NTAP
Neurofibromatosis Therapeutic Acceleration Program at Johns Hopkins
Neurofibromatosis Type 1 (NF1)

Neurofibromatosis type 1 is an autosomal dominant genetic condition that can affect any organ system of the body. Almost all patients with NF1 have dermatologic manifestations, including dermal neurofibromas, café au lait spots and skin fold freckling.

Dermal neurofibromas  
Café-au-lait spots  
Skin fold freckling
Neurofibromatosis Type 1

NF1 is characterized by multiple peripheral and central nervous system tumors including neurofibromas and gliomas.

Many people with NF1 will have cognitive deficits, bone dysplasias, ophthalmologic abnormalities, vascular anomalies, cardiovascular abnormalities and an increased risk of non-nervous system malignancies.
Neurofibromatosis Type 1

- Incidence: 1/2500-3000 births
  - >700 patients known to the Johns Hopkins clinic
  - An estimated 100,000 people in the US currently have NF1.
- Autosomal dominant
  - ~50% of cases are de novo
  - Symptoms within a family can vary widely
- Gene is neurofibromin on 17q11.2
  - Tumor suppressor gene
- Molecular testing available
  - Detects greater than 95% of mutations in individuals meeting NIH criteria
- Symptoms are progressive over time

Among the Most Common Single Gene Inherited Conditions
- Familial combined hyperlipidemia
- Familial hypercholesterolemia
- Dominant otosclerosis
- Adult polycystic kidney disease
- Multiple exostoses
- Huntington’s disease
- Fragile X-syndrome
- Neurofibromatosis
- Cystic Fibrosis
- Duchenne muscular dystrophy
Neurofibromatosis Type 1

Diagnostic Criteria

• Cafe-au-lait spots
  • 6 or more
  • ≥5 mm child (~dime), ≥15 mm adult (~quarter)
• Axillary or inguinal freckling
• 2 or more Lisch nodules
• Optic glioma
• 2 or more neurofibromas OR 1 plexiform neurofibroma
• Distinctive bone lesions
  • i.e. sphenoid dysplasia, tibial pseudoarthrosis
• 1st degree relative with NF1

Other Manifestations

• Macrocephaly
• Astrocytomas
• Scoliosis
• Short stature
• Neurofibrosarcoma/MPNST
• Pheochromocytoma
• Learning disabilities
• Vasculopathy (renal, brain, heart)
• Endocrine abnormalities
  • Thyroid, growth hormone
• Increased risk of breast cancer in women <50
Plexiform Neurofibromas

The most common tumors of NF1 are peripheral nervous system tumors, including nodular and plexiform neurofibromas\(^1\). An estimated 30-50% of patients with NF1 develop plexiform neurofibromas\(^2\).

These tumors can both cause significant neurologic disability, structural deformities, and in some cases transform into malignant sarcomas called malignant peripheral nerve sheath tumors (MPNST)\(^3,4\).

There are no known effective therapies for plexiform neurofibromas.

\(^1\)McGaughran et al, Journal of Medical Genetics, 1999
\(^2\)Ferner et al, J Med Gen, 2007
\(^3\)Evans et al, Journal of Medical Genetics, 2002
\(^4\)Huson et al, Brain, 1988
Plexiform Neurofibromas

Plexiform neurofibromas are benign tumors that are made up of a variety of cell types including neuronal axons, Schwann cells, fibroblasts, mast cells, macrophages, perineural cells and extracellular matrix materials such as collagen\(^1\).

These tumors grow along the nerve sheath spreading the axons as the abnormal cells proliferate and increased extracellular matrix is deposited. They may involve multiple branches of nerve and can involve nerves in any region of the body.

The most common location for plexiform neurofibromas in NF1 patients is the trunk including the paraspinal region (41%), followed by neck/upper trunk (24%) and the extremities (17%). Between 15-30% of plexiform neurofibromas are isolated to the head and neck region\(^2,3\).

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1 Le et al, Cancer Research, 2011
2 Waggoner et al, American Journal of Medical Genetics, 2000
3 Huson et al, Brain, 1988
Plexiform Neurofibromas

For patients in whom a plexiform neurofibroma progresses to require treatment, the most common symptoms are pain, structural disfigurement, airway compression, spinal cord compression, and loss of neurologic function.

Given their prevalence and their potential morbidity and mortality, developing therapeutics for plexiform neurofibromas is a priority.
Neurofibromin

NF1 results from a mutation in the Nf1 gene\(^1\). This gene is large (350kB and 60 exons) and encodes the protein neurofibromin\(^2,3\). Neurofibromin is widely expressed in almost all tissues, but is most abundant in the brain, spinal cord and peripheral nervous system\(^4\).

Neurofibromin has a guanosine triphosphatase (GTPase) activating protein (GAP) –related domain (GRD). This region of the protein has been the most deeply investigated domain of neurofibromin, as GAP plays a key role in regulating Ras activity.

Ras represents a family of proto-oncogenes that can be transformed into oncogenes that are implicated in both cancers common to the general population and in NF1 associated benign tumors\(^5\).

\(^1\) Li et al, Genomics, 1995
\(^2\) Shen et al, Journal of Medical Genetics, 1996
\(^3\) Upadhyaya et al, Human Genetics, 1997
\(^4\) Daston et al, Neuron, 1992
\(^5\) Le et al, Oncogene, 2007
Neurofibromin and Ras

In healthy cells, Ras regulates proliferation, differentiation, transformation, and apoptosis. In the absence of neurofibromin, Ras-GTP is constitutively activated resulting in excessive stimulation of multiple pro-growth pathways\(^1\).

Mutations in various Ras genes are found in almost all known solid tumors. Moreover, mutations in Nf1 have been linked to several malignancy syndromes that often occur outside of NF1\(^2,3,4\).

Therapeutics developed to address neurofibromin dysfunction may impact both NF1-related tumors as well as many common, treatment resistant, cancers.

1 Le et al, Oncogene, 2007
2 Harris et al, Nat Rev Clin Oncol, 2010
4 Bos et al, Cancer Research, 1989
The Schwann Cell

The cell of origin for plexiform neurofibromas is thought to be the $Nf1^{-/-}$ Schwann cell\textsuperscript{1,2}. Schwann cells differentiate from neural crest cells to either Schwann cell precursors, skin derived precursors, or boundary cap cells.

There is increasing pre-clinical evidence that loss of $Nf1$ in the early Schwann cell lineage is associated with the highest risk of neurofibroma formation.

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\textsuperscript{1} Wu et al, 2008
\textsuperscript{2} Zheng et al, 2008
Beyond the Schwann Cell

The microenvironment plays a key role in tumorgenesis of plexiform neurofibromas. Plexiform neurofibromas are composed of multiple cell types including bone marrow derived cells such as mast cells, fibroblasts, and endothelial components\(^1\).

In mice that routinely develop plexiform neurofibromas, daily treatment with the c-kit inhibitor imatinib decreased tumor size, mast cell infiltration and proliferation\(^2\). This dramatic result was the rationale for the ongoing clinical investigation of imatinib.

The growing understanding of these cell-cell interactions provide opportunities for therapeutic development.

\(^1\)Le et al, Cancer Research, 2011
\(^2\)Yang et al, Cell, 2008
### Animal Models

Multiple animal models have been developed for the investigation of plexiform neurofibromas.

<table>
<thead>
<tr>
<th>MODEL</th>
<th>PLEXIFORM TUMORS?</th>
</tr>
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<tbody>
<tr>
<td><em>Nf1Δ31/Δ31</em> (Jacks et al, Nature Genetics, 1994)</td>
<td>No; hyperplasia in sympathetic neurons</td>
</tr>
<tr>
<td><em>Nf1Δ31/+</em> (Jacks et al, Nature Genetics, 1994)</td>
<td>No</td>
</tr>
<tr>
<td><em>Nf1−/−</em> embryonic stem cells into <em>Nf1+/−</em> C57BL/6 blastocysts (Cichowski et al, Science, 1999)</td>
<td>Yes, NF1−/−</td>
</tr>
<tr>
<td><em>Nf1floxFlox; Dhh-Cre</em> (Wu et al, Cancer Cell, 2008)</td>
<td>Yes, in a WT microenvironment</td>
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<tr>
<td><em>Nf1floxFlox; Krox20-cre</em> (Zhu et al, Science, 2002)</td>
<td>Yes, in a microenvironment with haploinsufficient cells</td>
</tr>
<tr>
<td><em>Nf1floxFlox; Krox20-Cre + RT + NF1+/−</em> bone marrow transplant (Yang et al, Cell, 2008)</td>
<td>Yes, with donor mast cells</td>
</tr>
<tr>
<td><em>Nf1floxFlox; Poa-Cre</em> (Zheng et al, Cancer Cell, 2008)</td>
<td>Yes, 15-20 months</td>
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The two preclinical models in widest use for therapeutic testing are *Nf1floxFlox; Krox20* and *Nf1floxFlox; Dhh-Cre*, as they reliably produce plexiform neurofibromas that can be imaged with FDG PET or MRI.
In the past 10 years, there have been 16 clinical trials examining the impact of various agents on plexiform neurofibromas.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Agent</th>
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<tbody>
<tr>
<td>Angiogenesis</td>
<td>Thalidomide (Gupta et al, Neurology, 2003), 13-cis-retinoic acid plus interferon alfa-2 (unpublished data)</td>
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<tr>
<td>Farnesyl transferase</td>
<td>tipifarnib (Widemann et al, Journal of Clinical Oncology, 2006)</td>
</tr>
<tr>
<td>Mast-schwann cell-cell signaling (c-kit/PDGFR inhibition)</td>
<td>imatinib (Robertson, ASCO, 2011), sorafenib (Kim et al, Journal of Clinical Oncology, 2010), nilotinib (ongoing)</td>
</tr>
<tr>
<td>Immune modulation</td>
<td>PEG-Intron (Jakacki et al, Neurology, 2011)</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>pirfenidone (Babovic-Vuksanovic et al, Neurology 2006)</td>
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<tr>
<td>mTOR</td>
<td>sirolimus (unpublished data)</td>
</tr>
<tr>
<td>MEK</td>
<td>AZD6244 (ongoing)</td>
</tr>
<tr>
<td>Antihistamine</td>
<td>Ketotifen fumarate (Riccardi, Arch of Dermatology, 1993)</td>
</tr>
<tr>
<td>Tumor tissue disruption, pro-inflammatory</td>
<td>Photodynamic therapy (unpublished data)</td>
</tr>
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</table>

Most of these agents were either poorly tolerated or had little activity.
Alternate Therapeutic Approaches

altering gene expression

modulation of immune response

miRNA based therapies
Many Potential Therapeutic Targets
• The wider NF1 community of investigators, patients, patient advocates and funding agencies, have created organized pre-clinical and clinical research networks.

• **Pre-Clinical Consortium**: Seven (three specific to plexiform neurofibroma) expert laboratories sponsored by the Children’s Tumor Foundation to use robust mouse models and mechanistic insight to identify, test, and refine therapies for NF-associated tumors.

• **NF Clinical Trials Consortium**: sponsored by the United States Department of Defense Congressionally Directed Medical Research Program in Neurofibromatosis
The Neurofibromatosis Therapeutic Acceleration Program

NTAP was founded with a $27 million philanthropic gift and the vision of complementing the existing efforts in NF1 research with a specific focus on identifying and supporting the development of therapeutics for plexiform neurofibromas. The goals of NTAP are to:

1) Facilitate productive collaboration among the established stakeholders in NF1 (academic, industry, government, foundation)

2) Strategically “fill the gaps” in funding such that projects critical to the development of effective therapeutics for plexiform neurofibromas are prioritized and expanded

3) Attract new scientists to the investigation of plexiform neurofibroma in order to accelerate the rate at which new therapeutics are identified and tested

4) Provide the resources and support for research collaborations focused on developing effective therapies for plexiform neurofibromas
NTAP Mission

Accelerating the development of effective therapeutics for plexiform neurofibromas.

- **Focusing on Therapeutics**
  We are vigilant about advancing the most promising ideas for plexiform neurofibroma therapeutics. The pathway from the lab (where researchers are making discoveries) to the clinic (where therapies are needed) is often lengthy and convoluted. NTAP facilitates the timely translation of innovative discoveries into treatments for plexiform neurofibromas.

- **Fostering Collaboration**
  NTAP recognizes that developing treatments requires collaboration. We foster relationships across a broad research community including the pharmaceutical industry, regulatory agencies, private foundations and academic centers to increase the pace at which promising research can be converted into patient therapies. Advisory boards with diverse membership ensure that NTAP complements the ongoing efforts of the greater neurofibromatosis research community.

- **Open and Timely Sharing of Results**
  We require that data from funded projects be shared openly in real time. The NTAP leadership facilitates communication among collaborators and enables dissemination of data appropriately. In addition, research progress is monitored closely by the NTAP directors and the scientific advisory board to ensure that projects with the most potential for translational success are advanced.

- **Streamlining the Research Process**
  We work hard to identify critical gaps in knowledge that limit the development of therapeutics for plexiform neurofibromas and then recruit scientists with innovative ideas to address these gaps. The grants process is targeted to specific research questions and designed to provide quick feedback and funding. NTAP is poised to provide funding and other resources to investigators, allowing basic science discoveries to be aggressively translated into therapeutics for plexiform neurofibromas.
NTAP at Johns Hopkins

NTAP is established as an entity within the Johns Hopkins University School of Medicine. Johns Hopkins has a long history of promoting and coordinating collaborative research to find innovative solutions to disease.

Through the established collaboration with the Brain Sciences Institute (Bsi) Neurotranslational Program, NTAP has direct access to an internal translational research unit run by an experienced biopharmaceutical industry team. Bsi leadership both serve as advisors on therapeutic research projects and assist in drug development, drug optimization and building relationships with industry partners.

NTAP’s leadership team has a combined experience of over 25 years working within the infrastructure of Johns Hopkins running multi-institutional clinical trials, developing drugs, partnering with industry representatives, and managing a thriving clinical care practice to allow rapid translation of discoveries into the clinical arena.
# NTAP Leadership

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Jaishri Blakeley</td>
<td>Director</td>
<td>Johns Hopkins University</td>
</tr>
<tr>
<td>Amanda Bergner</td>
<td>Director of Operations</td>
<td>Johns Hopkins University</td>
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## Scientific Advisory Board

<table>
<thead>
<tr>
<th>Name</th>
<th>Research Area</th>
<th>Institution</th>
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<tbody>
<tr>
<td>Jaishri Blakeley</td>
<td>Adult Neuro-Oncology, Clinical Investigation</td>
<td>Johns Hopkins University</td>
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<tr>
<td>Barbara Slusher</td>
<td>CNS Drug Development and Drug Discovery</td>
<td>Brain Science Institute</td>
</tr>
<tr>
<td>Brigitte Widemann</td>
<td>Pediatric Oncology, Neurofibromatosis Clinical Experimental Therapeutics</td>
<td>Pediatric Oncology Branch, National Institutes of Health</td>
</tr>
<tr>
<td>John P. Clancy</td>
<td>Pediatric Pulmonary, Cystic Fibrosis Translational Research</td>
<td>University of Cincinnati</td>
</tr>
<tr>
<td>Charlotte Sumner</td>
<td>Neuro-Muscular Translational Science, Spinal Muscular Atrophy</td>
<td>Johns Hopkins University</td>
</tr>
<tr>
<td>David Loeb</td>
<td>Pediatric Oncology, Sarcoma Signaling Systems and Pre-clinical models</td>
<td>Johns Hopkins University</td>
</tr>
<tr>
<td>Karen Cichowski</td>
<td>Cancer genetics, Pre-clinical Experimental Therapeutics</td>
<td>Harvard Medical School, Brigham and Women's Hospital</td>
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NTAP Current Projects

• Investigation of metabolic modulation of the microenvironment in plexiform neurofibromas focused on glutamate carboxypeptidase activity in tumors

• Validation of targets driving tumorigenesis in plexiform neurofibromomas for biomarker development

• Implementation of therapeutic trials based on the most compelling pre-clinical data
Summary: NF1 and plexiform neurofibromas

Compelling Therapeutic Area

- Neurofibromatosis Type 1 is a common rare disease
- 1 / 3,300 people (100,000 in US); 30 – 50% develop plexiform neurofibromas
- Well-characterized disease pathogenesis
- Monogenic, autosomal dominant disorder
- Urgent unmet medical need with high potential for expansion
- No available drug therapies; symptomatic progressive tumors
- Ready for application of therapies already in development or in clinic with clear targets
- Overlap with many Ras driven disorders; potential for expansion to other larger market indications
Summary: NF1 and plexiform neurofibromas

Available Research and Development Resources

- Animal models that recapitulate human tumors
- Coordinated pre-clinical efforts through the Neurofibromatosis Preclinical Consortium
- Extensive regulatory precedent, with standardized endpoints via the experience through 16 prior clinical trials and the REiNS (Response Endpoints in Neurofibromatosis and Schwannomatosis) efforts

Accessible Patient Community

- Well organized patient registries for natural history data and to enhance enrollment:
  - 120 patients with NF1 are followed through a comprehensive natural history study sponsored by the NIH
  - >4000 patients with NF1 are followed through a Canadian registry
  - >1400 patients with NF1 are followed through a United Kingdom registry
- Department of Defense Clinical Trials Consortium
  - 15 clinical sites with expertise in NF1 and performance records for accrual to clinical trials
  - Operations center for statistical design and analysis; budgets, MTA, trial coordination

Well Situated Research Partners

- NTAP, CTF, DoD, NIH all with substantial commitment of resources to finding treatments for patients with NF1 and plexiform neurofibromas