlin suppresses tumorigenesis by translocating to the nucleus to inhibit the Cul4 E3 ubiquitin ligase-dependent gene expression.

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Scoliosis in NF1 and Idiopathic Scoliosis: Common genetic significance on chromosome 17q11

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Scoliosis, a lateral structural curvature of the spine, is the most common osseous manifestation of NF1 (10%-30% of patients). Scoliosis curvatures carry significant cosmetic and functional morbidity, defy bracing, rapidly progress, and may demand surgical intervention. Idiopathic scoliosis (IS) is the most common spinal deformity of unknown etiology in otherwise normal children (2-3%). It is a complex genetic disorder associated with several genetic loci on multiple chromosomes. An initial population of IS families (202 families; 1198 individuals) had blood DNA harvested, genomic screening, fine mapping, and analysis by model-independent linkage. A phenotypic subset, families with males having undergone spinal fusion for scoliosis (17 families, 147 individuals), was linked significantly to chromosome 17q11.2. The most prominent marker, D17s975, \( P = 0.0003 \) at 25.12 Mb is adjacent to the NF1 deletional region. A custom panel of fine-mapping SNPs extending from 18.30-31.47 Mb was analyzed for linkage. Two regions with ≥2 contiguous SNPs of significance \( P < 0.05 \) confirm significant linkage adjacent to the NF1 locus (see Table). Elucidation of shared genetic variations within this critical region by two disorders marked by scoliosis will elucidate molecular pathogenesis and guide future counseling and prognoses.

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SPRED1 Mutations in Patients from the Neurofibromatosis Clinic at The University of Utah

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Legius syndrome is a RAS pathway disorder shown to have an overlapping phenotype with neurofibromatosis type 1 (NF1). Legius syndrome, however, has not been shown to result in the more severe complications associated with NF1, specifically tumorigenesis. We developed a Sanger sequencing panel to test mutations in \( SPRED1 \). Patients with the clinical diagnosis of NF1 were enrolled from the NF clinic and tested for \( SPRED1 \) mutations. The aims of this study are to identify the phenotype(s) and frequency of \( SPRED1 \) mutations within our neurofibromatosis clinic. We sequenced 60 patients and identified 2 prepubertal individuals (3%) with \( SPRED1 \) mutations. The observed phenotype for the first patient, age ten, included greater than ten café-au-lait spots, axillary and groin freckling, learning disabilities, and no neurofibromas. The observed phenotype for the second patient, age twelve, included ten to twenty café-au-lait spots, intertriginous freckling, a single reported Lisch nodule by slit lamp examination, no learning disabilities, and no neurofibromas. Both patients have novel nonsense mutations (p.R18R/X and p.Q194Q/X). In addition, a high frequency haplotype was identified in 9 individuals (15%). The haplotype included four single nucleotide polymorphisms (SNP) [two were intronic, one within exon 4, and one within exon 8]. Eight of the nine patients exhibiting the haplotype showed all four SNPs, while one showed three of the four SNPs. This study is ongoing to better identify the frequency and phenotype of these disorders.

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Auditory Processing Disorders in a NF1 patient

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Auditory Processing Disorders (APDs) refer to difficulties in the perceptual processing of auditory information in the auditory nervous system as demonstrated by poor performance in one or more of the following skill areas: auditory discrimination, auditory pattern recognition, temporal aspects of audition, auditory performance in competing acoustic signals, and auditory performance with degraded acoustic signals. Aim: The purpose of this study was to describe the
results obtained in the auditory processing (AP) evaluation in a 31 year-old NF1 patient with learning disorder. Method: The patient has been evaluated through different objective and behavioral auditory tests, including audiometric test, immittance and auditory processing evaluation. The patient was required to pass a pure-tone, hearing screening and also to pass a tympanometry screening to ensure normal hearing and normal functioning of eardrum and middle ear system. Results: The diagnosis of APD was confirmed by normal performance on audiometric test battery and auditory processing evaluation which have shown the presence of severe hearing processing disorder, characterized by the alteration of integration, selective attention, auditory closure and memory process, and significant difficulties in supra-segmental and decoding. Conclusion: This NF1 patient present an impairment of central neurological processing that may be detected by auditory processing tests, which could contribute to the cognitive function and academic performance. References: American Speech-Language--Hearing Association. (2005). (Central) Auditory Processing Disorders—The Role of the Audiologist [Position Statement]. Available at www.asha.org/policy

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Vitamin D levels correlates with short stature but not with macrocephaly in NF1 patients

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Short stature (SS), macrocephaly (MC) (1), bone abnormalities (2) and lower levels of 25-OH-vitamin D (VitD) (3) have been observed in NF1 patients. It was hypothesized whether VitD would be correlated with SS and MC in NF1 patients. Height, head circumference (HC) and serum VitD levels (chemiluminescence) were measured in a group of randomly selected volunteer NF1 patients (n=27) and in healthy controls (n=19) matched by age and sex. The individual (x) height and HC were plotted into the WHO tables for height (4) and HC (5) for normal growing and calculated the number of SD the x value was away from the expected mean for sex and age [z score = (x-mean)/SD]. Twenty two of 27 NF1 patients were under the mean expected height (negative z values) and 20 of 27 were above the expected mean HC (positive z values). There was a positive correlation (r=0.4538; p<0.05) between lower VitD levels and height reduction in the NF1 group with SS, but not in the healthy controls nor in the NF1 patients without SS. There was not correlation between HC and VitD levels. The results suggest a relationship between SS and VitD levels in NF1 patients deserving of further investigation. These height and head circumference different patterns are in agreement with previous report indicating that SS and macrocephaly are independent phenomena of the NF1 disease (1). Conclusion: Lower levels of VitD correlated with short stature but not with increased head circumference in a group of NF1 Brazilian patients. References: 1) Riccardi VM. Skeletal system. In Neurofibromatosis: phenotype, natural history and pathogenesis. Edited by Friedman et al. JHU Press, 1999; 2) Stevenson DA et al. Bone mineral density in children and adolescents with NF1. J Pediatr 2007; 150: 83-88; 3) Lammert M et al. Vitamin D deficiency associated with number of neurofibromas in neurofibromatosis 1. J Med Genet 2006; 43: 810-813. 4- http://www.who.int/growthref/who2007_height_for_age/en/index.html ; 5) Bushby KM et al. Centiles for adult head circumference. Arch. Dis. Ch. 1992; 67:1286-1287.
OSU-03012 and HDAC-42: Novel Small Molecule Inhibitors of the AKT Pathway for the Treatment of Vestibular Schwannoma and Malignant Schwannoma

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Inactivating mutations in the Neurofibromatosis 2 (NF2) gene encoding the tumor suppressor protein merlin commonly cause vestibular schwannomas (VS). Treatment options for merlin-deficient tumors are currently limited to surgical resection or stereotactic radiation. However, serious post-operative complications can arise, including deafness, facial nerve paralysis, or radiation-induced malignant transformation. Moreover, there are no accepted chemotherapeutic options for these tumors since they resist conventional chemotherapy. We are investigating two novel potential therapeutics for these tumors: (1) OSU-03012 inhibits phosphoinositide-dependent kinase 1 (PDK1), which phosphorylates and activates the pro-survival protein AKT; (2) HDAC-42 is histone deacetaylase (HDAC) inhibitor and also inhibits AKT activation as well as inducing cell cycle inhibitors. We tested OSU-03012 and HDAC-42 on patient-derived VS cells, normal human Schwann cells, normal and merlin-deficient mouse Schwann cells and schwannoma cells, and human malignant schwannoma HMSC-97 cells. We first determined the efficacy of both inhibitors on cell proliferation and viability using MTS assays. We found that OSU-03012 and HDAC-42 potently decrease the proliferation of human VS and mouse NF2-/- schwannoma cell proliferation with IC50 values in the low micromolar to high nanomolar range, respectively. We next investigated whether these drugs induce apoptosis using TUNEL assays and Western blots for caspase-9 activation. OSU-03012 potently and selectively induces apoptosis in VS and HMSC-97 schwannoma cells at doses that spare normal Schwann cells, and appears to do so through AKT inhibition and activation of the intrinsic pathway as determined by caspase-9 cleavage. Moreover, merlin-null mouse Schwann cells were more sensitive to OSU-03012 than genotypically normal Schwann cells. HDAC-42 appears to inhibit schwannoma cell proliferation by inhibiting AKT and possibly inducing cell cycle arrest through p21 induction. Finally, we tested the in vivo efficacy of orally delivered OSU-03012 and HDAC-42 in xenografted SCID mice. We found that OSU-03012 reduced the size of HMSC-97 xenografts by 55% as determined by small-animal MRI, and the tumor histology showed massive areas of necrosis and decreased phospho-AKT staining. VS-xenografted SCID mice fed with HDAC-42 also showed a roughly 50% reduction in tumor size. Thus, OSU-03012 and HDAC-42 are two novel drugs with the potential to treat VS and malignant schwannomas, two tumors that have resisted conventional chemotherapy. Additionally, we are investigating potential synergistic effects by these two drugs.

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“Simple” Disorders, Complex Traits: A Search for Genetic Modifiers in NF1

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Background. The cause of the variation in phenotypic severity in neurofibromatosis type 1 (NF1) is unknown and may be due to genetic modifiers. A differentially expressed transcript that correlates in a statistically significant way with a measurable phenotype is referred to as a “quantitative trait transcript” (QTT). Identification of QTTs (and any corresponding expression quantitative trait loci (eQTL)) is a novel and emerging technique to identify modifier genes in animal models and humans.1,2 We hypothesized that variation in germline gene expression of certain genes correlates with variation in the severity of quantifiable phenotypic features ("sub-phenotypes") of NF1. Methods. We performed whole-genome transcriptional profiling (Illumina HumanRef-8 arrays) in lymphoblastoid cell lines from 79 individuals affected with NF1 and 23 controls. A single observer quantified severity in multiple NF1 sub-phenotypes, including height, head circumference (OFC), burden of cutaneous neurofibromas (CNF), café-au-lait macules (CALM), Lisch nodules (LN), and cherry hemangiomas (CH). We examined the correlation of the 6 NF1 sub-phenotypes with the level of each of the 22,177 transcripts. To control for multiple testing, we calculated a False Discovery Rate (FDR), in addition to a nominal P-value of the significance of the regression. We filtered for FDR (< 0.3), expression range (~2x) and expression level (mean log2 > 6.0). Results. From the statistical analysis, we identified 32 unique transcript-phenotype pairs (QTTs). These included 6 genes whose expression level significantly correlated with CALM burden, 2 with CH burden, 8 with LN burden, 1 with CNF burden, 8 with height and 7 with OFC. We then validated 22 QTTs by quantitative PCR on low-density microfluidic arrays (ABI). By qPCR, 9 QTTs remained statistically significant (nominal P-value < 0.05). Many QTTs were gender-specific, even for traits not known for sexual dimorphism (e.g. café-au-lait macule burden). Conclusions. We identified 9 putative genetic modifiers (QTTs) of severity in NF1. On-going investigation of these 9 QTTs includes genotyping known eQTLs and gene-specific functional validation. References: 1) Passador-Gurgel G, Hsieh W, Hunt P et al. Quantitative trait transcripts for nicotine resistance in D. melanogaster. Nat. Genet. 2007; 39: 264-268. 2) Morley M, Molony CM, Weber TM et al. Genetic determinants of genome-wide variation in gene expression. Nature 2004; 430: 743-747.

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Children with Neurofibromatosis type 1 show contrast sensitivity deficits compatible with abnormal inhibitory transmission in visual brain areas

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Neurofibromatosis type 1 (NF1) is a genetic disorder characterized by an increased risk of tumor formation, mainly neurofibromas and optic gliomas, and impaired cognitive functioning. Importantly, the cognitive function of these patients can be affected even in the absence of brain tumors suggesting that these deficits are a consequence of abnormal neuronal physiology. The development of NF1 mice models lead to the proposal of brain mechanisms that might underlie the cognitive deficits associated with this disease. The learning deficits observed in NF1 mice are a consequence of abnormally high levels of neuronal inhibitory transmission. These animal studies suggest that a similar neuronal anomaly might underlie the cognitive deficits found in NF1 patients. As abnormal inhibitory transmission might cause alterations in visual perception, including contrast sensitivity, we have therefore investigated if contrast sensitivity, chosen as a biomarker of inhibition, is indeed affected in NF1 children. Three distinct neuronal pathways transmit information from the retina to the visual cortex, the magnocellular, parvocellular and koniocellular pathways. We analysed these pathways separately by choosing visual stimuli that preferentially activate only one of these pathways. Interestingly, we found a deficit in the contrast sensitivity of the parvocellular pathway. In contrast, the magnocellular and koniocellular pathways are not affected in NF1 children. This result is consistent with similar observations in long-term users of Lorazepam, a drug that enhances GABAergic transmission, suggesting that alterations in inhibitory neurotransmission might affect preferentially the parvocellular pathway. Future work will attempt a correlation analysis between genotype and the inhibitory phenotype. So far 10 patients have been genotyped.

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Recent Progress in the Structural Analysis of the Neurofibromin

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Neurofibromatosis type 1 (NF1) is caused by alterations of the NF1 gene, leading to a missing or nonfunctional protein product neurofibromin (320 kDa). Using structural/biochemical approaches, we have previously characterized the RasGAP domain and recently discovered a novel bipartite module consisting of a lipid-binding Sec14 homology domain and a previously undetected pleckstrin homology (PH) like domain. Numerous trials to obtain additional fragments allowing biochemical analysis were unsuccessful either because of insolubility or aggregation problems. Focusing on the full-length protein we are currently using single particle and cry electron microscopy approaches in order to obtain structural information about the protein giant. First 3-dimensional views of neurofibromin will be presented and implications discussed.

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Vitamin D and calcium serum levels in NF1 patients

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In our previous study, it was hypothesized that 25-OH-vitamin D (VitD) and calcium metabolism (Ca++) could be possibly involved in reduced muscular force observed in NF1 patients (1), because low serum VitD levels were previously reported in NF1 patients and it was correlated with the number of neurofibromas (2) and bone abnormalities (3). Methods: VitD (chemiluminescence) and calcium levels (total: colorimetric; ionic: selective electrodes) were measured in a group of Brazilian NF1 patients (n=28) and healthy controls, matched by age and sex (n=20). Mean serum VitD and calcium levels in patients with NF1 and controls were compared using t Student’s test. Results: No difference in VitD levels was observed comparing NF1 patients (24.1 ± 9.3 ng.dL-1) and healthy volunteers (25.3 ± 9.3 ng.dL-1) (P=0.582). NF1 patients presented higher total serum calcium level (9.8 ± 0.6) than healthy volunteers (9.5 ± 0.4) (P=0.015). However, serum ionic calcium levels only presented a tendency to be different between NF1 group (4.8 ± 0.2 mg.dL-1) and healthy individuals (4.8 ± 0.1 mg.dL-1) (P=0.053). Discussion: Our observation of similar serum concentrations of VitD between NF1 patients and healthy volunteers could be due to higher solar radiation in Brazil compared to Germany. Moreover, the results suggest peripheral calcium participation in the mechanisms responsible for reduced muscular force observed in NF1 patients and it deserves further investigation. Conclusion: Total calcium serum levels were higher in NF1 patients but VitD levels were similar to healthy volunteers. References: 1) Souza JF, Guedes ACM, Rodrigues, Rezende NA. Some under-reported clinical complications in NF1 patients. 2008 NF Conference Abstract 140)2 Lammert M, Friedman JM, Roth HJ, Friedrich RE, Kluwe L, Atkins D, Schooler T, Mautner V-F. Vitamin D deficiency associated with number of neurofibromas in neurofibromatosis 1. J Med Genet 2006; 43: 810-813 3) Stevenson DA, Moyer-Milieur LJ, Murray M, Slater H, Sheng X, Carey JC, Dube B, Viscochil DH. Bone mineral density in children and adolescents with neurofibromatosis type 1. J Pediatr 2007; 150: 83-88.

Additional Authors: Passos RLF, Ferreira MCM, Toledo LL, Rezende NA, Rodrigues LOC - Neurofibromatosis Outpatient Reference Center, School of Medicine, Federal University of Minas Gerais, Brazil
Prevalence and Spectrum of Osseous Anomalies in Neurofibromatosis Type 1

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Objective: We retrospectively reviewed the prevalence and spectrum of osseous anomalies in a large cohort of Neurofibromatosis type 1 (NF-1) patients. To date, there is only limited data in this area. Methods: Medical records of all patients (n = 388, M=F) with NF-1 seen between 2000 and 2007 were reviewed for the presence of radiographic and/or clinical bony anomalies. Results: Bony anomalies were present in 114/388 (29.4 %, M=F). 42/388 (10.8 %) patients had multiple abnormalities. (i) Scoliosis/kyphoscoliosis (n=50) was commonest followed by, (ii) localized bone erosions including scalloping of vertebral (n=15) and bony dysplasia (n=15) of spine, ribs, pelvis, long bones, cranium and sphenoid wing, and (iii) localized osteopenia (n=13) and benign bone lesions (n=13): tibial non ossifying fibroma, femoral osteochondroma, aneurysmal and unicameral bone cysts. Other anomalies included pectus excavatum (n=11), degenerative spine disease (n=10), pseudoarthrosis of tibia/fibula/other bones (n=10), widening of neural foramina (n=9), long bone deformity including bowing/angulation (n=9), macrocephaly (n=6), leg-length discrepancy (n=5), spinal canal stenosis (n=3), and compression deformity/fracture of vertebral bodies (n=3). In addition, fused vertebral, advanced bone age, decreased bone mineral density, and short stature were noted (n=8, 2 each), while mandibular hypoplasia, pectus carinatum, and delayed bone age were noted only once in the cohort. Conclusions: In our NF-1 cohort, a wide spectrum of bony anomalies was noted in 30% of patients, a larger number than previously reported. Spinal deformity was the predominant bony manifestation in 13% of the cohort. Clinicians should carefully screen for osseous anomalies in all patients with NF-1. Future prospective studies should include screening for bone density, bone overgrowth and undergrowth and secondary bony defects. Earlier recognition and treatment will prevent complications.

Effects of NF1 haploinsufficiency on stochastic gene expression

Douglas R. Stewart
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Background: Using a computer model, Cook et al (PNAS 95: 15641-15646, 1998) predicted that haploinsufficiency syndromes may result in increased stochasticity (“noise”) in gene expression. Studies of the effects of NF1 haploinsufficiency on morphological phenotypes in keratinocytes and melanocytes were congruent with these predictions (Koivunen et al. J Invest Dermatol 114: 473-479, 2000 and Kemkemer et al. PNAS 99: 13783-13788, 2002). We investigated differences in gene expression stochasticity in NF1-haploinsufficient and control cells. Data Set and Analyses: Whole-genome transcriptional profiling of lymphoblastoid cell lines from 13 females affected with NF1 (A) and 10 unrelated female controls (U) was performed under similar conditions in February and June. Raw expression data was background-subtracted, quantile normalized and log2 transformed. To measure noise, we calculated the correlation between the 2 time points for each of the 10,757 expressed transcripts for both groups (A and U). We then calculated the mean of the correlation coefficients across all transcripts in A and U, as well as the difference in mean correlation between the 2 groups. We tested the latter using a permutation test. We also examined the variability of the change in expression between February and June for all 10,757 expressed transcripts in the 2 groups. Results: In our first analysis, the average correlation for all transcripts was significantly greater in the unaffected group than the affected group (U = 0.549; A = 0.428; difference = 0.121, P = 0.003, 1000 permutations). The differences were observed across all genes; no single transcript was identified with sufficient evidence of a difference in correlation to remain significant after multiplicity adjustment. In the analysis of variability of change over time, there were no statistically significant differences between the 2 groups. Discussion: In the unaffected cells, we observed a statistically significant higher average correlation between time points when compared to the NF1-haploinsufficient cells. To the extent that the comparison of mean correlation over 2 time points approximates expression noise, our observations are consistent with the predicted effect of haploinsufficiency. However, this effect was not observed in our analysis of variability of change in expression over time. The causes of this discrepancy and the ontology of the top transcripts from each analysis are subjects of investigation.

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Incidence and Clinical Characteristics of MPNSTs at the University of Chicago

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Using our NF database cross referenced with the Univ. of Chicago Cancer Database, and the University of Chicago Orthopedic database, we identified 47 patients with NF1 and MPNSTs that have been evaluated since 1989 for an overall incidence of 3.7% in our NF clinic. Approximately 25% of these patients presented for evaluation of possible/probable tumors at the first visit. The patients were equally divided between male and female. The mean age of presentation was 33.4 years (range 2 -59 years of age). The most common locations were extremities, followed by pelvis, neck, and retroperitoneum. All but one of the MPNSTs arose within plexiform neurofibromas but the clinical phenotype of the patients varied considerably: 33% had isolated discrete plexiform tumors on a background of mild NF1, in 28% the tumors arose within large internal plexiform masses, 11% in multinodular nerve lesions, and 15% in superficial tumors. Whatever possible, tumors were subjected to wide resections. Most were given radiation therapy prior to resection, and most received chemotherapy (using various agents). Tumor samples were examined pathologically for malignancy as well as reactivity with specific Schwann cell transcription factors. Less than 5% of the tumors were perceived to be low grade. Our sample included a small number of unusual tumors including 2 Triton tumors and 2 with myoid or lymphoid features. The mean length of survival was 7.9 years (median 5 years, range 1 month – 40 years). Survival was longest...
in patients with MPNSTs within superficial lesions. Seven of our patients had multiple malignancies, including 3 with MPNSTs separated by several years. A strong family history of cancer was present in several patients and 12% of patients had a family history of MPNSTs. Specific Schwann cell transcription factors Fox D3, AP-2alpha were upregulated in all the MPNSTs and a subset had upregulation of Pax7 and Sox5. No single chemotherapy combination clearly enhanced survival. The incidence of MPNSTs in our population is probably increased on the basis of ascertainment bias, suggesting that MPNSTs in our population are less common than previously reported. The clinical phenotype was not clearly helpful in identifying patients at risk, although there may have been an increase in incidence in patients with multinodular plexiform neurofibromas and familial MPNSTs.

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Are “Screening” MRIs for asymptomatic NF-1 patients Necessary or Cost Effective?

Sahithya Wintrich
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Introduction: There is significant debate about the value of baseline MRIs in asymptomatic NF-1 pediatric patients. There is no consensus regarding this practice among physicians. Objective: The purpose of our review was to determine whether the abnormalities found in baseline MRI scans were significant enough to impact clinical care. Methods: IRB approval was obtained and no sedation complications were encountered. A retrospective chart review of patients under 22 years, evaluated from 1995-2008 at the Cleveland Clinic with MRI scans dating from 1989-2008 was conducted. Inclusion criteria were: 1) Absence of neurological symptoms (excluding mild behavioral problems, ADD and LD) 2) a normal neurological exam. Patients scanned for other reasons: mental retardation, cerebral palsy, seizures, significant headache, impaired vision or hearing loss, movement disorders, autism or pre-existing tumors, trauma or vascular abnormalities were excluded. Results: 183 patients were reviewed of which 61 patients were excluded. 48 had neurological symptoms or signs and 13 had incomplete medical records. 122 (ages 3 months – 21 years) presented with no significant neurological symptoms or signs and had screening MRI scans. There were 64 (52.46%) males and 58 (47.54%) females with a mean age of 5.94 years. We found 35 (28.69%) without any abnormalities and 87 (71.37%) of the 122 with abnormalities. The abnormal MRIs included 41 (47.13%) that had UBOs as the only abnormality. 28 (32.18%) had abnormalities such as enlargement of optic apparatus, enlarged brainstem, arachnoid cysts, ChiarI I, neurofibromas, thickened corpus callosum, that would need clinical follow-up only on presentation of symptoms. 18 (20.69%) had significant abnormalities including: enhancement of the optic apparatus, optic gliomas, brainstem glioma, cortical dysplasia, hydrocephalus, astrocytoma of the corpus callosum and distal left ICA stenosis requiring definite clinical follow-up or intervention. Conclusion: Significant abnormalities requiring clinical and radiological follow up were noted in 18 (20%) patients, with screening MRIs. In addition, 41 (47%) had UBO’s only and 28 (32%) had various less significant abnormalities not requiring follow up. The above data are significant and suggest the need for a prospective case controlled multi-center study. If pre-symptomatic identification of significant lesions results in earlier treatment, fewer complications and an improved outcome, then a screening MRI can be justified in the evaluation of patients with NF-1.

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Inhibition of the VEGF pathway can alleviate growth of NF2-associated schwannomas

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Neurofibromatosis type 2 (NF2) is an autosomal dominant genetic disorder characterized by the presence of non-malignant tumors in the central and peripheral nervous system, including schwannomas, meningoima, and epedynomias. The role of the tumor microenvironment in these tumors has remained largely unexplored. We recently reported high levels of VEGF in both NF2 and spontaneous schwannomas from a series of patient archival specimens (Plotkin et al, 2008, submitted). These findings led us to postulate that VEGF and its downstream signaling may also contribute to the progression on slow-growing tumors. In the current study we investigate the potential benefit of targeting the VEGF pathway to control schwannoma progression. To mimic the microenvironment of schwannomas, we implanted tumors between the pia and arachnoid mater in nude mice bearing transparent cranial windows suitable for longitudinal studies using intravital microscopy with a multi-photon laser scanning microscope. Two cell lines were used: human HEI193 cells (a kind gift of X. Breakefield) and murine Nf2-/- Schwann cells. Tumors were treated with a human monoclonal antibody specific to human VEGF (bevacizumab, 10mg/kg/week i.v.) or a tyrosine kinase inhibitor of the VEGF and EGF receptors (vandetanib, 50mg/kg/day p.o.), respectively. We found that bevacizumab reduced HEI193 vessel density by 30% and decreased the vessel permeability to BSA by 36% 24 hours after the first injection. This effect was sustained and permeability was still reduced by 52% 6 days after treatment. Vandetanib treatment showed similar results, reducing tumor vessel density by 34% 6 days after treatment and suppressing vessel permeability by 65% at 24 hours and 74% after 6 days. In addition, when HEI193 tumors were implanted in the sciatic nerve of nude mice, 9 days oftreatment reduced tumor size by almost 90% with bevacizumab and by 70% with vandetanib. The permeability effect was stronger in the murine Nf2-/- tumors treated with vandetanib, most likely due to the fact that bevacizumab is unable to block host-derived VEGF. Also, EGFR is expressed by tumor endothelium and the dual blockade induced by vandetanib might be beneficial. However, bevacizumab induced a greater increase (by 85%) in the lifespan of nude mice bearing HEI193 tumors than vandetanib in mice bearing Nf2-/- (only 37%) suggesting that VEGF might activate receptors other than VEGFR2 alone. In conclusion, this study clearly shows for the first time that targeting the VEGF pathway in benign schwannomas can be effective in controlling the microenvironment and ultimately overall tumor growth.

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Expression of EGFR modifies neurofibroma number and promotes MPNST formation

Jianqiang Wu
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Neurofibromatosis type 1 (NF1) is an inherited disease predisposing to benign neurofibromas and malignant peripheral nerve sheath tumors (MPNST). Neurofibromas develop from Schwann cells and/or their precursors, while MPNST are believed to derive from glial cells that dedifferentiate to a neural crest-like cell, or directly from neural crest cells. We used gain- and loss-of-function experiments to test the relevance of EGFR to peripheral nerve tumor formation and progression to malignancy. Nf1-/-; EGFR+ cells isolated from E12.5 dorsal root ganglia (DRG) form colonies and resemble Schwann cells precursors. The Waved-2 (Wa2) hypomorph has reduced EGFR activity. Nf1flox/flox;DhhCre;Wa2/+ DRG cells have diminished colony formation compared to those of Nf1flox/flox;DhhCre, which together with previous data (Williams et al. 2008) supports a role for EGFR in precursor maintenance or expansion. Nf1-/- precursors were immortalized in vitro by exposure to EGF. Athymic nude mice were injected subcutaneously with Nf1-/- precursors (no EGFR) or EGFR-immortalized cells. We detected large GEM-PNST in mice engrafted with EGF-treated cells whereas small benign lesions were present in mice injected with progenitor cells naïve to EGF, indicating progenitor malignant transformation. To inspect tumor formation in the context of decreased EGFR signaling, we used the Nf1flox/flox;DhhCre;Wa2/+ mouse line. Mice show slightly increased survival time as compared to Nf1flox/flox;DhhCre mice but form similar numbers and sizes of GEM-neurofibroma. To investigate effects of increased EGFR on peripheral nerve tumor formation in vivo, we crossed CNPase-hEGFR/EGFR mice with Nf1flox/flox;DhhCre mice to obtain Nf1flox/flox;DhhCre;EGFR mice. Tumor number was increased following EGFR over-expression compared to the Nf1flox/flox;DhhCre mice (p<0.01). Tumor size was also increased in Nf1flox/flox;DhhCre;EGFR mice (p<0.05). Tumors from Nf1flox/flox;DhhCre mice demonstrate classic neurofibroma histology of a heterogeneous cell population containing both S100_+ and S100_- cells (Wu et al., 2008). On an EGFR background, tumors developed features of high grade GEM-PNST and 6 of 15 mice developed fast-growing tumors, some with features suggestive of muscle differentiation, which expressed human EGFR. Thus reduced EGFR diminishes Schwann cell precursor number and perhaps rate of neurofibroma formation, while increased EGFR expression increases neurofibroma number and promotes malignant transformation.

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Natural history and therapeutic effects of RAD001 on a neurofibroma mouse model

Jianqiang Wu
Cincinnati Children’s Hospital Medical Center

Neurofibromas are benign tumors that can cause disfigurement, nerve compression, and distortion or overgrowth of adjacent structures and can compress vital structures causing mortality. Several molecularly targeted agents that aim to slow the growth of plexiform neurofibromas (PN) are currently being tested in clinical trials. Using the Nf1flox/flox;DhhCre mouse model of PN in preclinical drug testing could facilitate the selection of promising agents for human trials. We used magnetic resonance imaging (MRI) to monitor neurofibroma development in the Nf1flox/flox;DhhCre mouse model. Mice (n=5) were imaged at 4, 6, 8, 10, 12, and 14 months using a Bruker 7.0T MRI. Tumor growth was determined by volumetric measurements. Mice developed measurable tumors as early as four months of age (n=2). Four of 5 (80%) mice developed visible neurofibromas by 6 months of age (>20mm³). All of the mice developed neurofibromas by 12 months. Based on these kinetic data and previous studies implicating mTOR signaling in NF1 mutant cells we tested the therapeutic effect of RAD001 (Novartis), an mTOR inhibitor, in this model. In a preliminary experiment 5 mice were treated for 2 months with 10mg/kg/day RAD001; all mice survived the treatment without significant side effects. Volumetric measurement showed that the tumor burden was decreased by more than 25% compared to the vehicle control group. Western blots showed that RAD001 inhibited its predicted target, pS6K. A larger cohort of mice (n=16) has now been imaged by MRI at 6 - 7 months of age and at 7 – 8 months to obtain tumor growth rates. RAD001 is being administered by daily oral gavage for 2 months and mice will be imaged by MRI at 6-7 months of age (n=2). Four of 5 (80%) mice developed visible neurofibromas by 6 months of age (>20mm³). All of the mice developed neurofibromas by 12 months. Based on these kinetic data and previous studies implicating mTOR signaling in NF1 mutant cells we tested the therapeutic effect of RAD001 (Novartis), an mTOR inhibitor, in this model. In a preliminary experiment 5 mice were treated for 2 months with 10mg/kg/day RAD001; all mice survived the treatment without significant side effects. Volumetric measurement showed that the tumor burden was decreased by more than 25% compared to the vehicle control group. Western blots showed that RAD001 inhibited its predicted target, pS6K. A larger cohort of mice (n=16) has now been imaged by MRI at 6 - 7 months of age and at 7 – 8 months to obtain tumor growth rates. RAD001 is being administered by daily oral gavage for 2 months and mice will be imaged by MRI at the end of treatment. We will use volumetric measurement to measure the tumor volume and final results will be reported. Our preliminary data suggest that RAD001 might have clinical therapeutic effects on neurofibromas.

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Examination of Key Signaling Pathways in Clinically Aggressive Vestibular Schwannomas

Charles W. Yates
Ohio State University

Vestibular Schwannomas (VS) are usually slow-growing tumors that originate from cranial nerve VIII. Currently, there are no known medical therapies for these tumors. Surgical excision or stereotactic radiation remain the only viable treatment options. Unfortunately, complications from treatment, such as hearing loss, facial weakness, intracranial bleeding, and stroke, remain major concerns. Recurrence after surgical excision is infrequent and occurs in <1% of our patients. When tumors recur, they generally grow slowly at 1-to-2 mm per year. A rapidly growing recurrent tumor would imply that they acquire altered cellular mechanisms for growth advantage. The objective of this study was to identify the molecular changes associated with the rapid growth of
recurrent VS. Initially, we identified a patient who underwent a complete translabyrinthine excision of a 2.4-cm primary VS of the left cerebellopontine angle, but experienced unusually rapid regrowth. Surveillance MRI four years after the initial excision showed a recurrent mass measuring 2.8 cm. Neuropathology confirmed a benign-appearing schwannoma. Excision of both the primary and recurrent tumors showed tenacious adherence of both masses to their surrounding environment, specifically to the facial nerve. Prolonged surgical times were required for successful anatomical salvage of the seventh cranial nerve; however, recovery of facial function was partial and prolonged. Facial function three months after excision of the recurrent schwannoma was still House-Brackmann grade VI/VII. Both the primary and recurrent tumors carry a four-nucleotide deletion in exon 10 of the NF2 gene, and showed little merlin protein staining. Low Ki-67 immunoreactivity was also seen; however, the recurrent tumor showed a slight increase in PCNA reactivity. Interestingly, immunoreactivity to phospho-PTEN and p53 was stronger in the recurrent tumor than the primary tumor, while phospho-AKT, phospho-PAK2, and b-Catenin staining remained high in both the primary and recurrent tumors. Based on these results, we identified additional six cases where there was a surgical excision attempted for complete removal of a tumor and a recurrence of the tumor requiring surgical excision. Two of the patients had neurofibromatosis type 2 and four had sporadic VS. Additionally, six patients with grossly cystic-appearing tumors on MRI and during surgery were identified. Cystic tumors were also chosen due to their clinically aggressive behavior. All tumor specimens were confirmed as benign schwannomas by the neuropathologist. Immunostaining of the primary and recurrent tumors revealed a difference in E-cadherin expression in one of the samples. Further examination of p53, phospho-PTEN, and other key signaling protein expression that might explain the clinical aggression of these otherwise pathologically benign tumors is ongoing.

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Therapeutic intervention of preclinical symptoms in a NF1 mouse model

Huarui Zheng
University of Michigan Medical School

We previously generated a mouse model by using Schwann cell lineage-specific ablation of Nf1, which recapitulates human plexiform neurofibroma with high penetrance throughout the peripheral nerves including the sciatic nerves. Although it takes more than 12 months for these mutant mice to develop frank neurofibromas, we have identified pre-neoplastic phenotypes in mutant sciatic nerves at as early as 3 months of age. In sciatic nerves of mutant mice at post natal day 90 (P90), we identified a subset of non-myelinating Schwann cells (nmSCs) as early stage neurofibroma cells, which exhibit a spectrum of abnormalities. These abnormal nmSCs are either dissociated or dissociating from axons, often accompanied by axonal degeneration and mast cell infiltration. Proliferation of these abnormal nmSCs leads to significantly increased cellularity in mutant nerves. Altered mTOR (mammalian target of rapamycin) activity was also observed at this stage. Based upon these observations, we propose that deregulated mTOR activity may be underlying molecular mechanism of neurofibroma initiation. To test this hypothesis, we are using rapamycin, a mTOR inhibitor, to treat mice starting from P30 until P90, at which time point they are analyzed. The extent of pre-neoplastic phenotypes at P90 can serve as assays for the efficacy of this treatment. Detailed results will be presented at the meeting.

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### ABSTRACTS

**Poster Presentation: Session 2 (even numbers)**

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Timing of Nf2 Inactivation Differentiates between Merlin’s Roles in Neural Tube Development and Tumorigenesis

Elena M. Akhmametyeva
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The NF2 tumor suppressor gene is frequently inactivated in neurofibromatosis type 2 (NF2)-associated vestibular schwannomas and encodes a protein named Merlin that plays an important role in regulating actin cytoskeleton-mediated processes, adherens junction formation, and cell proliferation. Studies of Nf2 knockout in mice have revealed that Merlin function is essential during early development; however, its exact role is not understood. In addition, whether Merlin plays any roles at later stages of development is not known. Previously, we reported a dynamic change in NF2 promoter expression during neural tube closure and neural crest cell migration. While little NF2 promoter activity was detected in premigratory neural crest cells, significant activity was seen in the neural crest cells already migrating away from the dorsal neural tube. To examine Merlin’s function during neural tube development and tumorigenesis, we employed two conditional gene knockout approaches: (1) Wnt1-Cre to inactivate Nf2 in the dorsal neural tube and (2) the tamoxifen-inducible Cre/LoxP recombination system to inactivate Nf2 in neuroprogenitor cells at different stages of mouse development. We found that the Wnt1-Cre,Nf2\(^{\text{flox2/flox2}}\) embryos were smaller in size than the wild-type embryos and displayed defects in neural tube closure. Neural tube explants from the mutant embryos adhered poorly to the fibronectin-coated substratum, and mutant neural crest cells were unable to migrate. These results indicate that Merlin plays important roles during neural tube closure and neural crest cell adhesion and migration. To confirm these findings and to further examine Merlin function at other stages of development, we generated Nestin-CreER;Nf2\(^{\text{flox2/flox2}}\) mice. Upon tamoxifen induction, Nf2 inactivation in neural progenitor cells during early gestation resulted in defects in neural tube closure and development. Immunohistochemical analysis revealed that Merlin was essential for the apical-basal polarity of the neuroepithelium and that Merlin-deficient neuroprogenitor cells are devoid of cadherin complexes and β-Catenin. Consequently, Nf2 inactivation affects neural stem cell proliferation, differentiation, and apoptosis. In contrast, when Nf2 was inactivated in neuroprogenitor cells during mid-to-late gestation, survival pups were obtained. Interestingly, these mice developed schwannomas and/or lymphomas at a high frequency (>50%). Although Merlin inactivation resulted in the loss of cadherin expression, Merlin-deficient tumor cells readily expressed Nf2 and its downstream signals. Collectively, our results show that Merlin plays important roles during neural tube development. Nf2 Inactivation in neuroprogenitor cells at mid-to-late gestation leads to tumorigenesis. The Nestin-CreER,Nf2\(^{\text{flox2/flox2}}\) mice could serve as an improved model for NF2-associated tumors.

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Perception and Imitation of Vowels in NF1 – Preliminary Findings in Adult Speakers

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Aim: NF1 may be accompanied with speech problems, such as abnormal phonation, difficulties in regulating intonation, deviant nasality and problems with articulation and fluency. As imitation plays a fundamental role in speech acquisition and language learning, we wanted to investigate whether the ability to perceive and imitate acoustic differences in vowels is reduced in speakers with NF1. The aim was to shed light on the etiology of speech problems in NF1. Material and methods: A total of 28 Finnish speakers with NF1 (age range 21–66 years) including 12 men and 16 women participated phonetic tests. The mean age of the patient groups were 49.4 and 46.2, respectively. The control groups of Finnish speakers (age range 25–63 years) included 9 men (mean 45.0) and 9 women (mean 42.6) with no history of speech deficits. The experiment consisted of perception and imitation tasks. The presented stimuli, 70 in total, formed a vowel continuum /ä–a/. (/ä/ and /a/ are contrastive phonemes in Finnish with no other vowel category in between). Firstly, the participants labeled the randomly arranged stimuli either as /ä/ or /a/. Secondly, they evaluated the goodness of the stimuli as category members. Thirdly, the participants imitated the same vowel stimuli, and their responses were recorded for acoustic analysis. Results: All the groups could label the vowels according to the Finnish orthographic symbols either as /ä/ or /a/. The control groups performed better than the patient groups in the goodness rating task. Also the imitation task proved to be more difficult for the patient groups. However, individual variation was remarkable. Conclusion: The preliminary results suggest that both perception and imitation abilities may be reduced in speakers with NF1. This may offer an explanation for some of the speech problems accompanied with NF1. For example language learning may be problematic if the ability to perceive and imitate phonetic differences in speech is reduced.

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Feedback from Hacettepe NF Study Group

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Neurofibromatosis type 1 is a complex disease which affects multiple organ systems; therefore multidisciplinary evaluation and timely approach are crucial to facilitate the delivery of optimal care. Hacettepe University, Faculty of Medicine is a well known reference center for neurological diseases in Turkey. To organize the multidisciplinary management of NF1 patients “The NF Study Group of Hacettepe University” has been established in 2005. The study group is composed of physicians from all related departments covering both pediatric and adult patient care and also researchers of basic sciences covering molecular studies of NF1. A national NF database with 242 probands and their 183 affected relatives, and a website providing information about the disease and ongoing research have been established as concrete products of this study group. Molecular genetic testing is provided by Medical Biology Department, and genetic counseling is given by specialists. Segregation analysis for familial cases and mutation detection by DNA sequencing for sporadic cases are available. High Resolution Melting Curve Analysis (HRMA) is used for prescreening, especially for exons smaller than 300bp. A number of known and novel mutations have been identified, which will be listed during the presentation. Beside diagnostic investigations, research projects are carried out to unravel the mechanisms of NF1-related learning disabilities and the development of neurofibromas.

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A high-throughput screen to identify small molecule inhibitors of NF1-deficient cell growth

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Neurofibromatosis 1 (NF1) is a common autosomal dominant disorder in which affected individuals are prone to the development of both benign and malignant tumors. Current treatments for tumors arising in the neurofibromatosis type 1 (NF1) inherited cancer syndrome are often not specifically targeted to the unique molecular and biochemical changes associated with NF1 gene inactivation, but involve the use of therapies that are effective in other cancers. To identify potential inhibitors of NF1-deficient cell growth, we employed a high-throughput chemical library screen (HTS) method using NF1-deficient ST88-14 malignant peripheral nerve sheath tumor (MPNST) cells. We identified a novel candidate compound, which specifically inhibits ST88-14 cell proliferation. Next, we sought to define the optimal dose of this compound capable of inhibiting ST88-14 cell and Nf1-/- murine astrocyte proliferation. We found that 10 nM of this compound could induce apoptosis in MPNST cells as well as in Nf1-/- astrocytes without any significant effect on wild-type astrocytes. Current studies are aimed at defining its molecular target and mechanism of inhibition in NF1-deficient cells.

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Rhabdomyosarcoma as a late complication of Stereotactic Radiotherapy in the patient with NF2

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Neurofibromatosis type 2 (NF2) is a rare autosomal dominant disorder characterized by the development of benign tumors of the peripheral and central nervous system (CNS) including schwannomas, meningiomas, and ependymomas. The gene responsible for the development of NF2 (schwannomin or merlin) acts as a tumor suppressor gene. Disease expression is quite variable resulting in a spectrum of different phenotypes. While there is an association between NF1 and malignant neurofibroma, the development of malignant tumors is not considered a feature of NF2. The hallmark of NF2 is development of bilateral vestibular schwannomas with a consequent hearing loss. Patients often have a large burden of intracranial tumors, particularly meningiomas, and require frequent interventions. Surgery has been a standard therapy, but stereotactic radiotherapy (SRT) has been increasingly used for the management of these tumors. It offers some enticing advantages over surgery particularly in regards to short-term risk.

We present the first documented case of rhabdomyosarcoma following SRT for a NF2-associated vestibular schwannoma. Patients with NF2 have a tumor suppressor gene defect and may be more vulnerable for development of secondary malignancy after treatment involving radiation, when compared to patients with isolated tumors. Decision about selection of therapy in NF2 patients, particularly ones in young age, must include consideration of long term complications, particularly radiation-induced malignancies.

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Preclinical Evaluation of HDAC Inhibitors and an EGFR Inhibitor on Human Vestibular Schwannoma and Meningioma

Matthew Bush
Ohio State University

Introduction: Neurofibromatosis Type 2 (NF2) is caused by mutations in the NF2 gene, which encodes a tumor suppressor protein, merlin. This mutation leads to the formation of bilateral vestibular schwannomas (VS) and meningiomas. Options for NF2 patients include surgical excision and radiation; however, no medical therapies exist. Therapeutics targeting common molecular pathways in NF2 tumors would benefit patients. Two drug classes, histone deacetylase (HDAC) inhibitors and epidermal growth factor receptor inhibitors, have gained interest in the treatment of NF2 tumors. Objective: The objective of this research is to identify novel therapeutics that inhibit the growth of VS and meningiomas. Specifically, two HDAC inhibitors, a novel drug, (S)-HDAC42, and SAHA, as well as, an EGFR inhibitor, erlotinib, were used in the in vitro treatment of human VS and meningiomas. Methods: We cultured five surgically-resected human VS. Primary cultures were treated with various concentrations of (S)-HDAC42 and erlotinib to determine the 50% inhibitory concentration (IC50). Similarly, two primary meningioma cell cultures were developed and treated with (S)-HDAC42, SAHA, and erlotinib. We also utilized BenMen1, a benign human meningioma cell line that was immortalized by telomerase. The status of the NF2 gene in BenMen1 cells was analyzed using PCR, RT-PCR, and DNA sequencing. BenMen1 cells and two malignant cell lines, NF2+/- KT21 and NF2+/- IOMM-Lee, were treated with (S)-HDAC42 and erlotinib to determine the IC50. Results: (S)-HDAC42 inhibited the growth of human VS cells with an average IC50 of 3.5 micromolar. Erlotinib-treated VS cultures had an IC50 of 8.5 micromolar. Mutation analysis in BenMen1 cells revealed a frame-shift mutation in exon 7 of the NF2 gene. This leads to premature termination 4 amino acids downstream of the mutation. (S)-HDAC42 treated benign and malignant meningioma cell lines produced an IC50 of approximately 7.5 micromolar. Primary meningioma cell cultures had an IC50 of 2.5 micromolar when treated with (S)-HDAC42, whereas, SAHA treated cells had an IC50 of 8.5 micromolar. Mutation analysis in BenMen1 cells revealed a frame-shift mutation in exon 7 of the NF2 gene. This leads to premature truncation of approximately 7.5 micromolar. Conclusion: Human VS and meningiomas are sensitive to in vitro treatment with (S)-HDAC42; however, both types of tumor cells were less sensitive to low concentrations of erlotinib. Additionally, BenMen1 is a unique merlin-deficient benign meningioma cell line that useful in therapeutic testing. Further investigation is needed to elucidate the mechanisms behind the drug response in NF2-related tumors and to further develop safe chemotherapeutic agents capable of potent inhibition of these tumors.

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Novel Murine Models of Malignant Peripheral Nerve Sheath Tumors

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Malignant peripheral nerve sheath tumors (MPNSTs) are one of the most lethal consequences for Neurofibromatosis type I (NF1) patients. Although NF1 patients have approximately a 10% lifetime risk of acquiring MPNSTs, these tumors are highly aggressive, with a 5 year survival rate of approximately 50%. Currently, surgical resection is the primary treatment option, with complete excision of small and early stage tumors correlated with higher survival rates. Unfortunately, no diagnostic markers consistently differentiate MPNSTs from neurofibromas, and few chemotherapies have been demonstrated to be efficacious in the treatment of MPNSTs, largely due to the relative rarity of these tumors. In this study, we present two novel murine models of NF1 that generate MPNSTs with high penetrance. These two models differ in their timing of NF1 inactivation, where one model losset NF1 in conjunction with p53, while our other model inactivates NF1 first with subsequent loss of p53. While both models produce MPNSTs, if NF1 is inactivated prior to p53 loss, neurofibromas develop, mimicking NF1 patients. As both these models utilize Schwann cell lineage specific ablation of a single p53 allele, they generate predominately MPNSTs. Comparison of these two mouse models enables elucidation of the role of neurofibromas in MPNST development. Finally, p53 ablation is only of the DNA transcription activation domain, allowing identification of malignant tumors through staining of stabilized p53 protein. These novel murine models enable us to generate large numbers of MPNSTs, allowing testing for diagnostic markers to differentiate between benign and malignant peripheral nerve sheath tumors, and offer improved NF1 models for pre-clinical therapeutic trials.

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Presenting Symptoms in Children Referred for Evaluation of NF-1

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The initial manifestations of NF-1 are usually café-au-lait (CAL) macules that can be present at birth or develop over the first few years of life. Often additional NF-1 diagnostic criteria do not emerge for several years. Early diagnosis of NF-1 is important for the management of non-cutaneous manifestations including learning disabilities, hypertension, and optic pathway gliomas. Method: The charts of all patients referred to the Division of Genetics & Metabolism in 2008 for evaluation of possible neurofibromatosis were reviewed. The age of the patient, source of the referral, and diagnostic outcome was recorded for each patient. Results: Seventy-eight patients were referred for evaluation. CAL macules were the most common reason for referral (63%). Eight patients (10%)
had a positive family history. Three patients were referred for unusual freckling, one had severe scoliosis, and one had a malignant nerve sheath tumor. Two patients had an optic nerve glioma, two had plexiform neurofibromas, and one had a schwannoma. Thirty-nine patients were male and 39 were female. The vast majority of patients (90%, 70/78) were referred by their primary care provider with the remainder referred by neurology (8%, 6/78) and one patient each coming from hematology and orthopedics. Twelve of the 78 were known to have NF-1 and were seeking a second opinion. Of the 78 patients, 34 (44%) met diagnostic criteria for NF-1. An additional 8 (10%) were felt by experienced NF clinic faculty to have NF-1 but did not meet NIH consensus criteria. All of these patients were under the age of three years. Six patients had segmental NF. In 24 patients (31%) NF-1 was excluded. Conclusions: Although the majority of patients with NF-1 present with CAL macules other clinical manifestations such as pseudoarthrosis, scoliosis, and plexiform neurofibromas, need to be considered. A number of patients who have NF-1 do not fulfill diagnostic criteria at their initial visit, thus potentially delaying important physical and neurocognitive interventions. A modification of the clinical diagnostic criteria for NF-1 should be considered for young children.

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The impact of neurocognitive disabilities and resiliency on quality of life in children with NF1

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Objectives: Attentional deficits and learning problems are common comorbid disorders in children with NF-1. An estimated 30 to 65% of children with NF-1 are diagnosed as learning disabled (LD), while 30 to 50% meet criteria for Attention-Deficit/Hyperactivity Disorder (AD/HD). No research to date has evaluated the impact of resiliency on outcomes in children with NF-1. This study examines the impact of attention and learning on quality of life, and the moderating effect of resiliency in such outcomes. Methods: A preliminary group of thirty-five children, ages 11-17 years, from a larger cohort of campers with NF1, were evaluated for quality of life, using the Impact of Pediatric Illness scale (IPI), and resiliency, with the Connor-Davidson Resilience Scale (CD-RISC). Parents and primary caregivers completed a demographic questionnaire to identify NF related symptoms and neurocognitive diagnoses of AD/HD and LD. In the group, 54% of the children were females, 49% were Caucasian, 46% African American, and 6% Hispanic. A regression analysis was used to evaluate the relationship between disability status (NF1 only [31%] and NF with co-morbidities of AD/HD [17%], LD [20%), AD/HD+LD [31%]) and quality of life, and role functioning in children with NF1. Results: Disability status was a significant contributor to the model (p = .02; R^2 = .15) and disability status (p = .00; R^2Δ = .27). Resilience accounted for 44.6% of the variance (p = .00), moderating the relationship between gender and quality of life and eliminating the significance. However, disability status remained a significant contributor to the model (p = .001; R^2Δ = .62). Males were significantly more likely to have learning and attention disabilities (t = -3.4; p = .002) associated with NF1 and report less resiliency (t = 2.7; p = 0.12) than females. Conclusions: These findings highlight the impact of neurocognitive disabilities and resiliency on a child’s assessment of quality of life. Resiliency appears to moderate the relationship between disability and quality of life for genders differently, with males faring more poorly.

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Working Memory Contributions to Academic Achievement in NF1

Peter J. Duquette
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Objectives: Neuropsychological impairments are common sequelae for children with NF-1, with recent estimates approaching 60%, with executive dysfunction being prominent. There are few studies that have examined the impact of executive dysfunction on school performance in NF-1 despite the high rates of specific learning disabilities in NF-1. Working memory is an executive skill involved with temporarily storing and managing information required to for learning, reasoning, and comprehension. This study examines the relative contribution of working memory to performance-based measures of academic functioning in children with NF-1. Participants & Methods: Twenty patients involved with prospective and/or retrospective NF-1 studies participated in the current study. Extensive neuropsychological assessments were completed from which this data was extracted. Measures used in this study included: Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV); Wechsler Individual Achievement Test, Second Edition (WIAT-II); California Verbal Learning Test, Children’s Version (CVLT-C); Rey Complex Figure Test (RCFT); Behavior Rating Inventory of Executive Functions (BRIEF) Parent form. Participants were categorized based on performance on WIAT-II subtests (Word Reading and Numerical Operations) falling >1 standard deviation below the mean, generating four groups – NF-1 with (NF1+R) and without (NF1-R) underachievement in reading; NF-1 with (NF1+M) and without (NF1-M) underachievement in math. Regression analyses were conducted to determine the relative contributions of working memory tasks (WISC-IV Digit Span Backward; CVLT-C Short-Delay Free Recall; RCFT Immediate Recall; BRIEF Working Memory scale) to academic achievement. Results: The ability to hold and retrieve words from working memory after a brief delay (CVLT-C Short-Delay Free Recall) was a significant predictor (p < .01) of underachievement in reading and math, accounting for 52% and 39% of the variance, respectively. The ability to hold and retrieve digits from working memory (WISC-IV Digit Span Backward) approached significance (p = .09) in predicting math underachievement. Conclusions: These findings highlight the possible contribution of working memory factors on academic underachievement in children with NF-1, and highlight the importance of intervening with executive functions when academic deficits are of concern in children with NF-1.

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Comprehensive SNP-array analysis of multiple tumors of a single NF1 patient to uncover additional constitutive and/or somatic genetic alterations that contributes to the development of malignancies

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Here we report a case of a 63-years old man diagnosed of NF1 at the age of 16 years. Mutation analysis revealed a frameshift mutation in exon 33 of the NF1 gene (c.6226delG) also present in his affected son. Patient had a severe phenotype and developed multiple malignancies during his life that can be summarized as follows: (1) A pheochromocytoma, removed in 1992; (2) A malignant peripheral nerve sheath tumour (MPNST) localized in the inferior right extremity, removed in 1998 recurrent in 1999 producing lung metastasis later on and (3) A colon adenocarcinoma with liver metastasis detected in 2006 causing his death few months after. Patient decided to donate his body for research use and a set of different lesions were collected and frozen-stored: normal stomach, GIST (gastrointestinal stromal tumour); normal colon, adenocarcinoma; normal duodenum, tumoral duodenonum; normal liver, tumoral liver; neurofibroma and normal fibroblasts. Additional tumor samples are available in formalin-fixed paraffin-embedded blocks. The aims of the present study are: 1) To identify genomic alterations causative of tumor development; 2) To compare genomic alterations between different tumor types to identify whether the same or different pathways are involved in their development; 3) To search for constitutional genetic alterations (in addition to the NF1 mutation) that can explain the high predisposition of this patient to develop malignancies. To achieve these aims we have analyzed the different samples using an array of single nucleotide polymorphism (SNP) (Illumina 370k) that allows the simultaneous measurement of DNA copy number and detection of copy neutral Loss of Heterozygosity (LOH). We are also planning to compare this data with FISH and immunochemistry on paraffin sections.

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Retrospective Analysis of NF1-Associated Optic Glioma Visual Outcomes

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Optic pathway gliomas (OPGs) arise in 15-20% of children with NF1; nearly half of these tumors will become symptomatic. Although the clinical characteristics of NF1-associated OPGs are well-defined, disagreement persists regarding indications to initiate treatment. Agreed upon indications include documented decline in visual acuity or MRI progression with associated symptoms. Other proposed indications include tumor progression without associated symptoms, development of enhancement on MRI, tumor extent and location, progressive proptosis, optic disc swelling/pallor, and severe visual impairment at diagnosis. In addition, despite nearly two decades of experience in treating NF1-associated OPG with chemotherapy, there exists a paucity of information regarding the ophthalmologic outcome of these children. Much of the literature on chemotherapy has focused on radiographic outcomes with 5-year EFS of 68.7% in a large Children’s Oncology Group study using carboplatin with vincristine. A retrospective multi-center study was therefore undertaken to define reasons for treatment and visual outcome following chemotherapy for NF1-associated OPG, and to identify the factors that place children at greatest risk for visual loss. Subjects included children with NF1 who underwent initial treatment for an OPG with chemotherapy between January 1997 and December 2007. Preliminary data on 47 subjects from three of nine participating sites is available for review. Approximately 1/3 had tumors confined to the anterior visual pathway (optic nerves and/or chiasm). The most common reasons to initiate treatment included visual acuity loss (26), tumor growth on MRI (24), tumor location (12), tumor size/extent (5), inability to obtain reliable visual acuity (5), and progressive proptosis (4). For most subjects, more than one factor spurred treatment (mean 1.96, range 1-5). Thirteen subjects were treated for a single reason: visual acuity decline (7), tumor growth (5), progressive proptosis (1). Thirty-nine subjects were evaluable for visual acuity outcomes. At completion of chemotherapy, acuity improved (28%), remained stable (36%), or declined (36%). Of note, acuity improved in 43% of subjects with anterior OPGs, but in only 20% of subjects with posterior pathway (hypothalamus and/or optic tracts/radiations) tumors. In contrast, acuity worsened in 44% of posterior OPGs and in 21% of anterior OPGs. In conclusion, visual acuity decline and tumor progression were the primary reasons to initiate treatment for OPG at these three institutions. Of concern, over 1/3 of subjects had a decline in visual acuity by the end of treatment, and outcomes for subjects with posterior pathway tumors were particularly poor. Data from additional participating institutions, as well as data on visual fields, proptosis, and optic disc swelling/pallor, is forthcoming and may provide greater insight into addressing the study aims. These data will be used to plan a prospective longitudinal study of the treatment of these tumors.

Genes controlling homologous recombination as modifiers of the number of neurofibromas

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Neurofibroma development requires the double inactivation of the NF1 gene in a Schwann cell. Somatic NF1 inactivation is due to mutation. We believe the number of neurofibromas developed by a patient is highly correlated with its somatic mutation rate. One way to evidence somatic mutation is to perform Loss of Heterozygosity (LOH) analysis. In neurofibromas a large percentage of LOH is caused by homologous recombination events between the centromere and the NF1 gene, involving chromatids of homologous chromosomes. The result is somatic uniparental disomy from the recombination point to the telomere and a homozygous NF1 mutation. We want to investigate whether genes implicated in homologous recombination are modifiers of the number of neurofibromas, due to their contribution to the total somatic mutation rate of the patient. Our final aim is to perform association studies between a cohort of well characterized NF1 patients and different candidate genes influencing somatic recombination. In order to do that, we are characterizing a cohort of NF1 patients for: 1) the number of neurofibromas developed and their age; 2) the percentage of neurofibromas carrying LOH due to homologous recombination;
3) their somatic mutation rate. We performed somatic mutation characterization in neurofibromas to dissect LOH according to the different mechanisms involved in its generation. We have used a Multiplex Microsatellite PCR (MMP) approach, together with MLPA. We have analyzed around 550 neurofibromas belonging to more than 100 patients. In addition SNP-array analysis has been performed for several neurofibromas. LOH is mainly caused by mitotic recombination followed by mechanisms leading to deletion. We have obtained the overall prevalence of LOH in neurofibromas for our cohort of patients, and also the prevalence of mitotic recombination. In addition, SNP-array data also indicates the presence of other genonomic alterations, beyond NF1 inactivation, in some neurofibromas. Each detected alteration is unique to a single neurofibroma. The percentage of tumors with LOH caused by homologous recombination varies among the cohort of NF1 patients.

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The NF1 Health Check Reminder Card – A Memory Tool for Adults

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The Yorkshire Clinical Genetics NF1 Team has designed a practical tool to encourage adults with NF1 to manage their own health. Clinical Guidelines recommend that all individuals with NF1 should be medically assessed at least once a year. Children are routinely seen by Paediatricians and receive an annual appointment. Adults however have to proactively manage their own health by booking an appointment. Our experience from Genetic Clinics in Yorkshire is that many do not do this. Some patients with NF1 may have problems with short-term memory. Memory aids are already used by some health professions for certain groups of patients. We have designed a memory aid in the form of a plastic card, about the size and shape of a credit card. It can be personalised and contains essential information about neurofibromatosis, as well as the recommended annual medical review and a list of symptoms needing immediate attention. It also contains details of where to access help and advice. Having had the card peer reviewed in the UK and adjustments made, we are now at the stage of asking adults with NF1 what they think of the card – do they find it acceptable and easy to use. To pilot the card we have started to offer it to adults with NF1 who attend the NF1 Clinic in Leeds, Yorkshire. Our aim in submitting this poster is to introduce the NF1 Card to a wider ‘expert’ audience – both professional and lay attendees to gauge opinion and encourage comment.

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DNA methylation analysis of mismatch repair gene promoters MLH1, MSH6, PMS2 and MSH2 in Neurofibromatosis type 1 (NF1) patients with respect to neurofibroma expression

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Neurofibromatosis type 1 (NF1) is a common genetic disorder caused by mutations of the NF1 gene. The NF1 phenotype is highly variable, and “modifiers” have been discussed as potential determinants of NF1 phenotype variability. Mismatch repair deficiency has been shown to cause NF1 mutations, but constitutional mutations of mismatch repair genes have only once been identified in a NF1 patient. The aim of this study was therefore to analyze whether DNA methylation of mismatch repair gene promoters, which is known to lead to transcriptional silencing, is associated with an increased tumour load in NF1 defined by the number of neurofibromas. Leukocyte DNA of 79 control probands and of 79 NF1 patients were investigated for promoter methylation of the mismatch repair genes MLH1, MSH2, MSH6 and PMS2 by methylation specific PCR (MSP) and pyrosequencing. MLH1, MSH6 and PMS2 gene promoters were not found to be methylated. By contrast, we found promoter methylation of MSH2. Statistical analysis revealed a higher methylation in NF1 patients as compared to controls. Furthermore, comparing NF1 patients with <60 neurofibromas vs. patients with >100 neurofibromas, a significant difference of methylation in two out of six CpG dinucleotides was found. In conclusion, enhanced methylation involving the transcription start points of a mismatch repair gene such MSH2 in NF1 has not been described so far and could be the first evidence for a genetic modifier in NF1.

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Cause of death in neurofibromatosis 2: a 12 year experience from a single specialist unit

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Introduction: Long-term survival rates in patients with NF2 have been documented and mean actuarial survival has been reported to be 62 years. However, there is a large range of disease severity and it is apparent that age of onset is one of the predictors of long-term survival, with patients diagnosed at a younger age doing less well. In a large database analysis of 368 UK patients, 74 (20%) died during follow-up, and in 51 of these cause of death was reported as ‘tumour burden’. Aim: To identify cause of death in patients managed within the specialist multi-disciplinary NF2 clinic at Guy’s and St Thomas’ NHS Trust and to compare this cohort to previously published data. Methods: The database of patients with a confirmed diagnosis of NF2 patients managed in the specialist clinic between 1992 and 2008 was analysed as part of a major quality of life study. Causes of death amongst patients in this group were investigated. Results: A total of 61 patients with a diagnosis of somatic or mosaic NF2 were identified. 32 were male, 29 were female. Mean age at the time of database analysis was 40 years, with a range of 6-81 years. A total of 5 patients had died during follow-up (8.2%). Of this group, no one of our patients died as a direct result of tumour burden. One patient died post-operatively following the excision of a vestibular schwannoma. One died of a lower respiratory tract infection which was secondary to a suspected radiation-associated encephalopathy, many years after irradiation of a frontal meningioma. One patient died of vascular disease, one of lower respiratory tract infection. One patient died due to malignant osteosarcoma. Discussion: Management of the NF2 patient is complex and challenging. It has been previously reported that around half of patients die due to tumour burden. In our series of patients managed in a single unit, our experience differed. Two patients died as a direct consequence of treatment for their disease and it is necessary to carefully balance risks and benefits of intervention in this group of patients.

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Seizures in NF2

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Introduction: Seizures have been reported in NF1, affecting 4-6% of patients, and in some cases is likely to relate to an underlying cortical dysgenesis. The incidence of seizures in individuals with NF2 has not previously been reported, however their development has the potential to greatly impact an individual’s quality of life. There are a number of potential causes for seizures in NF2, including meningiomas and meningioangiomatosis. Sporadic meningiomas commonly present with seizure as the first symptom and the site of the tumour is important in development of epilepsy. Meningioangiomatosis may lead to seizures in sporadic cases, but is not thought to cause seizures when found in NF2. Aim: To determine the frequency of seizures in our cohort of NF2 patients and identify any predisposing features. Methods: The case notes of patients with a confirmed diagnosis of NF2, managed in the specialist clinic between 1992 and 2008 were analysed. Patients who had a history of seizures were identified and clinical, neurophysiological and radiological characteristics were reviewed. Results: Sixty-one patients with a diagnosis of somatic or mosaic NF2 were identified. 32 were male, 29 were female. Mean age at the time of database analysis was 40 years, with a range of 6-81 years. 6 patients had a confirmed history of seizures (9.8%). Mean age of this group was 37.8 yrs, with a range of 27-52 years. 5 were male and 1 female. Seizure was the first presentation in two patients. None of the group had meningioangiomatosis. All six patients had meningiomas, compared to 22 patients who had meningiomas without seizures (40%). Discussion: To our knowledge, there have been no systematic studies of seizures in NF2. We have found that 9.8% of our patients had seizures. None of our patients had meningioangiomatosis and all individuals with seizures had meningiomas, in varying sites. Calcification was seen in four cases. The potential aetiology of seizures in NF2 will be discussed, including the site of the meningioma or an underlying dysgenesis.

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Relationship Between Executive Functioning Skills and Adaptive Behavior in Children with NF1

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Objective: Studies of children with NF1 have documented increased risk of cognitive difficulties, including deficits in executive functioning skills. However, adaptive functioning has not been fully explored among children with NF1. Adaptive skills are everyday, practical skills that allow one to function and meet the demands of the environment. As executive functions represent skills that contribute to purposeful, goal-directed behavior, it may be expected that deficits in executive skills could interfere with independent, daily functioning. The current study sought to explore the relationship between executive functioning and adaptive behavior in children with NF1. Methods: Parents of 60 children with NF1 (males n=31; mean age= 10.55) completed the Behavior Rating Inventory of Executive Function (BRIEF) and the Adaptive Behavior Assessment System (ABAS). The BRIEF provides ratings of eight domains of executive functioning, including skills related to behavior regulation (Inhibit, Shift, Emotional Control) and metacognition (Initiate, Working Memory, Plan/Organize, Organization of Materials, Self-Monitor). The ABAS rates everyday adaptive skills in nine areas: Communication, Community Use, Functional Academics, Home Living, Health, Leisure, Self-Care, Self-Direction, and Social. Results: Moderate to strong correlations (r= -.51 to -.70; p < .01) were found between all BRIEF scales and five of the ABAS domains: Communication, Community Use, Functional Academics, Self-Care and Self-Direction. The BRIEF Working Memory scale showed the most significant associations, correlating with 7 of the 9 ABAS scales. The Organization of Materials scale of the BRIEF did not correlate with any ABAS scale. Furthermore, the Leisure, Home Living, and Social scales of the ABAS did not correlate significantly with any BRIEF scale. Conclusion: This study found a relationship between executive functioning and aspects of adaptive skills in children with NF1. Both regulatory and
metacognitive skills correlated with academics, receptive and expressssive communication, and self-directed tasks. Working memory was most strongly associated with adaptive behavior. When evaluating children with NF1, it is important to consider the impact of their cognitive skills, particularly their executive functioning, on everyday behavior. These findings have implications for treatment, as improving executive skills through intervention will also likely help to improve everyday adaptive functioning.

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The Use of Class III beta-tubulin as a Biomarker in Neurofibromas and MPNSTs

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Class III beta-tubulin is one of the seven beta-tubulin isotypes existing with different tissue distribution in man. Class III beta-tubulin has been widely used as a neuron-specific marker molecule but it has been detected also in association with selected malignancies, such as in breast cancers and other malignant epithelial tumors. Our novel finding was the detection of class III beta-tubulin in an MPNST. Examination of this MPNST with higher resolution revealed class III beta-tubulin in a subpopulation of cells. Furthermore, confocal laser scanning microscopy revealed a fibrillar class III beta-tubulin (Tuj-1 antibody) immunoreaction in association with mitotic spindles. In benign neurofibromas with no mitoses, a positive immunoreaction for class III beta-tubulin was exclusively found in axons. In vitro cell culture studies of normal human skin fibroblasts and keratinocytes, Tuj-1 antibodies localized class III beta-tubulin to the mitotic spindle and showed a colocalization with alpha tubulin. The spindle specific immunoreaction lasted throughout the mitosis. The findings demonstrate that class III beta-tubulin is a component of the mitotic spindle in multiple cell types. Thus, class III beta-tubulin is not entirely a neuron-specific marker. In neurofibromas derived stem cell cultures, class III beta-tubulin expression can be detected in the mitotic spindle of dividing cells, but it can also be reliably used as a biomarker for differentiated neurons. References: Eeva-Mari Jouhilahti, Sirkku Peltonen and Juha Peltonen (2008) Class III beta-tubulin is a component of the mitotic spindle in multiple cell types, J Histochem Cytochem 56(12):1113-9.

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Uncharacterized brain abnormalities on MRI in patients with NF2

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PURPOSE: To describe gray and white matter lesions on magnetic resonance imaging (MRI) in patients with neurofibromatosis type 2 (NF-2). SUBJECTS: We reviewed all consecutive MRI scans obtained with patients with NF-2 obtained at our institution between 2004 and 2008. Scans were available for 15 patients. All patients met clinical diagnostic criteria for NF-2 upon chart review. RESULTS: Twelve of fifteen patients had white matter and/or gray matter lesions unexplained by NF-2 related tumors or post treatment changes. Three patients showed non-enhancing gray matter lesions suggestive of cortical dysplasias. T2 bright white matter lesions were seen in twelve patients. These lesions were similar to areas of myelin vacuolization seen in neurofibromatosis type 1 (NF-1) in that the lesions demonstrated no mass effect or enhancement but were not in locations typically associated with NF-1 myelin vacuolization. Similar lesions in NF-2 have only been described in a single case report to best of our knowledge1. CONCLUSIONS: In a small group of patients with NF-2, we found a high prevalence of gray and white matter lesions on brain MRI distinct from known NF-2 related pathology. There may be an increased prevalence of cortical dysplasia in NF-2 whereas the white matter lesions may represent a similar process as seen with myelin vacuolization seen in association with NF-1. Reference: R. N. Sener, S. Dzelzite, A. Migals, U. Raits, A. A. Veinbergs, Prominent myelin vacuolization in neurofibromatosis type 2, Clinical Imaging, Volume 27, Issue 1, January-February 2003, Pages 11-13.

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Neurofibromin, CRMP-2 and presynaptic calcium channel proteins control synaptic transmission in a mouse model of NF1

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Neurofibromatosis type I (NF1) is a common autosomal dominant disease characterized by the formation of multiple benign and malignant tumors. People with NF1 often have learning disabilities and other cognitive symptoms. The mechanisms by which mutations of the neurofibromin gene (NF1) cause these deficits are not known. Learning and memory deficits also are observed in mice with a heterozygous mutation of the NF1 gene (NF1+/-) and these deficits can be rescued by genetic and pharmacological manipulations that decrease Ras function. Our goal is to understand the molecular mechanisms that contribute to learning and memory deficits in NF1. Recently, direct interactions of collapsin response mediator protein 2 (CRMP-2) – a protein involved in neurite outgrowth, guidance and axonogenesis – and neurofibromin have been demonstrated. In addition, we have demonstrated that CRMP-2 associates with and influences the function of presynaptic calcium channels involved in transmitter release. We also have demonstrated that CRMP-2 influences axonal outgrowth and synaptic connectivity of neurons in the brain. Based on these results, we hypothesize that a lack of neurofibromin in NF1 alters CRMP-2 function leading to altered axonal connections and transmitter release that results in altered learning and cognitive function. Immunoprecipitation studies from brains of
wildtype and Nf1+/- mice reveal a direct protein-protein interaction between neurofibromin and CRMP-2. In addition, both proteins are enriched in cholesterol and sphingolipid rich, “lipid raft-like” microdomains within the plasma membrane. These rafts are critical for organizing specialized signaling transduction platforms within membranes. Notably, a significantly lower percentage of total neurofibromin is raft-associated in brain tissue from Nf1+/- mice compared to wildtype mice (31.4 ± 0.9% vs. 44 ± 1.1%). Treatment of wildtype sensory neurons with short interfering RNA (siRNA) to CRMP-2 causes an 80 ± 5% knockdown of protein expression and a subsequent decrease in release of the peptide transmitter, calcitonin gene-related peptide, of 52 ± 4% compared to neurons treated with scramble siRNA. CRMP-2 knockdown also causes an 80 ± 5% reduction in Ca2+ currents in hippocampal neurons compared to those treated with scramble siRNA. These data demonstrate an altered interaction of CRMP-2 and neurofibromin in Nf1+/- mice. Reduced raft sequestration of neurofibromin may impair CRMP-2 from freely interacting with calcium channels resulting in altered calcium channel density and transmitter release. By understanding the molecular mechanisms underlying the signaling involved in NF1-CRMP-2-Calbindin channel interactions, we hope to unravel new targets for the development of therapeutics for the treatment of learning deficits in NF1.

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Xenografting plexiform neurofibromas in nude mice and testing efficacy of Gleevec

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We xenografted small pieces of freshly operated plexiform neurofibroma onto the sciatic nerve of athymic nude mice. Xenografts persisted for at least 60 days and maintained major features of the original tumours. Newly formed blood vessels of murine origin were found indicating induced angiogenesis. Enlargement of the grafts was found 2 to 3 weeks after the implantation, which is likely due to inflammation rather than to tumor growth as revealed by immunohistochemical studies. Treatment with Gleevec at a daily dose of 75mg/Kg for 4 weeks resulted in significant reduction of grafts size by more than 80% (P=0.013), while grafts size did not change significantly in the control mice (P=0.2). Also in vitro, Gleevec reduced vitality of primary cultured Schwann cells derived from plexiform neurofibroma cell at an IC50 of 10 μM. Therefore, xenografting plexiform neurofibromas into nude mice provides an in vivo model for this tumor and Gleevec may be a potential drug therapy.

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Cognitive profile and behavioral phenotype of patients with NF1 – predictors of social adaptation or maladjustment

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Background: The most common “complications” in patients with NF1 are cognitive and learning disability (e.g. visuospatial deficits, problems with motoric functioning) as well as conductive disorders (e.g. disruptive behavior, impulsivity, tendency to overreactions). Therefore, we searched for predictors of good social adaptation or maladjustment. Patients: In the study repeated psychological assessment was undertaken in 60 children with NF1 consecutively admitted to our Center, to determine the effect of NF1 on cognitive and emotional outcome. Methods: Patients were examined using a battery of standardized psychological and neuropsychological methods, e.g. Wachslager Intelligence Scale and Benton Visual Retention Test. Emotional functions were assessed based on analysis of medical history (especially psychiatric and neurological data), interview with parents, psychological observation and investigation of the patients as well their self reports. Results: In our sample we observed specific cognitive development, as well numbers of emotional problems and difficulties in social adaptation. Analyzed group of patients showed also psychological adjustment difficulties and socially competent deficits. They may exhibit deficits in age-appropriate social competence and peers relations. In a pilot study we discovered that critical for good social adaptation was low rate of problems with structured material like: time, planning, organizing of activity; good relations in family and adequate self-esteem as well insight. Unfortunately these factors are hardly affected by NF1 and major part of this cohort have problem with that. Risk factors for maladjustment and limitations of social development and psychotherapy of patients with NF1 will be also presented. Practical applications: psychological rehabilitation and counseling for patients with NF1 and their families; prevention of maladjustment of patients with NF1 and improve quality of their life.

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The Vasculopathy of NF1: Literature Review, Analysis and Recommendations for Further Study

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To review the literature of NF1 vasculopathy; to examine the relationships between patient age, sex, anatomic location, lesion number and pathologic characteristics; to determine patterns that might aid in earlier detection, treatment, prevention of complications and better understanding of the pathogenesis of NF1-vasculopathy. Evidence indicates that vasculopathy in NF1 is congenital, progressive and that it may contribute to increased morbidity and mortality.

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A thorough characterization of NF1-vasculopathy could lead to earlier diagnosis, treatment and prevention of complications. Neurofibromin is expressed in vascular endothelium and smooth muscle and a specific genetic mutation in neurofibromin for NF1-vasculopathy has been proposed by Tang, et al. A pubmed search in the English language from 1970 to present, using predefined search words. Data were extracted and processed in a spreadsheet. Results: 315 patients (127M/188F/2NA); average age 26; 53% age <=20 were identified in 124 articles. The major anatomic locations were renal/mesenteric (43%, 85% age <=20). Extracranial/Carotid/Vertebral (21%; 2:2:1 M: F; M: age >40), Intracranial (15%; 3:1 M: F) and Aorta (15%). The most common types of lesions were stenosis (46%; average age 11.2), aneurysm (37%; average age 42.5), and arteriovenous fistula/AVM (10.8%; average age 36.5) Arteriovenous fistulae and AVMs were found almost exclusively in extracranial vasculature and comprised nearly 50% of these cases. Stenotic, occlusive and coarctation type lesions comprised 51% of all lesions with an average age of 11. Vasculopathy is a prominent component of NF1-1, but its prevalence remains unknown. The authors feel that vasculopathy is more common in NF1 than previously reported and often unrecognized until a life threatening or fatal event occurs. Further study of NF1-vasculopathy is warranted as it often leads to early morbidity and mortality. Many lesions predominantly stenosis, coarctation and occlusion were identified prior to age 20. Newer intra-vascular therapies make these lesions amenable to surgical treatment. Additional data concerning presenting symptoms, size of involved vessels, pathologic and genetic analysis of involved tissues and methods and effects of early intervention is needed to facilitate earlier diagnosis, earlier intervention and to decrease morbidity and mortality.


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Familial Cardiovasculopathy in Neurofibromatosis Type 1 (NF1): A Report of Four Patients in Two Families.

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To present the clinical features and investigations of two families with NF1 each having two members with NFI cardiovascular pathology. Of the multiple manifestations of NF1, vascular disease accounts for a significant percentage of early morbidity and mortality, yet its prevalence still remains unknown. Neurofibromin is expressed in endothelial and smooth muscle cells of blood vessels and is likely involved in the pathogenesis of vasculopathy. With the increased use of echocardiography in NF1 patients the importance of cardiovascular pathology is becoming apparent. A retrospective chart review of 2 families with NF1, each having two members with NF1 cardiovascular pathology seen at the Cleveland Clinic are discussed. Family A: 2 brothers currently aged 7 and 9 ½ years and their mother have confirmed NF1. The younger brother had a screening echocardiogram that revealed an anomalous right coronary artery that originated from the left sinus of Valsalva which was repaired surgically. He had a normal brain MRI and MRA. The older brother has pulmonary stenosis with mild pulmonary regurgitation and is being followed by cardiology. His brain MRI/MRA is normal. Family B: Father and daughter with clinically diagnosed NF1. The daughter died suddenly at age nine when an aneurysm of her left vertebral artery ruptured. Her father also died suddenly from a ruptured internal iliac artery aneurysm at age 38. The vasculopathy associated with NF1 was first described in 1945 and since then several reports suggested an incidence of approximately 10%. Cardiovascular pathology is also being reported in increasing numbers but to the best of our knowledge this represents the first report of familial cardiovascular pathology in 2 separate families with NF1. The presence of this complication in members of these 2 families may be significant regarding the pathogenesis of NF1 vasculopathy. In such patients mutation analysis may uncover a genotype/phenotype correlation, with particular attention to the mutation put forward by Tang et al. Vascular disease is an important cause of premature death in patients with NF1 and its presence in many patients is only discovered following death or irreversible damage to vital organs. Therefore if a family member is noted to have NF1 cardiovasculopathy, increased surveillance of other affected relatives should be undertaken.


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Ritalin and the treatment of the neurological and cognitive deficits associated with neurofibromatosis type I

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The key pathophysiological mechanism underlying NF1 mutations in both mice and humans is increased p21Ras activity, Therapeutic interventions designed to inhibit p21Ras function have been proposed as treatments of NF1. The mice studies suggested that alternation of Ras/MAPK activity might rescue the learning deficits and attention problems. Studies showed a high incidence of ADHD in NF1 and supported an association between ADHD and learning and social problems in children with NF1. A clinic report suggested that these NF1 children with ADHD might benefit from the use of Ritalin but the mechanism is unclear. Here we report the effect of Ritalin treatment on p44/42 MAPK phosphorylation and preliminary behavioral tasks in nf1 +/- mice.

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Immortalization of NF1 Neurofibroma-Derived Schwann Cells

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Schwann cells (SC) are a key cell type in neurofibroma formation, and culturing these cells is an indispensable technique for NF1 research. We have established SC-enriched cultures from NF1 tumors under conditions that favor NF1-/- cells over the constitutional heterozygous Schwann cells (Muir et al, 2001), but it is difficult to get pure cultures and most normal SC and neurofibroma-derived SC enter replicative senescence after 5-8 passages. These latter two phenomena often impede cell-based research in NF1. Through expression of mouse cyclin-dependent kinase (mCdk4) to overcome p16INK4a mediated stress response, in combination with expression of the human telomerase reverse transcriptase protein (hTERT) to overcome replicative senescence (Tamirez, et al 2004), we have been able to successfully immortalize some neurofibroma-derived Schwann cells and one normal Schwann cell culture thus far. Initial experiments employed MSCV-based retroviral expression vectors containing these genes, and stable lines were obtained by antibiotic resistance selection. This vector did not have a high efficiency of infection, however, and only one plexiform neurofibroma culture (pNF95.11b) was successfully immortalized, expands over 40 passages thus far, compared to a maximum of 21 passages for the original SC-enriched tumor culture. At this point we moved to using lentiviral vectors containing these genes due to their increased infection efficiency in SC. We tested this in several additional neurofibrom-derived SC cultures and a normal SC culture. There were no selectable markers in the lentiviral vectors, so infected cells were simply passaged until well beyond the capability of the original cultures to divide, such that only immortal cells would survive. In one case (pNF95.11b) we obtained an immortal culture using a combination of the two viruses (lenti-hTERT, retro-Cdk4), which interestingly became neuregulin-independent (but still required a laminin substrate). Transfection by either gene alone was not able to produce cells that divided beyond 12-20 passages. There were no obvious differences in outcome if the two infections were done serially (within a few passages of each other) or at the same time. In addition to the pNF95.11b cultures, we now have cultures at 20-25 passages for normal human SC, two other plexiform neurofibroma SC, and a dermal neurofibroma SC. In addition, we have isolated and propagated single cell clones from several of these cultures. With immunocytochemistry and molecular genetics, we have shown that these clones are derived from the tumor-initiating somatically-mutated Schwann cells (positive for S100 SC marker, and have both NF1 SC. In addition, we have isolated and propagated single cell clones from several of these cultures. With immunocytochemistry and molecular genetics, we have shown that these clones are derived from the tumor-initiating somatically-mutated Schwann cells (positive for S100 SC marker, and have both NF1 gene “hits”). We are testing these cells to see how faithfully they replicate the original culture cell phenotype (e.g. morphology, proliferation rate, expression of certain genes). These cultures will be valuable tools for cell biology work pursuing more basic research into NF1, and for in vitro screens of new therapeutics.

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Report of a Patient with Constitutional Missense Mutation of INI1/SMARCB1, Coffin-Siris Phenotype and Schwannomatosis

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INI1 (Integrase Interactor 1)/SMARCB1 is a subunit of the SWI/SNF ATP-dependent chromatin-remodeling complex, located at chromosome band 22q11.2. Mutations of the INI1 gene have been described in the context of familial schwannomatosis and, separately, in atypical teratoid/rhabdoid tumors of the brain and rhabdoid tumors of the kidney. No person with a constitutional INI1 missense mutation, schwannomatosis and a complex genetic disorder has been previously described. The constellation of findings in our patient provides important clues to the common genetics of these complex and rare tumors and to an identifiable phenotype that may be associated with this genetic alteration. We examined a 27-year-old man who was assigned the diagnosis of Coffin Siris Syndrome early in life. His physical findings include moderate mental retardation, hypotonia, mild microcephaly, coarse facies, wide mouth with full lips, hypoplasia of the digits, and general hirsutism. Ophthalmologic findings include bilateral cataracts and unilateral retinal detachment. MRI imaging of the brain reveals hypoplasia of the corpus callosum, Dandy-Walker complex, bilateral cranial nerve schwannomas, and ex-vacuo dilatation of the ventricles. The patient came to medical attention as the result of acute spinal cord compression by a previously undetected schwannoma. Subsequently, multiple other schwannomas have been detected by MRI and physical examination in the tongue, neck, and extremities. INI1 gene testing reveals a missense mutation at exon 9 c.1121G>A (codon 374 Arg>Glu). NF2 gene testing (Birmingham, AL) showed no mutation. Comparative genomic hybridization showed a small copy number variation within 1p31.3, a finding also present in his normal father. Specific tumor examination is in progress. The INI1 germline mutation likely accounts for the presence of multiple schwannomas in this patient. However, it is unclear whether the simultaneous occurrence of Coffin-Siris syndrome is a coincidence, or is related to some common molecular genetic etiology.

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Brainstem Pilomyxoid Astrocytoma In A Child With Neurofibromatosis Type 1: A Never-Reported Observation

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INTRODUCTION: Children with neurofibromatosis type 1 (NF1) are at increased risk for the development of both optic pathway and non-optic pathway central nervous system (CNS) tumors, primarily gliomas. The most common histopathology is pilocytic astrocytoma (PA). Pilomyxoid astrocytoma (PMA) is an unusual histologic variant of PA, first described in 1999 as a more aggressive tumor, with propensity to dissemination, typically arising in the hypothalamic region in children less than three years of age. Only two cases of PMA have been reported in children with NF1, both arising in the suprasellar/third ventricle-area. Only one case of PMA in an adult with NF1 has been reported, a thoracic spinal cord lesion with associated leptomeningeal dissemination. PMA has never been reported as arising from the brainstem in children with NF1. REPORT: A 5-year-old boy with NF1 presented with a
Augmented Sodium Currents Contribute to the Enhanced Excitability of Sensory Neurons in Nf1+/- Mice

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Neurofibromin, the product of the Nf1 gene, is a guanosine triphosphatase activating protein (GAP) for p21ras (Ras) that accelerates the conversion of active Ras-GTP to inactive Ras-GDP. We previously reported that capsaicin-sensitive, small diameter sensory neurons from Nf1+/+ mice exhibit greater excitability than Nf1+/- neurons. Heightened and altered pain sensation in those with NF1 may be a result of the altered excitability of these neurons that are the first step in pain signaling. To further investigate the potential role of neurofibromin in excitability, isolated membrane currents critical in controlling neuronal firing were examined by using the whole-cell patch-clamp technique. Consistent with the enhanced excitability of Nf1+/- neurons, the average peak current density of the total sodium current (INa) is -104 ± 21 pA/pF, which is significantly larger than Nf1+/+ neurons (-48 ± 7 pA/pF; p < 0.05, t-test). Activation of total INa in Nf1+/- neurons appears to be unaltered because the voltage for half-maximal activation (V0.5) is -3.6 ± 1.3 mV, which is not different than Nf1+/+ neurons (-2.7 ± 1.3 mV). However, there is a significant depolarizing shift in V0.5 for the steady-state inactivation of INa in Nf1+/- neurons (-18.8 ± 0.4 mV vs. -26.1 ± 0.5 mV for Nf1+/+ neurons, p < 0.05, t-test). In either genotype, the peak INa is not significantly different before and after treatment with tetrodotoxin (TTX, 500 nM), suggesting that under our conditions (30 mM Na+ outside, cesium aspartate in pipette), the TTX-resistant (TTX-R) INa contributes to the enhanced excitability of Nf1+/- neurons and may be the dominant INa in small diameter, capsaicin-sensitive neurons. We also examined the potassium current, which is critical in setting the neuronal excitability. Surprisingly, neither delayed rectifier types (Ik) nor A-types (IA) of potassium currents appear to contribute to the augmented excitability observed of Nf1+/- neurons. The average steady-state current density of Ik is 512 ± 72 pA/pF and is not significantly different than Nf1+/+ neurons (498 ± 83 pA/pF). Similar to Ik, the average peak current density of IA in Nf1+/+ neurons is 307 ± 32 pA/pF, not significantly different than Nf1+/+ neurons (182 ± 50 pA/pF). These results demonstrate that the enhanced excitability of Nf1+/+ neurons results from a larger IA that may be TTX-R and support the idea that regulation of Ras activity through GAPs could be an important determinant of neuronal excitability and contribute to the altered pain sensitivity in those with NF1.

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The Control of Cell Differentiation in Merlin null Schwannoma Cells

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Loss of the Merlin tumour suppressor in patients with neurofibromatosis type 2 is associated with the development of many different kinds of tumours of the nervous system. The most common tumours involving Merlin loss are benign schwannomas, derived from Schwann cells, the myelinating glia of the peripheral nervous system (PNS). We have begun to study the relationship between Merlin loss and the transcription factors and signalling pathways that control the normal cell cycle exit and differentiation of Schwann cells during development. We have examined expression of the zinc finger transcription factor Krox-20, a critical regulator of Schwann cell proliferation and differentiation during peripheral nerve development. Preliminary findings show that the induction of Krox-20 is strongly inhibited in Merlin null schwannoma cells. We are now studying other transcription factors and signalling pathways known to regulate Krox-20 in Schwann cells, with a view to identifying novel potential therapeutic targets for schwannoma tumours involving Merlin loss.

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Search for DNA methylation and subchromosomal structural rearrangements as contributing factors in patients with schwannomas not carrying constitutive SMARCB1 and NF2 mutations

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Minor lesion mutations affecting the SMARCB1 gene have recently been reported as causal factors of schwannomatosis. However, according to currently available published data, a mutation in the SMARCB1 gene can only be found in ~30% of familial patients and ~7% of sporadic patients with schwannomatosis.

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Furthermore, constitutive mutations in this gene can predispose to the development of atypical teratoid/rhabdoid tumours and it is unclear whether the mutational spectrum in the latter disorder is different. Hence, the majority of patients with multiple schwannomas do not carry a constitutional mutation affecting the coding region of the SMARCB1 or NF2 gene and the other genetic causes are still largely unknown. DNA methylation abnormalities and/or chromosomal structural rearrangements may affect expression of this gene and/or other loci on chromosome 22q in a subtle way and contribute to the development of the disease. We want to analyze a cohort of schwannomatosis patients without constitutional SMARCB1 and INI1 mutations, using INI1 expression and methylation analysis as well as DNA copy number analysis initially for chromosome 22q and eventually for the entire genome. So far, we identified a cohort of 23 patients with multiple schwannomas (characterized by absence of vestibular schwannomas by MRI and presence of multiple schwannomas) and no germline mutation in the NF2 and SMARCB1 gene, including 3 familial cases. In 7/23 patients, we analyzed at least 1 tumor sample besides the peripheral blood.

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Metabolic Features of Cerebral Gray and White Matter Using Quantitative Single Voxel Proton MR Spectroscopy in Children with NF1

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Quantitative proton MR spectroscopy (MRS) is a noninvasive technique which has been used to improve our understanding of the cellular composition of brain tissue by measuring metabolic activity in specific brain regions. The purpose of this study was to assess neuronal-axonal integrity in children with NF1 and in healthy controls by comparing the metabolic concentration of specific metabolites in gray and white matter in these two populations. Single voxel 1H spectra were acquired using point resolved spectroscopy (PRESS) with a short echo time of TE = 35 ms, a repetition time of TR = 1.5 seconds and 128 signal averages. Measurements were made of N-acetyl-aspartate (NAA), creatine, total choline, myo-inositol, glutamate + glutamine = Glx, scyllo-inositol, taurine, lactate, alanine, and guanidinoacetate in parietal-occipital gray matter and periatrial parietal white matter. All spectra were measured in gray and white matter regions which demonstrated normal signal on conventional MR sequences. In gray matter, 9 measurements were made from 7 subjects with NF1 (average age = 11.33 years) and compared to 106 measurements made in 104 controls (average age = 10.66 years). No statistical significance was found between NF1 subjects or controls for any of the metabolite measurements in the gray matter. In white matter, 13 measurements were made in 11 NF1 subjects (average age = 11.6 years) and 76 measurements were made in 74 controls (average age = 10.68 years). The absolute concentration of NAA was 8.46 +/- 0.71 mmol/kg in NF1 subjects and 9.17 +/- 0.68 mmol/kg in controls which was a statistically significant finding (p = 0.01). The myo-inositol/creatinine ratio was 1.14 +/- 0.11 in the NF1 population and 1.0 +/- 0.15 in controls which was also statistically significant (p = 0.006). In conclusion, there were no significant detectable metabolite differences in gray matter between NF1 and control subjects. However, decreased NAA and an increased myo-inositol/creatinine ratio in the white matter of the NF1 population may represent a loss of neuronal-axonal integrity and increased astrogliosis respectively, which further supports evidence that NF1 is associated with significant white matter metabolic abnormalities.

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Dual targeting of AKT and mTOR: a potential therapeutic approach for malignant peripheral nerve sheath tumor

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The mTOR pathway may constitute a potential target for the treatment of malignant peripheral nerve sheath tumors (MPNST). However, investigations of other cancers suggest that mTOR blockade can paradoxically induce activation of pro-survival, pro-tumorigenic signaling molecules, especially upstream AKT. Consequently, we hypothesized that dual PI3K/AKT-mTOR blockade might be applicable for MPNST treatment. Expression of activated mTOR, PI3K downstream targets, and pAKT were evaluated immunohistochemically in a tissue microarray of human MPNSTs (n=96) and benign neurofibromas (n=31). Results were analyzed by Wilcoxon Rank Sum tests. Activation of mTOR and AKT pathways in human MPNST cell lines, and the effects of rapamycin (mTOR inhibitor), LY294002 (dual PI3K/mTOR inhibitor), and PI-103 (potent dual PI3K/AKT-mTOR inhibitor) on pathway activation were evaluated by western blot (WB). Agent effects on MPNST growth were evaluated via MTS and colony formation assays. Cell cycle progression and apoptosis were assessed by PI/FACS staining and annexin V assays. Acridine orange staining/FACS analysis, electron microscopy, and WB evaluated autophagy induction. We demonstrate that mTOR and AKT pathways are highly activated in MPNST and were significantly higher in MPNST versus neurofibroma (p<0.05 for all markers evaluated). MPNST cells were sensitive to rapamycin; however, paradoxically increased pAKT and p-eIF4E was observed. PI-103 abrogated MPNST cell growth and induced G1 cell cycle arrest, potentially through repression of cyclin D expression. PI-103 did not induce apoptosis, but significantly induced autophagy in MPNST cells. These results suggest that combined PI3K/AKT and mTOR inhibition might comprise a novel and efficacious therapy for patients harboring MPNST.

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Whole body tumor burden in patients with familial and sporadic schwannomatosis

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Background: The SMARCB1 tumor suppressor gene has recently been identified as a causative gene in schwannomatosis. Germline SMARCB1 alterations have been found in about 30-68% of familial cases and 5-10% of sporadic cases. Until recently, comprehensive phenotypic characterization of schwannomatosis patients was not possible since peripheral schwannomas may be asymptomatic and would not be identified on regional MRI scans. Whole body magnetic resonance imaging (WBMRI) is a technique that allows imaging of the entire body in a relatively short time and provides a global assessment of tumor burden. Methods: We carried out exon scanning of all 9 SMARCB1 exons in genomic DNA from a panel of 10 familial and 8 sporadic schwannomatosis patients who have undergone WBMRI. Each subject was imaged from head to ankles in the supine position using a 1.5 Tesla magnet, integrated body coil, and no intravenous contrast. Using five acquisitions, the entire body was imaged using a fat suppressed fluid sensitive STIR sequence. The images were then fused into a single whole body DICOM image. Tumors were identified by a board-certified radiologist and tumor volume was calculated using semi-automated analysis. Quality of life was assessed by visual analog scale (VAS) for pain and short-form 36 (SF-36). Results: Germline SMARCB1 alterations were identified in 9 of the 10 familial patients and in 2 of the 8 sporadic cases. All familial mutations were non-truncating, while one truncating and one non-truncating mutation were found in sporadic cases. The median number of tumors identified on WBMRI was 2.5 for familial patients and 6.5 for sporadic patients; the median tumor volume was 32 cc for familial patients and 320 cc for sporadic patients. Median VAS pain scale was 0.5 for familial patients and 5.5 for sporadic patients. The norm-adjusted physical component scale score of SF-36 was lower in sporadic patients (43.9) than in familial patients (51.9). The single subject with a truncating mutation had the greatest tumor burden (27 tumors, 913 cc tumor volume). Conclusions: Patients with sporadic schwannomatosis have a higher tumor burden and report greater pain/worse quality of life than patients with familial schwannomatosis. The finding of heavy tumor burden in a patient with a truncating SMARCB1 alteration raises the possibility of a genotype/phenotype correlation in schwannomatosis.


Identification of copy number changes affecting the SPRED1 gene using RT-PCR, Multiplex Ligation-dependent Probe Amplification and Quantitative PCR

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Neurofibromatosis Type 1 Like Syndrome (NFLS [OMIM 611431]) was identified by Brems et al in 2007 as phenotypically similar but genetically different from Neurofibromatosis Type 1. The phenotype consists of café-au-lait macules, axillary freckling and macrocephaly. So far, only heterozygous minor lesion mutations (i.e., frameshift, nonsense, splice and missense mutations) in SPRED1 have been identified as the cause of this syndrome. Using RT-PCR, intragenic copy number changes due to deletions can be identified, but duplications or deletions extending beyond the 5' and/or 3' end of the gene would not be recognized, hence an additional approach is needed. We have used Multiplex Ligation-dependent Probe Amplification (MLPA) on all SPRED1 exons in a cohort of 180 NF1-negative patients presenting with a phenotype compatible with the NFLS in whom no minor lesions were found using a cDNA/RT-PCR based approach for SPRED1 mutation analysis. Two deletions were identified by MLPA and confirmed using q-PCR: one was a deletion of the entire SPRED1 gene; the other was a deletion involving exon 1 and the SPRED1 promoter region. These results indicate the need for dosage analysis to complement sequencing-based SPRED1 mutation analyses.


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Motor Proficiency and Coordination in Children with Neurofibromatosis Type 1

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Objective: Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder. Our clinical experience suggests that NF1 children have poor motor coordination, and preliminary data show that 79% of parents (N=28) reported that their NF1 children were “uncoordinated”. We therefore designed an investigation to quantify motor function in NF1 children. Methods: NF1 children were recruited from the University of Utah NF1 Clinic and assessed at the Shriners Movement Analysis Lab. Children ≤4 yrs of age were excluded due to limitations of the instrument. All individuals were assessed using the Bruininks-Oseretsky Test (BOT-2) instrument, which quantifies motor coordination and proficiency in several categories, and compared to age and sex-matched validated control data. Groups were compared using Student t-tests (alpha=0.05). Summary of Results: Thirteen children with NF1 were enrolled (age: 5-14 yr, ave. 8 yr; 9M, 4F). Statistically significant decreases were observed in all subtest point scores except for manual dexterity (p=0.36) (fine motor precision (p=0.002); fine motor integration (p=0.034); upper limb coordination (p<0.001); bilateral coordination (p=0.049); balance (p<0.0001); run speed agility (p<0.001); combined strength and agility (p=0.004)). Well below or below average scores were observed in 11/13 for total motor composite (z-score: -1.5), 7/13 for fine manual control (z-score: -0.8), 6/13 for manual coordination (z-score: -0.7), 11/13 for body coordination (z-score: -1.3), 8/13 for strength and agility (z-score: -1.4). Conclusions: NF1 children display impaired motor proficiency and coordination. Gross motor tasks were worse than fine motor tasks with significant deficiencies in timing and strength. The effects of NF1 haploinsufficiency on motor coordination are not
well understood, but could be a function of abnormal muscle learning, hypotonia, and/or weakness.

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10 years experience in 38 patients with neurofibromatosis 1 (NF1) and optic pathway glioma (OPG) – Is treatment justified at all?

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Objective: Our aim was to describe the prevalence, mode of presentation and course/outcome of patients with OPG from our NF1 clinics in the light of the recommended guidelines from the 1997 Consensus Statement[1]. Methods: Retrospective case study. Patients: 356 children cared for by two NF1 referral clinics in Düsseldorf and Lüdenscheid, Germany, between 1997 and 2008. Results: 38 patients (11%) with an OPG could be identified in our cohort. Mean age at diagnosis was 4.7 years. 11 patients presented after the age of 6 but only two were symptomatic. In one boy (aged 9) the diagnosis of NF1 was only established after the discovery of an OPG, another child (aged 13) had just moved to Germany. Almost one third (11/38) of the children had visual symptoms leading to the diagnosis. This includes two children who were picked up by routine ophthalmological screening. 9 patients had an unrelated complain as a reason for a cranial MRI. In 18 cases the MRI was done as a routine work up before the referral to us. Cranial MRI showed involvement of the chiasma in 41%, of a single optic nerve in 44% and both optic nerves in 15% of the patients. There was no statistically significant difference between tumor location and symptoms. In over 80% there was no radiological tumor progression. 5 OPG continued to grow slowly before cessation. Mean time between first and last MRI was 40 months. Chemotherapy was used in four (10%) children. No visual improvement was noted but in fact deterioration in 2 patients. Limited tumor regression occurred in one child. None of these patients was older than 5 years at presentation. 25% (10/38) of the patients had some kind of visual impairment at the end of the study. Conclusion: In concert with the consensus statement and other published data our study confirms that most NF1-related OPG do not cause symptoms, rarely progress and do not require therapeutic interventions. Treatment results in terms of visual outcome were disappointing.


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Pilot study using a virtual analogue of the Morris Water Maze to assess spatial learning in children and adolescents with neurofibromatosis type 1

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Background: Cognitive deficits and learning difficulties are the most frequent problem that patients with NF1 and their families face in their daily life, with a prevalence of 35-65% of children. Hallmark features of neuropsychological impairment are visual spatial deficits and difficulties with complex motor tasks. In animal models, these spatial deficits are best characterized by performance in the Morris Water Maze, which can be reversed by restoring neurofibromin or by blocking Ras activity pharmacologically. Currently, there is no standardized test of spatial learning in children or adults in a comparable paradigm. The Computer-Generated Arena is a virtual environment task that has been developed as a “Human Morris Water Maze”. Participants and methods: Children and adolescents with NF1 age 10-16 years (mean 12.8 years) were recruited for this study. The task was completed after completion of standard neuropsychological testing performed in the course of clinical care. Participants used an X-box controller to navigate a circular “arena” to locate an invisible target on the floor, using the patterned walls of the arena as cues. The hidden target was located during each and subsequent trials. Time to target (latency), total distance/path length to locate target (accuracy) and total time spent in the correct quadrant during the last trial in which the platform was removed were measured. Results were compared to age-matched unaffected sibling control group. Results: Participants required less than 10 minutes to successfully locate the hidden platforms and to complete the task. Children with NF1 and their unaffected siblings were able to find the targets easily and both populations demonstrated improved accuracy (path length) over time; however, children with NF1 had more difficulty in the earlier search trials, suggestive of delayed spatial learning. In addition, there were two patterns of spatial learning identified in children with NF1: short latencies on all trials and long latencies on early learning trials with substantial improvements of subsequent trials. Children with NF1 had more difficulty learning the location of the invisible platform for the last trial with no hidden platform, participants with NF1 spent less time in the appropriate quadrant compared to siblings (66 versus 80%). Conclusions: Children with NF1 are able to complete a computerized visual spatial learning task and demonstrate learning over subsequent trials. We hope to validate this task further in the NF1 population to explore specific patterns of performance and to address the sensitivity of this task as a measure of spatial learning.

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Contribution of attention and executive function in the presentation of visuospatial deficit in children with NF-1

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Objectives: Neuropsychological impairments are among the most common sequelae for children with NF-1, most notably visuospatial, attention, and executive function deficits. Upwards of 50% of children with NF-1 exhibit regulatory control deficits. This study examines the relative contributions of visuospatial versus attention/executive dysfunction on error patterns on the Judgment of Line Orientation (JLO) in children with NF-1. Establishing the psychometric properties and utility of a novel scoring system for the JLO was also an objective. Participants/Methods: Nineteen patients involved in one of several prospective or retrospective NF-1 studies participated. All participants underwent extensive neuropsychological assessments from which the data for this study was extracted. Participants were categorized based on quantitative attentional deficits (TEA-Ch, Sky Search DT), generating two groups – NF1 with (NF1+AD) and without (NF1-AD) attention deficits. Group performance was compared on an expanded scoring system of the JLO that extended the score range by assigning credit for each response, as well as establishing error scores based on difficulty level and item location in the test. Results: After controlling for IQ and visuomotor function, a significant difference in error patterns was identified between the NF1-AD and NF1 + AD groups such that the latter exhibited significantly more errors on items requiring greater vigilance and time to differentiate, including higher difficulty items and those in the last third of the test. Conclusions: These findings highlight the possible importance of attention-related factors on a classic test of visuospatial ability in those children with poor regulatory control. A novel scoring system with more sensitivity and specificity is presented.

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Phase II trial of pirfenidone in children and young adults with neurofibromatosis type 1 (NF1) and progressive plexiform neurofibromas (PN)

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Background: Pirfenidone is an orally bioavailable antifibrotic agent in experimental clinical use for pulmonary fibrosis. We hypothesized that pirfenidone could alter growth of NF1-related PN by inhibiting fibroblast proliferation and formation of collagen. Pirfenidone was well tolerated in a previously conducted phase I trial for children with NF1 and PN. Methods: Pts. (3-21 yrs.) with NF1 and progressive PN received pirfenidone at the optimal dose (from the phase I trial) of 500 mg/m2/dose TID continuously (28 days = 1 cycle). Time to progression (TTP) defined as ≥20% increase in PN volume on MRI is the primary endpoint. Pts. who progressed on phase A crossed over from tipifarnib to placebo randomized at enrollment (phase A) to receive tipifarnib or placebo (double-blinded, 200 mg/m2 q12 h po daily x21 days, q 28 days). Time to progression (TTP) defined as ≥20% increase in PN volume on MRI was the primary endpoint. Results: MRI scans and quality of life (QOL) evaluations (parents of pts. 6-18 yrs completed Impact of Pediatric Illness Scale) were performed after 3, 6, and 9 mo and every 6 mo thereafter. Pirfenidone would be considered active if it doubled TTP (Kaplan-Meier analysis) compared to the TTP on the placebo arm (phase A) of an ongoing phase II trial of tipifarnib in pts. with NF1 and progressive PN. Results: Thirty-six pts. (10 f.), median age 8 yrs (range 3-18 yrs), with 49 PN (median PN volume 301 mL, range 12mL 5629 mL) were enrolled. Pirfenidone was well tolerated, and only 3 pts. required a dose reduction for toxicity. The worst toxicities observed in >1 pt. during any cycle were vomiting [grade 2 (n=11), grade 3 (n=1)], nausea [grade 2 (n=8), grade 3 (n=2)], diarrhea [grade 2 (n=3)], anorexia [grade 2 (n=2)], fatigue [grade 2 (n=2)], rash [grade 2 (n=2)], and neutropenia [grade 2 (n=1), grade 3 (n=1), grade 4 (n=1)]. Twenty-nine pts. were removed from the trial for PN progression, none for toxicity, and 5 for other reasons. Currently 2 patients remain on trial (49+ and 25+ cycles). The median TTP for pirfenidone was 13.2 months. In comparison, the median TTP on the placebo arm (phase A) of the tipifarnib phase II trial was 9.4 months. Results: The median TTP for pirfenidone was 13.2 months. In comparison, the median TTP on the placebo arm (phase A) of the tipifarnib phase II trial was 9.4 months. Conclusions: Pirfenidone did not result in a doubling of the TTP compared to the placebo arm of the tipifarnib phase II trial and does not warrant further evaluation in children with NF1 and progressive PN.

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Phase II randomized, flexible cross-over, double-blinded, placebo-controlled trial of the farnesyltransferase inhibitor (FTI) tipifarnib (R115777) in pediatric patients (pts) with neurofibromatosis type 1 (NF1) and progressive plexiform neurofibromas (PN).

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Background: PN cause substantial morbidity in patients with NF1. Tumorogenesis is attributed, in part, to dysregulation of RAS. Tipifarnib is an orally bioavailable FTI that inhibits Ras farnesylation and is well tolerated in children with NF1-related PN. Methods: Pts. (3-25 yrs.) with NF1 and progressive PN randomized at enrollment (phase A) to receive tipifarnib or placebo (double-blinded, 200 mg/m2 q12 h po daily x21 days, q 28 days). Time to progression (TTP) defined as ≥20% increase in PN volume on MRI is the primary endpoint. Pts. who progressed on phase A crossed over from tipifarnib to placebo or vice versa in phase B, followed for progression. MRI scans and quality of life (QOL) evaluations (parents of pts. 6-18 yrs completed Impact of Pediatric Illness Scale) performed at 3, 6, 9 mo. then every 6 mo. on both phases. Tipifarnib considered active if TTP (Kaplan-Meier analysis) 2-fold longer on
phase A than placebo. **Results:** Sixty eligible pts. (24 f.), med. age 8 yrs (range 3-21 yrs), with 80 PN (median PN volume 272 mL, range 21-5573 mL) enrolled. Tipifarnib and placebo well tolerated, no difference in worst grade of toxicity on phase A for tipifarnib (555 cycles) versus placebo (469 cycles) (two-sided p=0.28, Cochran-Armitage trend test). Seven pts. (6 on tipifarnib, 1 on placebo) removed from trial for reversible adverse events including neutropenia (n=3) and rash (n=2). Median TTP on phase A was 19.2 mo. for tipifarnib (n=31), and 10.6 mo. for placebo (n=29), one-tailed p = 0.117. On Phase B the median TTP was 13.3 months for tipifarnib (n=22) and 14.5 months for placebo (n=18), one-tailed p = 0.14. QOL analysis (repeated measures ANOVA) showed a significant group X time interaction (p < .05) in which the total mean scores from baseline to the last assessment on phase A were no different in the placebo group but increased significantly (p < .01) in the tipifarnib group, indicating an improvement in overall QOL on tipifarnib.

**Conclusions:** The randomized flexible cross-over trial design is feasible and allowed comparison of tipifarnib to placebo, while ensuring that all patients received the study drug. Serial volumetric MRI measurements sensitively detected PN progression, which allowed for assessment of drug activity within 12 to 18 mo. in most patients. Toxicity on the tipifarnib treatment arm was indistinguishable from placebo, and there was no evidence of cumulative toxicity. The median TTP for growing PN on placebo is 10.6 months. Tipifarnib did not result in doubling of this TTP on Phase A. The placebo arm on phase A (TTP 10.6 mo) will serve as historical control group for ongoing and future open label clinical trials directed at progressive PN.

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**Loss of the Saccharomyces cerevisiae neurofibromin homologues IRA1 and IRA2 modifies the cellular response to DNA damage**

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In response to DNA damage, eukaryotic cells initiate a signaling cascade that halts the cell cycle. This checkpoint response prevents the replication of damaged DNA and the segregation of damaged chromosomes, thereby maintaining genetic and genomic stability. Altered signaling pathways that modify or attenuate DNA damage checkpoint signaling can have important consequences for the process of carcinogenesis. In the budding yeast Saccharomyces cerevisiae, several types of DNA damage cause a preanaphase cell cycle arrest. Proper initiation and maintenance of this cell cycle arrest are essential for cells to cope with genotoxic stress. Once the DNA damage has been repaired, cells must terminate the DNA damaging signal in order to resume cell division. Failure in the initiation, maintenance, or termination of the DNA damage signaling pathways leads to sensitivity to several kinds of DNA damage. Budding yeast carries two genes homologous to the mammalian NF1 gene, called IRA1 and IRA2. The IRA gene products act as GTPase-activating proteins for the budding yeast Ras homologues. Deletion of one or both IRA genes leads to increased Ras activity and a number of phenotypes including sensitivity to heat shock, poor tolerance of nutrient starvation, and increased sensitivity to oxidative stress. Here, we show that loss of the budding yeast NF1 homologues modifies the yeast cell response to DNA damage. Yeast strains lacking the IRA genes are sensitive to pharmacologic and genetically-induced DNA damage. In IRA deficient strains, activation of the DNA damage response is intact, but cells are defective in recovery from genotoxic stress. These results suggest an interaction between Ras signaling pathways and the DNA damage response in yeast. If conserved in mammalian systems, this interaction could contribute to malignant progression of plexiform neurofibroma in the neurofibromatosis type 1 patient population.

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**MicroRNA-10b Represses Neurofibromin and Regulates NF1 Tumorigenesis**

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Maine Institute for Human Genetics & Health

Patients with neurofibromatosis type 1 (NF1) commonly develop benign peripheral nerve sheath tumors known as neurofibromas. Of these, approximately 10% develop malignant peripheral nerve sheath tumors (MPNST), a highly aggressive form of Schwann cell neoplasm that most often arises from plexiform neurofibromas. MicroRNAs (miRNAs) regulate messenger RNA translation and are frequently deregulated in tumors. We hypothesize miRNAs contribute to tumorigenesis and tumor progression in NF1. Our aim was to determine NF1 specific miRNA profiles and to investigate the role of NF1-related miRNAs in tumorigenesis. We profiled miRNAs in NF1 MPNST cell lines (ST8814 and T265p21) and tumor tissues versus a non-NF1 MPNST cell line (STS262T) and tumor tissues. We also investigated miRNAs in primary Schwann cells from normal adult sciatic nerves, and NF1 dermal and plexiform neurofibromas. MiRNA microarray and real-time RT-PCR analysis revealed a specific miRNA signature in NF1 MPNST cell lines compared to the non-NF1 MPNST cell line. The largest, significant differences were in higher levels of miR-10b, miR-155, miR-335, and lower levels of let-7a and let-7b. The differences in miR-10b and miR-335 were verified in tumor tissues. Of these miRNAs, miR-10b was the only one that was also significantly higher in primary Schwann cells from human NF1 dermal and plexiform neurofibromas compared to normal Schwann cells. Bioinformatics analysis showed miR-10b directly targets NF1 3’UTR, and this was confirmed experimentally. Over-expressing miR-10b in 293T cells suppressed neurofibromin and activated RAS signaling. Antisense inhibiting miR-10b in NF1 MPNST cells decreased RAS signaling, and decreased cell proliferation, migration and invasion. Our research suggests that miR-10b plays an important role in NF1 tumorigenesis. The roles of deregulation of miR-155, miR335 and let-7 in NF1 tumorigenesis need to be investigated further. Understanding the role miRNAs play in NF1 carcinogenesis will give new insights into mechanisms of the disease, and suggest targets for diagnostic biomarkers and therapies.
Hyperactivation of mTOR Critically Regulates Abnormal Osteoclastogenesis in NF1

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Maine Institute for Human Genetics & Health

Individuals with neurofibromatosis Type 1 (NF1) frequently suffer a spectrum of bone pathologies, which include abnormal skeletal development (scoliosis, congenital bowing, and congenital pseudoarthroses, etc), as well as a juvenile osteoporosis with increased fracture risk. These skeletal problems may result, in part, from abnormal osteoclastogenesis. The NF1 protein, neurofibromin, contains a highly conserved GTPase-activating protein (GAP)-related domain (GRD) that converts active RAS-GTP into inactive RAS-GDP in RAS signal transduction. Elevated RAS/PI3K activity has been reported in Nf1-heterozygous (Nf1+/−) osteoclasts, supporting the concept that deregulated RAS signaling contributes to abnormal osteoclastogenesis in NF1. Mammalian target of rapamycin (mTOR) is the downstream effector pathway for RAS. The mTOR pathway is essential in the stimulation of osteoclast survival, differentiation and apoptosis. Recent research has shown mTOR is constitutively activated in NF1-deficient primary cells and NF1-associated tumors. We hypothesize that mTOR is a key downstream effector responsible for abnormal osteoclastogenesis in NF1. Osteoclasts were induced from bone marrow of Nf1 wildtype (Nf1+/+) and Nf1+/− mice with RANKL and M-SCF. Compared to wild type controls, there were 20% more osteoclasts induced from Nf1+/− mice. In addition, these osteoclasts were larger and contained more nuclei. These findings indicate neurofibromin may directly regulate osteoclast formation and function. Compared to wildtype controls, there was hyperactive mTOR signaling in Nf1+/− osteoclasts, indicated by high levels of mTOR mRNA and ribosomal S6 activity. Inhibition of mTOR signaling by rapamycin in Nf1+/− osteoclasts abrogated abnormalities in osteoclast size and number, indicating that hyperactivation of mTOR underlies the pathogenesis of abnormal osteoclastogenesis in NF1. These results suggest that the mTOR signaling pathway plays an important role in NF1 osteoclastogenesis. Inhibiting mTOR may represent a viable strategy to treat NF1 bone diseases.

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</table>

Please note that any registrations received after May 12, 2009 are not listed here.

### NAME BADGE KEY

- **Red**: 2009 NF Conference Chair
- **Yellow**: Children’s Tumor Foundation Board of Directors
- **Pink**: Speaker
- **Blue**: Children’s Tumor Foundation Staff
- **Green**: 2009 NF Conference Attendee
Saturday: Welcome Dinner - Portland Spirit

Sunday: Cocktails/Presentation - Kells Irish Restaurant and Pub
Special thanks to the 2009 NF Conference Chairs

Kathryn North, Children’s Hospital at Westmead, Australia
Joe Kissil, The Wistar Institute

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