Requests for Applications: Understanding of Cutaneous Neurofibromas

Background and Overview

Cutaneous neurofibromas (cNF) are multicellular tumors involving the skin that are one of the hallmark findings of neurofibromatosis type 1 (NF1). They affect >90% of adults with NF1 and are a major source of emotional and social distress as well as intermittent but, chronic physical symptoms such as pain and itching (Huson S, et al, Brain, 1988). The tumors typically appear in adolescence and commonly increase in number and size over time. Although cNF are not medically predisposed to malignant transformation and are rarely associated with functional limitations, these tumors are highly damaging to people with NF1 *via* their disfigurement. In adults with NF1, perceived disease visibility is significantly associated with depression, psychosocial distress, quality of life (QoL) impairment and negative body experience for attractiveness/self-confidence (Page PZ, Am J Med Gen, 2006; Wolkenstein P, Arch Dermatology, 2001). There is currently no known way to prevent these tumors from developing and current treatments are limited to regional procedure based approaches that have uncertain efficacy. As this is an unmet need and patients with NF1 often identify these tumors as their greatest burden within the complex syndrome of NF1, the development of therapies to prevent, stabilize or reduce these tumors is a priority.

Current knowledge about the underlying mechanistic, structural, and genetic factors responsible for the formation of human cutaneous neurofibromas (cNF) is limited. This represents a major hurdle to understanding disease initiation and pathogenesis, and ultimately the ability to develop effective treatments for these tumors. To overcome this barrier, the Neurofibromatosis Therapeutic Acceleration Program (NTAP) is investing in cNF research and invites proposals focused on NF1 associated cNF pathophysiology with special focus sought in the following areas:

1. Identifying the human cells of origin for cutaneous neurofibromas and understanding how these cells influence and drive cNF initiation and pathogenesis.
2. Understanding how both nerve and tumor microenvironment contribute to cNF pathogenesis.
3. Identifying and elucidating the specific genetic and molecular factors that underlie cNF initiation and progression.
4. The generation of preclinical model systems that elucidate disease biology and enable preclinical therapeutic testing.

Award Details

Funding: NTAP anticipates funding up to 10 awards *via* this initiative. Awards are anticipated to be for projects lasting from 12-36 months, but with appropriate justification, projects can be outside of this timeframe. The anticipated funding is $300,000 per project with detailed budget justification. There is flexibility based on the project and appropriate budget justification. Investigators can be from academia, government, or private sector, and international candidates are welcomed. Indirect costs are not to exceed 10% of the total direct funds.

Next Steps: Investigators who are interested in submitting a proposal for any one of the cited research areas should first review the following detailed descriptions of the associated tasks for these research topics as noted in the 'Appendix' section, and then submit a two page letter of intent per the format described in the 'Submissions' section by 5p EST on May 12, 2017. If advanced for consideration of award, notification of a request for a full proposal will be made to the primary investigator by May 31, 2017.

Submissions

A. Letter of intent: The first step in the submission process will be to submit a letter of intent. This should consist of a two page document which outlines the proposed scientific strategy for the study (or studies) of interest. Please include the following in the letter of intent:

- Rationale
- Details about the specific question(s) to be addressed
- Schema of the experimental design
- Available preliminary data
- Project goals, milestones, and anticipated timeline

Please also include CV(s) for the lead investigator(s).

The letter of intent and CV(s) are due at 5:00p Eastern Standard Time on May 12, 2017. Please submit the letter of intent as a PDF file by email to **mstathi1@jhmi.edu** and **rjacks13@jhu.edu**. A confirmation email will be sent upon receipt of the letter of intent. The letter of intent will be critically reviewed to make the decision regarding which projects will be advanced for a full proposal based on the criteria listed above. If a project is selected for a full proposal, the submitting investigator will be notified by May 31, 2017 about the expected format and timeline for submission of a full proposal.

B. Submission of a solicited full proposal: The template for a solicited proposal will be made available to the investigator when they are notified that their application has been recommended for second stage review. The full proposal is due by 5:00p EST on August 18, 2017. Please submit the full proposal as a PDF file by email to **mstathi1@jhmi.edu** and **rjacks13@jhu.edu**.

C. Selection Process: All letters of intent will be screened by NTAP Leadership and based on the review criteria above, proposals will be selected to be advanced for the second stage of review. Applications submitted for the second stage of review will undergo detailed peer-review by a minimum of three independent reviewers with appropriate subject matter expertise. Proposals will be scored based on: 1) The scientific strategy and feasibility of the proposal; 2) The quality and innovation of the methods and research plan; 3) Clarity of the plan as applied to the stated categories of interest; 4) Investigator commitment, environment and resources; and 5) Appropriateness of budget. Projects that highlight collaboration and proposals from investigators new to NF1 research are encouraged. All reviewers will be under a confidentiality agreement with NTAP to ensure the privacy of ideas and data within applications. Investigators will be informed of the final decision for acceptance and funding by October 6, 2017. Please note that revisions may be requested by NTAP in order to proceed with confirmed funding (inclusive of SOW, timeline/deliverables, and budget/budget justification exhibits) as part of the process.

D. Proposal acceptance: If a proposal is advanced for funding, the primary investigator will be required to travel to Baltimore, Maryland, to participate in an investigators meeting on October 26, 2017 (please plan for 9:00a-4:00p). Members of NTAP Leadership, all investigators whose proposals were advanced for funding, and experts who participated in the review will be present at this meeting to discuss the scope and proposed milestones for each project and the overall research program goals. The purpose of this meeting is to establish communication between all funded investigators for this announcement and provide expert technical feedback with the goal of minimizing overlap and maximizing the chances for successful progress in identifying and understanding biological factors underlying the initiation and pathogenesis of cutaneous neurofibroma. Participation in this meeting

is required. The final research plan (the scope of work, budget and timeline) incorporating the details agreed to at the investigators meeting will be requested to be sent to NTAP by 5:00p EST on November 10, 2017. If the research plan is complete, funds will be available for release immediately upon completion of contract agreements with the investigators institution. A signed contract and completed material transfer agreement (MTA) between the primary investigator's institution and Johns Hopkins University are required prior to funds being released. Applications must meet all of the NTAP conditions for funding (www.n-tap.org; Research Opportunities). Applying to this RFA will not preclude an application to NTAP for other projects.

E. Overall Timeline:

RFA Release Date: April 18, 2017
Letter of Intent Submission Deadline: May 12, 2017 at 5:00p EST.
Notification of proposal solicitation: May 31, 2017
Proposal Submission Deadline: August 18, 2017 at 5:00p EST.
Notification of award: October 6, 2017
Planning Meeting Date: October 26, 2017
Final Research Plan Due: November 10, 2017 at 5p EST
Funds Available: TBD (pending contract and MTA)

F. For Questions or Concerns,
Please contact:

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Appendix: Proposal Topics

NTAP will be interested in receiving proposals for any of the studies listed below. Please clearly specify which study(s) is/are of interest for funding consideration. If there are modifications for a listed study of interest, or any questions, please feel free to contact us to further discuss.

1. Identifying the cells of origin in human cutaneous neurofibroma and understanding how these cells influence and drive cNF initiation and pathogenesis

Successful proposals will focus on identifying the specific progenitor cells responsible for the formation of the different types of human cNF with respect to any of the following areas: where these cells come from, elucidating the functional and mechanistic factors that underlie their tumorigenic potential, understanding their roles in disease initiation and pathogenesis, demonstrating methods which enable the isolation/analysis of pure populations of the cells of origin.

A. **Determining the cell(s) of origin for human cNF and their characteristics**.

Identification of these cells, understanding where they come from, and if there are more than one type contributing to disease initiation.

i. Studies that focus on exploration of Schwann cell development with respect to the location of derivitization, and timing of events. Detailed studies should be aimed at understanding the precise stage of Schwann cell differentiation and associated processes to ascertain what type of cell gives rise to a cNF (i.e. precursor cell, immature Schwann cell, or mature Schwann cell ?), and when this occurs (i.e. differentiation from a mature Schwann cell, or from related progenitor cells?).
   - These studies should account for different types of cNF with respect to size, visibility, growth rates, etc., and also address differences between mouse vs human.
   - Studies should aim to address if different cells of origin are in fact responsible for the initiation of cNF (vs. plexiform neurofibromas) rather than environmental factors as being responsible for the different behaviors of the two disease types.

B. Factors leading to tumorigenesis

Exploration of the molecular genetic events that enable the tumor cell of origin to progress or advance into a tumor.

i. Studies that focus on determining if there are "tumor initiation cells", and if so, reporting of their identities and neoplastic properties (e.g. angiogenic and invasive behaviors)
ii. Studies that explore if there are other cells within cNF that lead to/sustain the tumor mass, and if so, reporting of information around their identities and neoplastic properties.

C. Application of methods for isolation/analysis

i. Studies that evaluate methods which allow for the isolation of a pure population of the cells of origin.
ii. Studies that evaluate methods for assays that can assess the tumor cells *in vivo* (following their isolation).
   - Such methods should be highly reproducible and allow for robust isolation, assessment, and facilitate model development.

2. Effects of tumor microenvironment

Successful proposals will be those which focus on any of the following areas: defining how either the nerve tumor microenvironment, non-Schwann cells (e.g. mast cells, fibroblasts, macrophages, fibrocytes, endothelial cells, pericytes, lymphocytes, and perineurial cells), or non-cellular elements (e.g. antibodies, fats-lipids-adipocytes, hyaluronate) each play key roles in influencing and contributing to disease pathogenesis. The gaining of insights on approaches for therapeutic targeting and the identification of specific targets which support a preventative treatment strategy (or reversal of disease course) as a result of these investigations, will represent highly significant outcomes.

A. Nerve micronenvironment signaling and events

Exploration of what are the perineural signals that regulate cNF formation.

i. Studies that aim to identify the developmental steps which produce the tumorigenic phenotype with an understanding of the timing and events taking place in the nerve tumor microenvironment, and enable identification of a temporal window appropriate for therapeutic intervention.
ii. Studies that aim to address what is the cell source of the signals, requirement(s) of NF1 heterozygous status in these cells, and the timing and location of NF1 loss of heterozygosity (LOH) during neural crest migration.
iii. Studies that probe signaling in detail with respect to determining if: the signals are soluble or due to extracellular matrix or cell-cell contact signaling, if there is a signaling cascade or hierarchy of signals, and what are the signals that may influence peripheral nerves and cause collateral branching and expansion of the neural branching within the lesions.

B. Non-Schwann cell components contributing to cNF formation

i. Studies exploring the basic biology of mast cells (using available models, and human tissue), and also their role in cNF with respect to natural history, and pruritus.
ii. Studies exploring NF fibroblast differences with regard to basic biology, phenotype (keloid, embryonic, hypertrophic scar) and location (endonurial vs. skin).
iii. Studies exploring the roles of melanocytes, epidermal biology, and biology of senescence. Such studies should aim to elucidate the tumorgenic potential of 'Nf1 Schwann cells vs fibroblasts vs melanocytes' vs 'non-Nf1 cells', and the roles of these cells in impacting tumor behavior. These studies should further aim to identify why Schwann cells, and melanocytes do not undergo malignant transformations for cNF.
iv. Studies exploring nerve injury/recovery with respect to neurofibroma formation, and the roles of macrophages in these processes.
v. Studies exploring the basic biology of either fibrocytes, endothelial cells, pericytes, lymphocytes, perineurial cells, fats-lipids-adipocytes, or hyaluronate, in order to delineate their specific roles in cNF tumor formation and identify potential targets for intervention.
    - Modalities of consideration for targeting include (but are not limited to) : Druggable targets necessary for lesion initiation, progression, or senescence. (i.e. prevention based approach), mechanisms of fibrosis progression, reversal, and remodeling (i.e. treatment based approach), and also tumor cell and

microenvironmental targets (taking advantage of small molecule screening/synthetic lethal approaches).

Studies should utilize tools available (e.g. laser capture microdissection, single cell analysis).

3. Identifying and elucidating the specific genetic and molecular factors that underlie cNF initiation and pathogenesis

Successful proposals will be those which focus on any of the following areas: providing an improved understanding of the background and effects of the 2nd hit, identifying the biological factors that specifically configure the Schwann cell towards tumorigenicity (in the context of NF1 loss), enabling genotype-phenotype correlations through genetic analysis of multiple samples, providing an improved understanding of the structural, mechanistic, and functional properties of the large and relatively unexplored protein that is neurofibromin.

A. Impact of 2nd hits

i. Studies that explore the origin of the 2nd hit, including genetic type and mutational spectrum of inherited and 2nd hits.

ii. Studies focused on determining the timing of 2nd hit onset (and associated mechanism), and the initiation of NF1 cutaneous neurofibroma prior to the somatic mutation.

B. Microdeletion effects on cNF growth

Exploration of epigenetic signaling abnormalities to determine if the higher number of lesions observed in microdeletion populations are due to epigenetic factors.

i. Studies that probe histone methylation differences, and the influence of the heterozygous deletions (e.g. SUZ12 microdeletion) in amplifying growth factors, to ascertain if there is really a difference for cNF growth in the presence or absence of microdeletions.

C. Genetic analysis

Exploration of genotype phenotype correlations to characterize inherited and acquired hits within the gene

i. Studies that aim to correlate the clinical profile, genetic type and mutational spectrum of the 2nd hits, and also histone methylation abnormalities.
   - Such studies should involve assembly of a large database to include sufficient numbers of patients with the same recurrent mutation for correlation.

D. Neurofibromin and Molecular factors

Exploration of the cell biology of the NF1 protein and mutants, and neurofibromin (Ras GAP) on a broad scale with respect to functional and mechanistic factors.

i. Studies that examine the region in the cell membrane where neurofibromin is present, in addition to other regions, and seek to potentially identify additional binding partners and functions of neurofibromin.

4. The generation of preclinical model systems that elucidate disease biology and enable preclinical therapeutic testing

Successful proposals will focus on generating robust preclinical animal models which delineate the disease biology and are conducive to testing of therapies directed at specific targets that are predictive of therapeutic results in human.

A. Biology and Therapeutic Testing

i. Proposed model systems for development may include (but are not limited to) either human-mouse xenograft models, porcine models, 3D organotypic models, PDX/organ culture models, cell models (e.g. induced pluripotent stem (iPS) cell lines), and *in vitro* cNF models.

ii. Desirable features a model should have will include:

- The display of multi-state progress that recapitulates the progression of human cNF
- The ability to capture the many features of cNF and that of the tumor initiation cell
- Tumor histology and pathology features similar to human cNF and where genetically NF1 loss is associated with the tumors
- The display of altering effects in the same pathway(s) that are altered in human cNF (e.g. MAPK)
- A gene expression profile that is very similar to the cutaneous tumor
- Similar microenvironmental factors as in human cNF for the tumor to develop
- Suitability for therapy testing