NHLBI Programs of Excellence in Gene Therapy

Vector Application Request Checklist

The following items are required to complete an application.

1) Application summary sheet
2) Letter of agreement
3) Abstract (page 2, PHS 398)
4) Biographical sketch of principle investigators and key personnel
5) Summary of resources
6) Background and summary of preclinical data (includes pharmacology, toxicology, safety data)
7) Summary of clinical protocol
8) Letters of collaboration
9) Vector information
10) Regulatory approvals (innocentness, DSMB, IRB, IBC, IND) If available please include approval letters from indicated agency
## NHLBI Programs of Excellence in Gene Therapy

### APPLICATION SUMMARY SHEET

<table>
<thead>
<tr>
<th>Principal Investigator:</th>
<th>Institution:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Phone: FAX: E-Mail:</td>
<td></td>
</tr>
<tr>
<td>Study Title:</td>
<td></td>
</tr>
<tr>
<td>Vector/Gene:</td>
<td></td>
</tr>
<tr>
<td>Delivery System:</td>
<td>Adenovirus, Retrovirus</td>
</tr>
<tr>
<td>Target Cells:</td>
<td></td>
</tr>
<tr>
<td>Transduction:</td>
<td>in vivo</td>
</tr>
<tr>
<td>Proposed Duration:</td>
<td></td>
</tr>
<tr>
<td>Estimated Total Patient Accrual:</td>
<td></td>
</tr>
<tr>
<td>Date of IRB Approval:</td>
<td></td>
</tr>
<tr>
<td>Date of IBC Approval:</td>
<td></td>
</tr>
</tbody>
</table>

Has vector been previously produced by the PEGT?  Yes No Unknown

Facility preference: Cornell Pittsburgh

### CO-INVESTIGATOR COLLABORATION

"No" answers to any of the questions below require written explanation.

- Does the proposal list all scientists who will be involved with this study? Yes No
- Have all investigators listed in the proposal been consulted and are they in agreement to participate in this study? Yes No
- Does the proposal list all companies/institutions involved with this study? Yes No
- Have all companies and institutions listed in the proposal been consulted and are they in agreement to participate in this study? Yes No

### IND STATUS

- Has an IND been filed for the use of this agent in the submitted protocol? Yes No
- If yes, who is filing the IND? IND #
- If an IND has not been filed; has the investigator contacted the FDA and discussed the proposal? Yes No
- If Yes: FDA Contact

Principal investigator signature: ____________________________
NHLBI Programs of Excellence in Gene Therapy

PEGT GENE VECTOR LABORATORIES (PGVL) LETTER OF AGREEMENT

DATE:

TO: DIRECTOR OF THE PGVL PRODUCTION FACILITY

I have read the PGVL Policy and Procedures Document and as an Investigator submitting a "Request for Vector" application, agree to abide fully with the terms described in the Policy and Procedures document. An overview of these terms is outlined below:

a. All documents and requests in the application packet will be completed.

b. Documentation of all required provisional and final approvals will be supplied to the PGVL Production Facility.

c. I/we agree to perform vector stability studies and develop lot release vector characterization assays as required by the FDA.

d. I/we agree to meet the goals of post-distribution reporting as stated in the PGVL “Policy and Procedures” document. I/we agree to provide the PGVL with all amendments made to the clinical protocol following release of vector. I/we recognize that this is important since it may affect the resources originally approved through the PGVL.

e. Discussions with the PGVL will be held confidential, unless mutually agreed upon.

f. I/we agree that PGVL personnel may contact the FDA on issues related to the production of my clinical vector, I also agree that the PGVL may release records about my product to the FDA.

It is understood that failure to comply with these terms may serve as grounds for suspension of the PGVL support. It is also understood that vector production will be put on hold in synchrony with clinical holds put in place by the Food and Drug Administration (FDA), Institutional Biosafety Committee (IBC), Institutional Review Board (IRB), US Department of Health and Human Services Office for Human Research Protections (OHRP) or the NIH Office of Biotechnology Activities (OBA).
Any studies that are conducted in violation of regulatory guidelines will result in immediate suspension.

Sincerely,

Investigator
DESCRIPTION. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This description is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

PERFORMANCE SITE(S) (organization, city, state)

KEY PERSONNEL. See instructions on Page 11. Use continuation pages as needed to provide the required information in the format shown below.

Name      Organization      Role on Project
### BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2. Photocopy this page or follow this format for each person.

**NAME** | **POSITION TITLE**
--- | ---

**EDUCATION/TRAINING** *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE <em>(if applicable)</em></th>
<th>YEAR(s)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
</table>

**RESEARCH AND PROFESSIONAL EXPERIENCE:** Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**
RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under “Other,” identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

Clinical:

Animal:

Computer:

Office:

Other:

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.
NHLBI Programs of Excellence in Gene Therapy

6) Background and summary of preclinical data (includes pharmacology, toxicology, safety data)
6a) Background (include a list of up to ten relevant publications)
6b) Preclinical data including methods of assessing gene transfer, biological assays, summary of \textit{in vitro} and \textit{in vivo} experiments
6c) Pharmacology and safety data
NHLBI Programs of Excellence in Gene Therapy

7) Summary of clinical protocol
7a) Summary of clinical protocol (which should include objectives, methods of assessing gene transfer and efficacy, preclinical data, statistical section, and biological and clinical endpoints)
8) Letters of collaboration
A) Adenovirus

i) VECTOR INFORMATION

a. Type of vector: ____________________________________________________________

If adenovirus:
The virus contains the E4 region: Yes (E4+) _________ No (E4-) _________

b. Name of transgene: ______________________________________________________

c. If applicable, please list any references that describe the structure and properties of the vector:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

ii) CELL LINE INFORMATION

a. Name of packaging cell line required:________________________________________

b. Species of origin: _________________________________________________________

c. Will a Master Cell Bank (MCB) or Working Cell Bank (WCB) be used for this production lot?

Yes___________ No____________

d. If so, who was the cell bank produced by (name of company) and what safety testing was done? Please provide data summary.
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
iii) CELL CULTURE CONDITIONS
 Please note, cells are grown antibiotic-free. If culture media is considered proprietary or contains “unusual” supplements, the media should be defined by the client.

a. Growth characteristics: Adherent _________ Suspension _________

b. Suggested split ratio: _____________________________________________

c. Temperature: ___________ %CO₂: _______________

d. Type of media: ___________________________________________________
   Supplier: _______________________________________________________
   Catalog number: ________________________________________________

   Serum type (if special considerations are necessary): ___________________
   Concentration in media: __________________________________________
   Supplier: _______________________________________________________
   Catalog number: ________________________________________________

   Supplements and their respective concentrations in media: ______________
   ______________
   ______________

   e. Additional culture instructions / requests:
   ______________
   ______________
   ______________
   ______________
   ______________

vi) CLINICAL VECTOR PRODUCTION

a. Amount of product needed (fluorescent forming units [a measure of infectious viral titer], viral particles, or volume):
   ______________
b. Steps to purify (in order): For example 1-cell pellet, gradient centrifugation, DNase / freon treatment etc.

_________________________________________________________________

c. What type of buffer shall product be stored in?

_________________________________________________________________

_________________________________________________________________

d. Details of any special purification steps requested:

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

v) FILLING

a. Vector volume or particles / vial: ______________________________________

b. Type of vial: ______________________________________________________
Supplier: _________________________________________________________
Catalog number: ___________________________________________________

c. Cryopreservation medium (if applicable): _______________________________

d. Labeling as you would like it to appear on vial label:

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

_________________________________________________________________

vi) VECTOR MAP: Please provide a restriction map indicating all components of the proposed vector.
vii) **VIRUS SEQUENCING AND CHARACTERIZATION DATA**: Provide the full sequence of the seed adenovirus (if available) and a summary of all characterization, infectivity and transgene expression data:

viii) **LOT RELEASE TESTING**

Place a check mark by quality control tests that GTCF and / or contracted companies will perform.

- Sterility (final product)
- Mycoplasma
- *In Vitro* Adventitious Virus
- *In Vivo* Adventitious Virus
- Adeno-associated Virus
- Parvovirus B-19 Hybridization
- Epstein-Barr Virus
- Cytomegalovirus
- Porcine viruses
- HIV1/2
- HTLV I/II
- Hepatitis B
- Hepatitis C
- In vitro assay for bovine viruses
- Transgene Product Functional Assay
- General Safety Test
- Replication Competent Adenovirus
- Endotoxin
- Genomic Structural Integrity
- E1a Sequences PCR assay
- E4 sequence PCR assay
- Expression of Transgene
- Transgene Product Detection
- Transgene Product Functional Assay
NHLBI Programs of Excellence in Gene Therapy

B) RETROVIRUS

i) GENERAL INFORMATION

Clinical grade retroviral vector containing supernatant will only be produced from a certified master cell bank (MCB) of producer cells. Investigators may submit either a vial of producer cells from a MCB, along with all corresponding certificates of analysis, or they may apply to have a MCB generated from an individual clone of stably transfected producer cells that have been partially expanded. In either case, the cells must be shown to be free of microbial and mycoplasma contamination prior to being shipped and archived at the PGVL. In addition to these requirements, the investigator must provide and submit the information requested below:

ii) VECTOR CONSTRUCT

a. In adequate detail, describe the strategy used in construction of the vector and the reasons for including retroviral sequences, the specific transgene sequences, and any enhancer/promoter or other regulatory regions.

b. Describe in detail the following structures including the size of restriction fragments used and restriction enzyme cloning sites:
   b.1) origin of all retroviral sequences.
   b.2) notation of modifications (e.g., deletions, insertions, or mutations) in the retroviral genome or vector plasmid.
   b.3) origin and any modification of inserted regulatory elements (e.g., promoter, poly A signals).
   b.4) the size of restriction fragments used and restriction enzyme cloning sites:

c. Describe in detail the plasmid containing the vector.
   c.1) State the original source of the plasmid.
   c.2) The source, type of bacterium, and medium in which it was propagated.
   c.3) The type of resistance present.

d. Has the vector been sequenced? Provide sequence on disk if available.
   Has the plasmid been sequenced? Provide sequence on disk if available.

If the vector has not been sequenced, describe in detail the theoretical transgene DNA sequences (e.g. obtained from Genbank) and theoretical DNA sequences for transgene cassette regulatory elements as well as reference to the database where
NHLBI Programs of Excellence in Gene Therapy

these sequences can be found (include Accession numbers). Provide a map of the plasmid and the size of restriction sites which could establish the identity of the plasmid. Note: Since recent guidelines require that the identity of all vectors less than 40 kb will need to be established by sequencing, the PGVL will sequence the vector at the appropriate stage of production.

e. Is the investigator the originator of the vector? Are there any legal or contractual constraints on the use of vector?

iii. PACKAGING CELL LINE

a. Describe the package cell line utilized in generating the vector packaging. Include:

b. Original source of cells. Identify species. Include the results of any adventitious agent or identity testing if it has been performed.

c. Passage number at all intermediate steps if known.

d. Media composition used to culture the cells. Include supplier and source if available. (ie. DMEM, 10% Calf serum (Gibco BRL US Herds, Ampicilian?))

e. Provide inventory of available cells (ie 4 vials of Clone 8 @ 1 x 10^6 cells/vial)

f. In detail, describe how the packaging cell was generated. If intermediate packaging cell lines were used to create the producer cell line, describe their history according to 6a - 6d. If the packaging cells were produced by transfection, outline the procedure.

g. By definition, a MCB is defined as a collection of cells of uniform composition derived from a single cell. Therefore, cells of a MCB must be clonal in origin. In detail, describe how the packaging cells were cloned. Provide information about antibiotic use, FACS, or limiting dilution strategies.

iv. DESCRIBE THE CELL GROWTH KINETICS

a. What is the population doubling time?
b. What is the cell density at confluence?
c. What is the optimal temperature for cell growth?
NHLBI Programs of Excellence in Gene Therapy

d. What are other important parameters for cell growth (e.g., %CO₂, Relative humidity, etc.)

v. VECTOR PRODUCTION

a. In detail, describe how the vector is produced in the laboratory for pre-clinical study. Include media composition, supplements, time, temperature, relative humidity, %CO₂, and purification strategies. If comparative production studies have been performed, please provide a synopsis of the data. (Virus production at 32°, 34°, & 37°C, or in 5, 7.5, or 10% calf serum) If antibiotics were used as selection agents to establish the producer, have the producer cells been grown without antibiotics? If yes, for how many passages and has the ability of the producer cells to maintain vector production been established?

b. Describe the method used to titer the vector, include information concerning sensitivity, reproducibility, the positive and negative controls used, and whether or not the assay has been validated. If the assay is performed as a standard operating procedure, you may append a copy of the SOP. Describe the titer that is commonly obtained when the vector has been produced for pre-clinical study using the method described in section 8 above.

v. GENE EXPRESSION

a. The Potency (biological activity) of the vector will need to be established prior to lot release. How are gene transfer and expression of the pre-clinical vector determined? Describe the sensitivity, specificity, use of positive and negative controls, and length of time required to perform the assay. Has the assay been validated? If the assay is performed as a standard operating procedure, you may append a copy of the SOP.

vi. GENERAL VECTOR SAFETY

a. Does the vector show cytopathic effects following transduction of target cells in culture? Does the vector transform cells in tissue culture following transfection?

b. Have toxicity studies been done in animal models? What animal systems have been tested and how do these tests relate to the clinical design? What additional animal studies would be necessary for IND approval (refer to discussions with FDA)? If applicable, describe changes in the following parameters and the level of gene expression at which the changes occurred (organ pathology, serum biochemistry,
NHLBI Programs of Excellence in Gene Therapy

immune parameters, hematologic parameters).

c. What is the stability of gene transfer? Is gene expression transient, if so over what time period do expression levels change?

d. Has gene expression been monitored for spread outside the immediate site of gene transfer in animals?

e. What criteria will be used for determining toxicity in patients?

vii. SAFETY TESTING FOR A RETROVIRAL PRODUCER CELL LINE.

Place a check mark by the safety tests that the PGVL at the University of Pittsburgh/ or contacted companies will perform.

a. Producer Cell Bank

Characterization of cell morphology and growth
Cell Line Species Identity( isoenzyme electrophoresis)
Mouse Antibody Production (MAP) test
In Vitro Assay for Adventitious Viral Contaminants
In Vivo Assay for Adventitious Viral Contaminants
Sterility 21 CFR 610.12
Agar cultivable and non-cultivable mycoplasma
Co-Cultivation of test article cells and RCR detection
Supernatant amplification and RCR detection
Detection of Adventitious Bovine viruses in cells grown
Detection of Porcine viruses in cell preparations

b. End of Production Cells

Cell Line Species Identity( isoenzyme electrophoresis)
In Vivo Assay for Adventitious Viral Contaminants
Co-Cultivation of test article cells and RCR detection

viii. IN-PROCESS AND LOT RELEASE TESTING
NHLBI Programs of Excellence in Gene Therapy

a. Unprocessed bulk material

*In Vitro* Assay for Adventitious Viral Contaminants

Sterility 21 CFR 610.12
Agar cultivable and non-cultivable mycoplasma
Supernatant amplification and RCR detection

b. Purified Bulk Material

Sterility 21 CFR 610.12
Detection of contaminating DNA

c. Final Vialed Product

Endotoxin
Sterility 21 CFR 610.12
General Safety Test
Supernatant amplification and RCR detection