Open Application for Funding from NTAP

Please complete all of the following sections. The proposal is limited to 5 pages, there is no page limit for the addendums. Submit completed applications to mstathi1@jhmi.edu and rjacks13@jhu.edu. Open applications will be reviewed by the last week of each quarter (unless received within two weeks of the end of a quarter, or if additional information is needed in which case the PI will be notified).

PROPOSAL (limited to 5 pages)

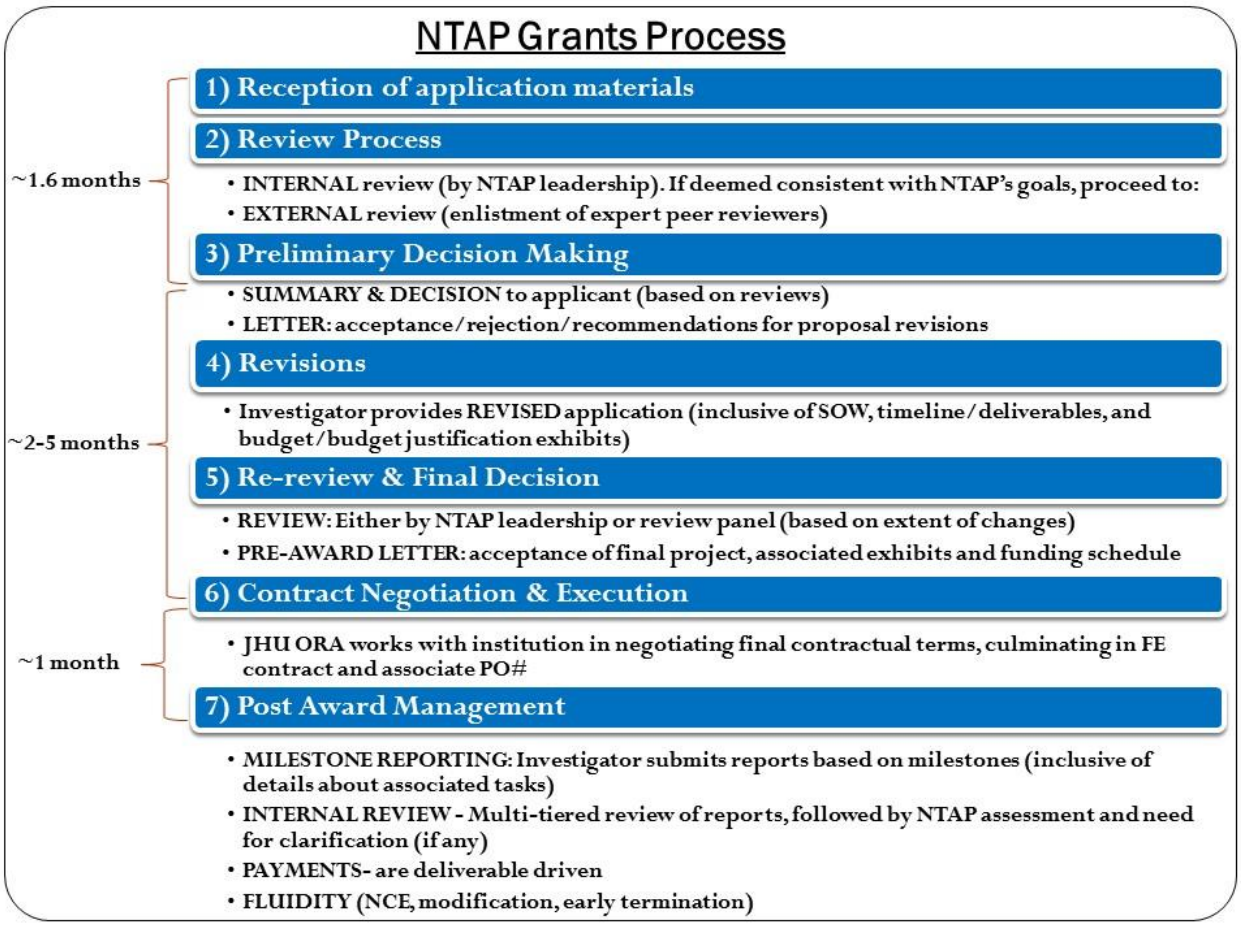
1. **Title of Project**
2. **Name of PI**
3. **Scientific Strategy** (Outline of proposed scientific strategy; typewritten, single-spaced in typeface no small than Arial 11-point and 0.5" margins).
   A. Background
   B. Rationale
   C. Preliminary Data (if applicable)
   D. Schema of the Experimental Design
   E. Project Goals
   F. Scope of Work (refer to example exhibit below)
   G. Milestones/Deliverables (refer to example exhibit below)
   H. Anticipated Timeline

ADDENDUMS (no page limit)

1. Contact information for the primary investigator (i.e., phone numbers, email, fax, and address)
2. References
3. Biosketch(es) of the primary investigator and key personnel (in NIH format)
4. Complete list of collaborators (and their roles in the project)
5. Proposed budget with detailed justification (including specific resources to be used and a description of any existing core support and institutional resources available to the primary investigator and collaborators relevant to the project)
6. Any relevant, overlapping/leveraged support
7. Letters of support (optional)

ADDITIONAL GUIDANCE (for applicants):

- Please refer to the figure below, which illustrates the typical flow and timeline of NTAP's funding process



- Please note that while this is a 'typical' example of the process per historical data, each application and situation is unique and therefore the timings as shown may be different in your experience.

- When several institutions are represented, a separate agreement for each institution may have to be created (especially for co-PI's), inclusive of distinct Scopes of Work, Milestone/Deliverables, and Budget/Budget justification schedules. **(see examples below). Accordingly, this may increase the amount of time needed for the granting process.**

- **Basic Definitions:**
  - **Scope of Work (SOW):**
    - The work, broken down, that is required to achieve the aims and goal(s) of the project. Can be described in either narrative or tabular format.
    - A good SOW statement should be preceded by (1) A brief background to the project or component and (2) The general objective(s) that justify its existence.
  - **Milestones/Deliverables:**
    - **Milestone:** A reference point that marks a major event in a project and is used to monitor the project's progress. The milestones for a project should present a clear sequence of events that will incrementally build up to the completion of the approved project. Milestones serve as unambiguous indicators of progress and are used to satisfy requirements that grants are paid on a reimbursement basis against agreed-upon work.
    - **Deliverable:** A concrete, discreet outcome or task that must be completed and delivered under the terms of an agreement or contract.

Exhibit A

TITLE: Transition to 5D and Sun screening of plexiform neurofibroma models in 96-well format
PI: Dr. Roger Will Achieve, PhD (Bestinclass State University, BSU)

Scope of Work

August 1, 2017 – July 31, 2018

INSTITUTION X previously performed a library screen of compounds against PN cells growing on 1536-well plates using Dark-n-Glo to assay proliferation. Based on this screen, it is known there is a group of compounds that show altered response in cells that express DGDP. In this current project, Dr. R. Will Achieve (from BSU) will work on leveraging these results.

INSTITUTION X will ship a set of 20 selected compounds to BSU with the expectation that BSU will perform 96 well (2D) and then Sunscreen (5D) assays, based on the results from the preliminary 1536 well (2D) screens. It is believed that this collaborative approach will provide important scientific knowledge and practical application. The latter will result from the development of a 96-well (2D) format Dark-n-Glo assay procedure that will be useful for general confirmatory testing. It is expected that these results should directly reproduce those of the initial screen, i.e., that the outcome is independent of the platform (1536-well vs. 96-well) or performance site (INSTITUTION X vs. BSU).

Next, a transition to a 5D culture, 96-well format assay will be executed, that is predicted to advance from confirmation to a secondary screen of initial results. It is hypothesized that cells growing in 5D matrices, as compared to those growing in 2D on plastic, will exhibit drug sensitivity that serves as a better predictor of eventual clinical effectiveness.

The primary goals are: (1) To perform an initial confirmatory 2D assay of 20 compounds (selected and provided by INSTITUTION X) against plexiform neurofibroma (PN) cells in a 96-well format, and then (2) To establish a secondary screening protocol of 5D PN cell cultures in a 96-well (5D sunscreen) format to assay 20 compounds (selected and provided by INSTITUTION X).

Exhibit B

TITLE: Transition to 5D and Sun screening of plexiform neurofibroma models in 96-well format
PI: Dr. Roger Will Achieve, PhD (Bestinclass State University)

Milestones/Deliverables

August 1, 2017 – July 31, 2018

Milestone 1 (Month 6)

1. Establish Dark-n-Glo assay in 96-well (2D) format at BSU (using adequate controls, Z' value determinations of assay plate conditions, etc).

2. Execute confirmatory 2D assay (in 96-well format), in order to (1) Define efficacy and potency for 20 selected compounds against PN cells, and (2) Compare results with those previously executed in 1536-well (2D) format (at INSTITUTION X).

Milestone 2 (Month 12)

Providing that 2D results in 96-well format confirm previously determined 2D results in 1536-well format, proceed with plans to transition to secondary (5D) screen for initial hits:

3. Establish Dark-n-Glo assay in 96-well (5D) format at BSU (using adequate controls, Z' value determinations of assay plate conditions, etc).

4. Execute secondary (96-well) screen of 20 selected compounds in 5D cultures of PN cells, in order to (1) Define efficacy and potency for 20 selected compounds, (2) Compare and contrast these values against those resulting from prior assays for the determination of drug sensitivity changes.

5. Work with Sage Bionetworks in preparation of Synapse data upload (for the purposes of NF1 community sharing)

Exhibit C

TITLE: Transition to 5D and Sun screening of plexiform neurofibroma models in 96-well format
PI: Dr. Roger Will Achieve, PhD (Bestinclass State University)

Budget & Justification

August 1, 2017 – July 31, 2018

I. Budget

Personnel	
Hellova Grate Tech, (40% effort)	$15,892
SUBTOTAL:	**$15,892**
Supplies	
Cell Culture Supplies	$7,350
96-well Dark-n-Glo assay Supplies	$9,390
SUBTOTAL:	**$16,740**
Equipment	
Eppendorf 5430R (centrifuge w/ buckets)	$3,878
SUBTOTAL:	**$3,878**
Publication Costs	
Standard fees, applicable	$1,500
SUBTOTAL:	**$1,500**
TOTAL due (Direct Costs):	**$38,010**
TOTAL due (10% maximum indirects):	**$3,801**
GRAND TOTAL:	**$41,811**

II. Budget Justification

Personnel

Ms. Hellova Grate Tech is an experienced cell culture technician who has become familiar with the culture of the PN cell models, including in 5D. She will commit 40% of her effort to complete the work on this project. Budget request: $12,553 salary; $3,339 fringe benefits; **total = $15,892**

Supply Costs

Cell culture supplies: (Note: Cell culture supply costs have been extrapolated from those incurred so far on this project)
media, serum, tissue culture plastic, reconstituted basement membrane, medical gases= **$7,350**

96-well Dark-n-Glo assay supplies: (Note: costs in 96-well format estimated from scaling the A MYSTERY INSTITUTION standard protocols)
Greiner Bio-One 96-well assay plates, Dark-n-Glo reagent, 2 multi-channel pipettors and racked repeater tips=**$9,390**
total = $16,740

Equipment Costs

We will need a refrigerated centrifuge that can accommodate 96-well microplates to generate even coating of reconstituted basement membrane for transition to 5D cultures. The BSU Office of Vice-President for Research has committed more than half ($4,290) toward the cost of this necessary equipment to support our efforts and to reflect that we expect the equipment to be useful beyond the term of the supplementary project.

Eppendorf model 5430R: refrigerated centrifuge with conventional rotor and swinging bucket rotor for 96-well microplates, quote price $8168 less institutional commitment of $4290; **total = $3,878**

Publication Costs

We expect to publish the results of this project and request funds to partially offset the costs associated with doing that.
Publication costs: **total = $1,500**