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