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REQUEST FOR PROPOSALS
GENE THERAPY STRATEGIES FOR NEUROFIBROMATOSIS TYPE I

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The **Gilbert Family Foundation's (GFF) Gene Therapy Initiative** is pleased to announce a Request for Proposal (RFP) for high-impact research on gene therapy strategies aimed at addressing the loss of **neurofibromin 1 *(NF1)* gene** function in **neurofibromatosis type 1 (NF1)** patients. Proposals will be accepted for **Team Science Awards** defined as collaborative research amongst investigators with experience in gene-targeting strategies in or outside the NF1 field.

INTRODUCTION

NF1 is an autosomal dominant, monogenic disorder that affects approximately 1 in every 3,000 individuals throughout the world. Hallmark features of NF1 include multiple café au lait (light brown) skin spots, neurofibromas (small benign growths) on or under the skin, and tumors on nerves that can lead to disfigurement, blindness, and cancer. NF1 can also result in cognitive disability, skeletal deformity, and cardiovascular malfunction. The course of the disease is both unpredictable and variable among individuals. Even within the same family, patients may experience vastly different symptoms with varying degrees of severity.

NF1 results from mutations or deletion of the *NF1* gene, which encodes a tumor suppressor protein called neurofibromin that is a negative regulator of the Ras signal transduction pathway. *NF1* is a large gene, comprised of 60 exons and spanning approximately 350 kb of genomic DNA. Its transcript is between 11 and 13 kb long with an approximate 8,500 bp open reading frame, encoding a protein of 2,818 amino acids. Thousands of different pathogenic mutations distributed along the entire *NF1* gene have been identified in NF1 patients. While haploinsuffiency is necessary to cause the disease, tumor formation requires alterations in both copies of the *NF1* gene. The somatic mutation (the 'second hit') can occur in various cell types and at various timepoints during an individual's lifetime and contributes to the pathologic variability observed in patients.

GFF is a private nonprofit foundation founded by philanthropists Dan and Jennifer Gilbert. GFF mission is to develop effective treatments and ultimately a cure for NF1. At present, its major research initiatives include (1), the Gene Therapy Initiative (GTI), aimed at developing innovative therapies that address the underlying genetic abnormalities in NF1 patients, and (2) the Vision Restoration Initiative with a goal of advancing vision enhancement and restoration therapies in patients with NF1. In December 2018, GTI funded nine Team Science Awards focused on developing gene targeting strategies such as gene editing and replacement, RNA editing, exon skipping, nonsense mutation suppression, and synthetic lethality.

OBJECTIVE AND AREAS OF INTEREST

The objective of this RFP is to identify promising therapies and drug-delivery mechanisms that have the potential to dramatically reduce pathologic burden in NF1 patients. Potential areas of interest include but are not limited to the following list.

- **Exogenous Delivery of Full-length *NF1* Gene**
  Inserting a full-length, wild-type *NF1* gene to compensate for mutant or deleted alleles could restore neurofibromin levels and functions. However, the *NF1* cDNA, at 8.5 kb, is large and represents a physical hurdle that would need to be overcome.

- **Gene Editing**
  Gene editing has the potential to 'fix' mutations in the *NF1* gene. Even though thousands of unique *NF1* gene mutations have been identified in patients with no major mutation hot spots, gene editing still represents a promising avenue of research with therapeutic potential. Proposals using gene editing strategies not currently investigated by GTI, such as base editing, would be preferred.

- **RNA Editing**
  RNA editing refers to post-transcription modification of RNA molecules to make changes to specific nucleotide sequences. These changes may include insertions, deletions, and base substitutions. RNA editing is relatively new and the technologies associated with it are just beginning to be developed; however, it may have advantages over more traditional DNA editing. For example, it doesn't require homology-directed repair machinery; therefore it can be used in non-dividing cells, and RNA editing avoids the risk of permanent gene editing such as indels, inverted insertions, foreign DNA insertion; as cells constantly make RNA, it can likely be reversible. The use of *adenosine deaminase acting on RNA* (ADAR) enzyme to perform post-transcriptional modification of mRNA is an example of RNA editing.

- **Regulation of NF1 Signaling Pathways**
  Neurofibromin is a large protein containing several functional domains. The most studied and well-recognized is a GAP-related domain (GRD) that mediates the protein's effects on the Ras/MAPK signaling pathway. Neurofibromin is a GTPase-activating protein and loss of function results in increased RAS activation. In addition to the Ras/MAPK pathway, the presence of other functional domains within the neurofibromin protein and protein-interaction studies have suggested that it is involved in other cellular processes via Akt/mTOR, ROCK/LIMK/cofilin, and cAMP/PKA pathways. Together, all these signaling pathways provide several potential downstream targets, including neurofibromin-interacting proteins, where a gene therapy approach could be used to compensate for the loss of normal NF1 function.

- **Preventing Loss of Heterozygosity**
  As an autosomal dominant disease, one copy of the *NF1* gene exists as a germ line mutation. However, tumorigenesis is believed to follow the two-hit hypothesis for tumor-suppressor genes

where a somatic mutation in the normal allele and subsequent loss of heterozygosity (LOH) is required for tumorigenesis. Despite the importance of LOH in NF1 pathogenesis, the mechanisms involved are not well understood. Studies investigating the mechanisms of LOH, including any potential role of the germ line mutation, could lead to therapeutic strategies designed to protect the cells and tissues involved in the formation of tumors and other manifestations from LOH.

- **DNA Repair Enhancement**
  The ability to repair DNA is strongly associated with cancer risk as DNA damage plays a role in LOH. While DNA repair has not been well studied in NF1, there is evidence that DNA repair capacity is diminished in NF1 patients (Gutierrez et al, *Biotecnología Aplicada* 2014;31:136) and that the *NF1* gene may be especially vulnerable in mismatch repair-deficient cells (Wang et al., Hum Genet 2003; 112:117-123). Reduced DNA repair capacity could lead to a greater chance of tumorigenesis and other disease manifestations in NF1 patients; therefore, strategies designed in enhance DNA repair could lead to therapeutics that protect the normal allele.

- **Delivery Systems**
  Targeted and efficient delivery of therapeutic payloads remains a major challenge for the treatment of NF1, for example, safe systemic delivery to Schwann cells. Proposals aimed at developing delivery platforms, viral or nonviral, capable of meeting these challenges are of significant interest.

KEY SELECTION CRITERIA

- Innovative and transformative research: Novel approaches with strong scientific rationale that could advance the development of a therapeutic strategy to 'cure' the disease.
- Potential for rapid progression to clinical testing: Proposals that articulate a clear path to NF1 clinical application will be strongly favored.
- Scientific merit: Outstanding and rigorous proposals as determined by peer review.

TEAM SCIENCE AWARD

Awards for team science are designed to foster a collaborative research process amongst researchers with complementary expertise and capabilities, who will work together to advance new therapeutic solutions for NF1. For multidisciplinary teams of two or more established Principal Investigators (PIs), GFF expects to provide up to $1.2 million over 3 years per team to undertake projects projects with a clear potential to lead to novel gene therapies for NF1 patients.

Teams may consist of investigators from the same institution, different institutions, and may be international. The designated Administrative PI is responsible for administrative leadership. All PIs on the team share authority for scientific leadership.

Team Science Awards have a collaborative and multidisciplinary emphasis, involving meaningful collaboration between participants. Applications therefore must include a description of the nature of and rationale for the proposed collaboration, the specific role of all PIs, and synergistic opportunities. Evidence of prior productive collaborations between members of the team is useful. However, new collaborations are welcome.

Applicant Eligibility

PIs must hold a full-time faculty or industry appointment at the level of Assistant Professor (or equivalent) or above at an academic, non-profit research institution, or industry organization whose primary mission is medical research within or outside the United States. PIs must be able to show clear evidence of an independent research program. Fellows or those in other training or research support positions are not eligible.

Investigators need not be specifically trained in the field of NF1 or have any documented experience with NF1 research. However, researchers who are new to NF1 are strongly advised to work closely with an NF1 research expert and to consult existing literature on the disease during the formative stages of the research plan.

An investigator may serve as PI on only one proposal submitted to GFF. Multiple applications will be accepted from a single institution, provided that each application has a different PI and represents a distinct hypothesis.

Applications from PIs who do not meet the eligibility criteria will not be reviewed. If there are any questions about eligibility, please contact GFF before submitting an application.

Application Instructions

There will be a two-stage peer-review application process:

1. In the first stage, Letters of Intent (LOIs) are due by February 28, 2021.

2. GFF will select LOIs to advance and notify applicants of their application status by April 1, 2021. For those applications selected to advance, full-length proposals will be due by May 31, 2021.

All applications are due by 5:00 PM Eastern Time on the dates specified above. LOIs and full proposals received after the applicable deadline will not be considered.

GFF utilizes the proposalCENTRAL online application tool and the document templates and requirements therein. Please carefully follow the instructions in proposalCENTRAL and below. Applications include the following steps and components.

Letter of Intent

All applicants must submit a one-page LOI to GFF via proposalCENTRAL, which can be accessed here. Only select applicants will subsequently submit a full proposal. The LOI application consists of the following components.

1. **Title Page:** Enter the project title.

2. **Applicant/PI Information:** Team Science applications must identify one PI for administrative purposes (the Administrative PI for the proposal). This is the 'Applicant'.

3. **Organization/Institution Information:** This is the Administrative PI's institution.

4. **Key Personnel Information:** Identify all individuals who will contribute in a substantive, meaningful way to the scientific development or execution of the project, whether or not salaries are requested.

5. **LOI:** 1-page maximum that includes (a) a description of the scientific aims and translational potential and (b) the nature of and rationale for the proposed collaboration, the specific role of each participant, and synergistic opportunities. Letters exceeding the 1-page limit will not be considered.

Full-length Application

Full-length applications will be invited from meritorious LOIs selected by GFF. Applications include the following steps and components.

1. **Title Page:** Enter the project title. For proposals involving multiple institutions, please include a total amount requested for each institution in the designated spaces provided.

2. **Templates and Instructions:** Download RFP and templates.

3. **Enable Other Users to Access this Proposal:** Allow others (e.g., institutional administrators or collaborators) to view, edit, or submit your proposal.

4. **Applicant/PI:** Key information about the applicant PI. This must be the Administrative PI on team science applications.

5. **Organization/Institution:** Key information about the Applicant/PI's institution, including name and email address of the signing official who, in addition to the PI, will be contacted if the award is selected for funding.

6. **Key Personnel:** List and provide contact information for key persons. Include all PIs on the proposal as well as any additional key personnel.

7. **Abstracts and Keywords:** Provide a lay audience friendly abstract and a technical abstract (2,000 characters maximum each) and key words. Please note: the lay abstract will become public if the award is selected for funding; therefore, it should not contain any proprietary information.

8. **Budget Period Detail:** Enter budget detail for each award period requested. GFF will not support indirect costs, overhead costs, or other similar institutional levies in excess of 5% of the total award amount. Fringe benefits for personnel salaries are allowable.

9. **Budget Summary and Justification:** A summary of the budget detail will be shown. In addition, the budget will provide sufficient detail for the evaluation of the major portions of the budget that are being requested. If more space is required than is provided in the proposalCENTRAL forms (2,000 characters), applicants may upload the budget justification in document form in step # 11. *For proposals involving more than one institution, do not include partner institution costs under contract costs but rather make it clear in your application which costs are appropriated to which collaborator. The contract cost category should only be used for contracts with outside facilities for performance of services.

10. **Organizational Assurances:** IRB and IACUC approvals, if applicable.

11. **Upload Attachments:** Upload the following:
    a. **Curriculum vitae for PIs and other key personnel:** Applicants may use the template provided or the NIH biosketch format.

    b. **Current and pending research support for the PIs:** Use the template provided in proposalCENTRAL, which includes a statement of overlap. Any overlap of current or pending support with the GFF proposal must be described and explained.

    c. **Project description:** Must be formatted in Arial 11-point or Times New Roman 12-point font with no less than ½ inch margins. 5 pages maximum, inclusive of the following: Background and specific aims, preliminary data, timeline, milestones, experimental design and methods, figures (which may be embedded within the above sections), and rationale/fit with key criteria, including the potential for clinical impact. Descriptions exceeding the 5-page limit will not be considered.

    d. **Literature references:** A list of up to 20 references (maximum) supporting the project description is allowed, in addition to the 5-page project description.

    e. **For multi-institutional proposals:** Attach a letter from the Administrative PI's institution confirming that if the award is made, the institution will execute the necessary sub-award agreements within 30 days of execution of the award agreement between GFF and the applicant institution and will transfer funds from their institution to the collaborating institution(s).

12. **Validate:** Check for any missing required information.

13. **Signature pages:** Print the signature page, which must be signed by the PI and the institution's signing official and uploaded as part of the application package.

14. **Submit:** Please note that no proposals will be able to be submitted past the deadline. Technical support for the online application system is not available after 5:00 PM Eastern Time.

TIMELINE

Activity	Date*
Letter of Intent	February 28, 2021; 5:00 PM Eastern Time
Teams of selected LOIs invited to submit full length proposals	April 1, 2021
Full length proposal due	May 31, 2021; 5:00 PM Eastern Time
Peer and organizational reviews/modifications of submissions	June-August 2021
Award letters/negotiate research agreements	September 2021
Projects commence	December 2021

*Please note that dates are subject to change

REVIEW MECHANISM

All proposals will undergo rigorous peer review by GFF, comprised of experts in NF1 and diverse areas of gene therapy research. Applications will be scored according to the Key Selection Criteria (above). GFF will provide summaries of reviewer critiques or evaluations to applicants through proposalCENTRAL. Depending on peer review and GFF program priorities, GFF may work with applicants to modify the submitted work plan and/or budget prior to award execution.