The Gene Therapy Resource Program: A Decade of Dedication to Translational Research by the National Heart, Lung, and Blood Institute

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Over a 10-year period, the Gene Therapy Resource Program (GTRP) of the National Heart Lung and Blood Institute has provided a set of core services to investigators to facilitate the clinical translation of gene therapy. These services have included a preclinical (research-grade) vector production core; current Good Manufacturing Practice clinical-grade vector cores for recombinant adeno-associated virus and lentivirus vectors; a pharmacology and toxicology core; and a coordinating center to manage program logistics and to provide regulatory and financial support to early-phase clinical trials. In addition, the GTRP has utilized a Steering Committee and a Scientific Review Board to guide overall progress and effectiveness and to evaluate individual proposals. These resources have been deployed to assist 82 investigators with 172 approved service proposals. These efforts have assisted in clinical trial implementation across a wide range of genetic, cardiac, pulmonary, and blood diseases. Program outcomes and potential future directions of the program are discussed.

Keywords: gene therapy, vector, lentivirus, adeno-associated virus, preclinical, clinical trial

INTRODUCTION

IN JUNE 2005, the National Heart Lung and Blood Institute (NHLBI) convened a working group to recommend the most effective means to translate potential genetic therapies funded by the NHLBI into the clinic. The specific recommendation was to establish the Gene Therapy Resource Program (GTRP) to fulfill the following objectives: (1) to provide support for preclinical and current Good Manufacturing Practice (cGMP)-grade vector production; (2) to support pharmacology/toxicology studies; (3) to promote multi-site gene therapy trials to improve patient recruitment; (4) to provide expertise in clinical and regulatory affairs; and (5) to organize a workshop to develop novel approaches to public-private partnering.¹

In order to implement these recommendations, the NHLBI-GTRP supported five Core Centers designed to provide National Institutes of Health (NIH)-supported contract manufacturing and contract research services to investigators on the cusp of clinical translation. In addition to the NHLBI support, specific protocols were supported in partnership with the National Center for Advancing Translational Science (NCATS) Bridging Interventional Development Grants (BrIDGs) program, and GTRP services were provided to researchers supported by other NIH Institutes via transfer of funds to the NHLBI. Each of the GTRP Core Centers amassed a remarkable track record of success over the 10 years that followed. The highlights of these accomplishments are presented here.

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GTRP STEERING COMMITTEE AND PROJECT IMPLEMENTATION

In order to provide oversight to the entire program, NHLBI established the Steering Committee (SC) of the GTRP consisting of leadership from each of the GTRP core centers, a number of experts from the NHLBI-funded gene therapy community, as well as *ex officio* representatives from NHLBI, the Food and Drug Administration (FDA) Centers for Biologics Evaluation and Research (CBER), and the NIH Office of Biotechnology Activities. In addition, the GTRP established a Scientific Review Board to evaluate selected Request for Service Applications (RSAs) and make recommendations to the SC and NHLBI. Furthermore, there were three RSAs that received support from the National Eye Institute (NEI) and two RSAs that received combined support from the National Institute for Neurologic Diseases and Stroke and the BrIDGs program.^{2–66}

A critically important aspect of the overall program was the ability of GTRP resources to span all aspects of translational research between basic, exploratory studies and clinical applications (Fig. 1). The number of times each particularly service was accessed is indicated Fig. 1. As was previously published, the GTRP-funded resources serve to accelerate preclinical to clinical application after initial proof-of-concept studies in animal models.²

Over the first 10 years of the GTRP, 154 investigators registered with the GTRP, and 118 of these submitted at least one RSA. Of those 118 principal investigators (PIs), 95 completed the RSA sufficiently for review, and 82 of those 95 received service for at least one RSA. In total, 196 RSAs were reviewed and 172 were approved, an 88% approval percentage. The specific service support provided by each of the cores is presented below (see Tables 1 and 2).

Figure 1. Depiction of the translational pipeline demonstrating stages at which the Gene Therapy Resource Program may assist. NDA, new drug application; BLA, biologics license application; PCOL, pharmacology; PKIN, pharmacokinetics. [Adapted with permission from McDonald et al. Human Gene Therapy Clinical Development 2013;24:1–10].²

Table 1. *Scope of support offered by the NHLBI-GTRP*

<i>Point on the translational pathway</i>	<i>No. of investigators/ projects supported</i>	<i>Type and no. of RSAs/support for projects</i>
Proof-of-concept studies	2	<ul style="list-style-type: none"> • 5 preclinical vector production RSAs • 3 immunology testing RSAs
Pre-pre-IND meeting	5	<ul style="list-style-type: none"> • 4 regulatory support RSAs • 4 pharm/tox core consultations • 3 AAV vector core consultations
Pre-IND meeting	6	<ul style="list-style-type: none"> • 4 regulatory support RSAs • 1 AAV vector production RSA for study requested by FDA • 1 pharm/tox RSA for study requested by FDA • 1 lentivirus vector production RSA for study in preparation for meeting • 6 pharm/tox core consultations • 4 AAV vector core consultations • 1 lentivirus vector core consultation
Pharm/tox/biodistribution studies	10	<ul style="list-style-type: none"> • 10 pharm/tox testing RSAs • 4 AAV vector production RSAs • 1 preclinical vector production RSA • 1 lentivirus vector production RSA
IND	9	<ul style="list-style-type: none"> • 3 regulatory support RSAs • 3 pharm/tox testing results included • 4 AAV vector information for CMC section • 1 lentivirus vector information for CMC section
Clinical trial	9	<ul style="list-style-type: none"> • 7 clinical trial funding supports RSAs • 4 regulatory supports RSAs • 3 AAV vector production RSAs • 1 lentivirus vector production RSA

At each point in the translational pathway, the number of investigators and the specific type and number of RSAs supported is listed.

NHLBI, National Heart Lung and Blood Institute; GTRP, Gene Therapy Resource Program; RSA, Request for Service Applications; IND, Investigational New Drug; pharm/tox, pharmacology and toxicology; AAV, adeno-associated virus; FDA, Food and Drug Administration.

Table 2. *Diseases/disorders represented by the approved RSAs*

<i>Heart/cardiovascular (19 different diseases)</i>	
<ul style="list-style-type: none"> • Arrhythmia • Atherosclerosis • Atrial fibrillation • Cardiomyopathy • Congestive heart failure • Coronary artery disease • Critical limb ischemia • Danon disease* • Diabetic cardiomyopathy • Duchenne muscular dystrophy* 	<ul style="list-style-type: none"> • Fabry disease • Homozygous familial hypercholesterolemia • Hypertension • Hyperlipidemia • Ischemic heart failure • Myocardial ischemia • Peripheral artery disease • Pompe disease • Occlusive vascular disease
<i>Blood/hematologic (11 different diseases)</i>	
<ul style="list-style-type: none"> • ADA-SCID • Cyclic hematopoiesis • Fanconi anemia complementation group A • Hemophilia • Human immunodeficiency virus 	<ul style="list-style-type: none"> • Leukemia/lymphoma (NCI funding) • Sickle cell disease* • X-linked chronic granulomatous disease • X-linked severe combined immunodeficiency • Wiskott–Aldrich syndrome* • T-cell acute lymphoblastic leukemia
<i>Lung/pulmonary (5 different diseases)</i>	
<ul style="list-style-type: none"> • Alpha-1 antitrypsin deficiency* • Cystic fibrosis* • <i>Pneumocystis carinii</i> pneumonia* 	<ul style="list-style-type: none"> • Pompe disease* • Ventilatory failure
<i>Non-NHLBI related (3 different diseases)</i>	
<ul style="list-style-type: none"> • Parkinson's disease—2 RSAs (BrIDGS and NINDS) • Osteoarthritis (BrIDGs and NIAMS) 	<ul style="list-style-type: none"> • X-linked retinoschisis—2 RSAs (NEI)*

A total of 172 RSAs were approved, addressing 38 different diseases.

*Rare diseases.

PRECLINICAL VECTOR CORE

The preclinical vector core was located at the University of Pennsylvania. The core provided research-grade preparations of recombinant adeno-associated virus (rAAV), recombinant adenovirus, and lentiviral vectors to investigators whose RSAs were approved. The preclinical vector core fulfilled 71 RSAs during GTRP phase 1 (2007–2012),² which resulted in 34 publications^{3–36} (Table 1). The RSAs were predominantly focused on cardiac and cardiovascular diseases, with others focused on diseases of the lungs (*e.g.*, cystic fibrosis) or of the blood and bone marrow (*e.g.*, leukemia). The preclinical core fulfilled 36 RSAs, resulting in 13 publications to date during GTRP phase 2 (2012–2017).^{37,51–62} The distribution of diseases addressed by these projects was similar to that in phase 1 (Table 2).

CLINICAL-GRADE AAV VECTOR CORE

The clinical vector core at the Children's Hospital of Philadelphia (CHOP) Raymond G. Perelman Center for Cellular and Molecular Therapeutics served as the clinical-grade AAV vector core for the GTRP. Services included pilot-scale and large-scale vector and vector excipient manufacturing for both preclinical and clinical studies, including single-strand AAV1, AAV2, and AAV8, and self-complementary AAV2(triple Y-F), and support for Investigational New Drug (IND) applications. During the first funding period (2007–2012), the GTRP fulfilled four RSAs, of which three were for preclinical Pharm/Tox studies (Table 1). These were to support studies toward the treatment of Parkinson's disease (PI Krystof Bankiewicz, University of California, San Francisco), Pompe disease⁴² (PI Dwight Koeberl, Duke University), and X-linked retinoschisis (PI Peter Colosi, National Eye Institute, NIH). The fourth RSA provided clinical-grade vector to Krystof Bankiewicz for advanced Parkinson's disease in a Phase I dose-escalation study. During the second funding period (2012–2017), the GTRP supported five RSAs, including two preclinical and three clinical-grade vector manufacturing runs.² Preclinical vectors supported Pharm/Tox studies for ischemic cardiomyopathy^{36,37} (PI Charles Bridges, Carolinas Healthcare System) and homozygous familial hypercholesterolemia³⁰ (PI Daniel Rader, University of Pennsylvania). Clinical vectors generated were used for Phase I studies of X-linked retinoschisis (PI Paul Sieving, National Eye Institute, NIH), homozygous familial hypercholesterolemia³⁰ (PI Daniel Rader), and Leber hereditary optic neuropathy (PI John Guy, University of Miami; Table 2).

Although the clinical vector core has developed standardized procedures for the manufacturing of AAV serotypes 1, 2, 5, 6, 8, 9, and LK03, their experience in manufacturing of close to 100 products in support of more than 20 clinical studies has shown that each individual product comes with its own challenges. Vector plasmid design, including vector backbone, and transgene size is often overlooked and has a major impact on vector purity. New products, new capsids, and self-complementary vectors almost always require development or pilot runs to evaluate the existing process prior to GMP manufacturing. In some cases, several development runs were needed to identify the optimal plasmid ratio, determine vector yield, and verify performance of QC assays.

Three of the four investigators, for whom the AAV core manufactured clinical-grade AAV vector during either GTRP-1 or 2, had used the GTRP program previously for the manufacturing of Pharm/Tox materials. Since GMP process-comparable preclinical and GMP-grade clinical materials were manufactured at the same facility using the same manufacturing materials and methods, investigators were able to continue to clinical studies without development runs or additional comparability testing, saving significant time and effort. Dr. Koeberl, who had received preclinical vector from the GTRP previously, advanced to GMP manufacturing at the CHOP core facility supported by the NCATS BrIDGs, prior to transitioning to sponsor-manufactured material.

In summary, the AAV core laboratory provided preclinical and clinical-grade AAV manufacturing services for a total of nine projects, including manufacturing support for four clinical studies. Although the number of clinical studies supported by the AAV core over the past 10 years may appear small, the growing number of inquiries into clinical vector manufacturing are indicative of the growth of the gene therapy field, and underscore the need to invest in a robust infrastructure for manufacturing and qualification of preclinical and clinical-grade viral vectors to support future studies.

CLINICAL-GRADE LENTIVIRUS CORE

Indiana University has served as the GTRP site for lentiviral vector manufacture and release testing. The GTRP has funded vector production of nine large-scale lots. Two of the lots were for use in a porcine model of cystic fibrosis using lentiviral vectors pseudotyped with the gp64 envelope (PI Paul McCray, University of Iowa).⁴⁶ The first clinical lentiviral vector generated by the GTRP

was for the treatment of adenosine deaminase deficiency⁴⁷ (PI Donald Kohn, UCLA) and was also used by the GTRP Pharm/Tox center in safety analysis. Dr. Kohn *et al.* in London have now licensed their vector to Orchard Therapeutics, which is moving the product into Phase II/III trials. Subsequent clinical vectors include those for cancer immunotherapy targeting CD19 and CD30 (PI James Kochenderfer, NIH). A third immunotherapy trial directed at T-cell acute lymphocytic leukemia is completing production (PI Yupo Ma, iCell Gene Therapeutics). The latter investigators took advantage of NIH support for small businesses, which include access to resources such as the GTRP. The GTRP also coordinates efforts with other NIH programs, as typified by the assistance provided to David Wilcox (Medical College of Wisconsin) who is developing a platelet-directed therapy for hemophilia. Dr. Wilcox had GMP-comparable vector generated in the GTRP, which was then provided to the NCATS “Disseminating Curative Biological Therapies for Rare Pediatric Diseases” program at Boston Children’s Hospital, which developed the CD34-cell transduction protocol needed for his clinical trial. The GTRP subsequently funded the manufacture of the clinical-grade vector. Investigators have also utilized GTRP testing services to complement their own vector production activities. Punam Malik *et al.* generated a vector expressing beta globin in their production facility at Cincinnati Children’s Hospital. Subsequently, the GTRP at Indiana University performed vector release testing to certify the vector for clinical use. Given that the cost of release testing can approach one-third of vector production costs, the GTRP can be a resource for investigators with and without manufacturing capacity at their institution. GTRP also provided novel assay development. This service was utilized by investigators at St. Jude’s Children Research Hospital who were the first to manufacture vector using a lentiviral packaging cell line. The GTRP assisted in screening the cell line and clinical vector for replication competent lentivirus (RCL). This involved adaption and validation of existing RCL assays and subsequent certification of the cell line and final vector product as RCL free.

While lentiviral vector production within the GTRP has provided vector for a variety of different indications, the experience has also pointed out the challenge of moving vectors into GMP-compatible production. For example, lentiviral vectors pseudotyped with the gp64 envelope are less tolerant to the processing methods used for VSV-G pseudotyped vector. Certain vectors, particularly those with complex regulatory elements, produce

vector particles at much lower amounts, and it has not been possible to provide sufficient material for one GTRP investigator. The GTRP has funded a development project at Indiana University to assess alternate production methods in order to meet these challenges for future GTRP investigators.

PHARMACOLOGY/TOXICOLOGY CORE

The pharmacology/toxicology core (PTC) was located at the Lovelace Biomedical and Environmental Research Institute (Albuquerque, NM). The main service provided by the PTC was conducting pilot or pivotal IND-enabling preclinical safety studies prior to Phase I or Phase I/II clinical trials being conducted. In order for an RSA submitted by an investigator to be reviewed and approved by the GTRP prior to implantation of the safety studies, a pre-pre IND or pre-IND package needed to be reviewed by the FDA. Scientists of the PTC, in collaboration with the staff of the Clinical Coordinating Center and relevant vector production core, assisted six investigators with the development and review of the pre-IND packages prior to submission to the FDA. Preclinical safety and biodistribution study protocols were developed for inclusion in the pre-IND packages. The PTC staff also, when requested by an investigator, participated in pre-IND meetings with the FDA and responses to FDA questions regarding protocol details.

As shown in Table 1, the PTC provided seven preclinical studies covered under six RSAs in phase 1 of GTRP (2007–2017; Table 1). There have been eight publications associated with this work.^{38–45} Nine pilot or pivotal preclinical studies were conducted during GTRP phase 2 (2012–2017), with three additional RSAs developed but ultimately not funded. Three manuscripts resulted from the work conducted under GTRP phase 2.^{46–48}

Overall, these preclinical studies supported the development of gene therapies for a broad range of indications, including alpha-1 antitrypsin deficiency,^{38–42} Pompe disease,^{42,48,49} adenosine deaminase deficient severe combined immunodeficiency,⁴⁷ osteoarthritis,⁴⁴ cystic fibrosis,⁴⁵ congestive heart failure, and atrial fibrillation (Table 2). Animal models ranged from rodents to macaques, and delivery methods ranged from simple intravenous or intramuscular injection to more isolated limb infusions and intraarticular injections. Two of these therapies have advanced to clinical trials.

CLINICAL COORDINATING CENTER

The GTRP Clinical Coordinating Center (CCC) was staffed by Social and Scientific Systems, Inc.

Over the course of GTRP phase 1, the CCC supported 11 RSAs (eight regulatory and three clinical trial funding assistance), resulting in three publications.^{48–50} Likewise, in GTRP phase 2, the CCC supported 11 RSAs (seven regulatory and four clinical trial assistance; Table 1), resulting in one publication.⁶⁶ Taken together, CCC services have enabled investigators to conduct six clinical trials and allowed other investigators to move three more projects from basic science into IND-enabling studies.

Regulatory support offered by the CCC included the following:

- Drafted pre-pre-IND meeting materials and compiled information into an FDA submission package.
- Drafted pre-IND meeting materials and assisted in compilation of an FDA information package.
- Compiled and submitted IND and IND amendments.
- Provided Good Clinical Practice training and regulatory binder preparation.
- Compiled initial submission to the Data and Safety Monitoring Board.
- Provided templates and instructional guidance documents to the new study coordinator and regularly interacted with study coordinators at clinical trial sites that received GTRP funding.

Overall, these RSAs supported research in cardiovascular disease, Pompe disease, and a variety of genetic disorders of the blood and bone marrow (Wiskott–Aldrich syndrome, chronic granulomatous disease, Fanconi anemia, and sickle cell disease; Table 2). In many instances, the GTRP regulatory or clinical trial support provided crucial enabling funds and expertise to allow the program to move forward.

CONFERENCES AND PUBLICATIONS SUPPORTED BY GTRP

In addition to providing the aforementioned services, the GTRP helped to disseminate information and best practices regarding translation of gene therapy and public–private partnerships. This effect has taken the form of one previous publication in *Human Gene Therapy Clinical Development*,² two newsletters to the NHLBI research community, a glossy brochure for the NHLBI Advisory Council, and four presentations at the American Society for Gene and Cell Therapy (ASGCT) annual meetings:

- 2008 ASGCT meeting—overview of the GTRP.
- 2013 ASGCT meeting—GTRP: Perspective of an Investigator and the Program Cores.

- 2014 ASGCT meeting—Translational Resources: What We Have and What We Need.
- 2016 ASGCT meeting—Interface of Academia, the NIH, Foundations, and Biopharm/Biotech: Best Practices to Ensure the Continued Growth and Success of Gene and Cell Therapies.

In addition to these, the GTRP was one of four of NHLBI programs presented at the 2010 ASGCT meeting in a symposium (NIH Resources for Gene and Cell Therapy Investigators) describing resources from six NIH institutes/centers.

CHALLENGES AND FUTURE DIRECTIONS

While the success of the GTRP has been remarkable, both PIs and core centers continue to face challenges in moving gene therapy products forward to the clinic. These challenges, especially when unanticipated or more extensive than anticipated, can increase the cost and extend the time frame of critical segments of the product development pipeline.

In many cases, the transfer of technology from PI laboratories to the respective core facilities has presented a challenge. Vector constructs and cell lines that may serve a particular purpose at the proof-of-concept stage may require modifications to be suitable for further product development. In the area of cGMP manufacturing of clinical-grade vectors, the processes used for upstream production and downstream purification may need to be revised substantially in order to achieve the scalability and reproducibility necessary for clinical translation. In addition to the challenge of process development, there have also been challenges with developing and validating proper quality-control assays for both in-process and lot release testing of cGMP vector material. It seems prudent in the future to anticipate the need for substantial process development and quality-control work in the expectations regarding timing and expense of particular programs.

As the gene therapy community looks forward to the next decade of clinical translation, we can be certain that there will be many exciting advances. Genome editing tools, such as the CRISPR/Cas9 system and TALENs, have made it possible to accomplish highly precise alterations in the genome of the target cell population very efficiently, either by inducing non-homologous end-joining (NHEJ) or homology-directed repair (HDR). While NHEJ may make it possible to inactivate offending genes in autosomal dominant disorders or inactivate aberrant alternative splice sites, HDR could conceivably restore the wild-type sequence precisely at the mutation sites in cells carrying any deleterious

allele. The power of these tools is yet to be fully elucidated, but TALEN-based gene editing has already been used in the clinic (in a chimeric antigen receptor [CAR] T-cell trial in infant B-cell leukemia), and other genome editing tools are soon to follow. In such a dynamic environment of innovation, agile resource programs such as the GTRP will continue to play a pivotal role in moving the field forward.

Immunotherapy mediated by gene transfer is advancing rapidly on all fronts, with the introduction of CARs into patient T cells most often accomplished with lentivirus vectors. CAR T-cell therapy is also ripe for further expansion within the GTRP. Cancers that have been targeted by such immunotherapy in the clinic have included several forms of CD19-expressing B-cell leukemia and lymphoma, ovarian cancer, metastatic renal cell carcinoma, neuroblastoma, and colon carcinoma. Nonmalignant disease may also be amenable to gene therapy-mediated immunotherapy in the near future as the inflammatory and immune mechanisms of many disorders become better understood.

Currently, the GTRP is in a re-tooling phase, but it is anticipated that in 2018 it will be poised to resume the provision of resources needed to facilitate the clinical translation of NIH-funded research. By assisting cell and gene therapy researchers to advance their basic science discoveries

to the clinic, the GTRP illustrates the stewardship entrusted to the NIH by the American public to fulfill its mission to “enhance health, lengthen life, and reduce illness and disability.”

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AUTHOR DISCLOSURE

J.M.W. is an advisor to REGENXBIO, Dimension Therapeutics, and Solid Gene Therapy, and is a founder of, holds equity in, and has a sponsored research agreement with REGENXBIO and Dimension Therapeutics. In addition, he is a consultant to several biopharmaceutical companies and is an inventor on patents licensed to various biopharmaceutical companies. T.R.F. is an advisor to Editas Medicine, Dimension Therapeutics, and ApicBio, and is a founder (without equity) of AGTC and ApicBio, and has a sponsored research agreement from Voyager Therapeutics. In addition, he is an inventor on a number of patents potentially related to the work described here. K.C. is a consultant for PACT Pharma.

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