Report and Recommendations
of the Gene Therapy Working Group
National Center for Research Resources
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Introduction

Gene therapy offers a promising strategy for treating genetic defects and other diseases at their molecular source by replacing damaged or abnormal genes with healthy ones or re-programming cells to fight disease. These genes are transferred into the patient by a vector—a "molecular taxi"—that delivers genes to the cells. Vectors are either modified viruses or non-viral DNA termed plasmids.

Although still experimental, gene therapy is expected to evolve into a major therapeutic strategy in the coming years for patients who have genetic disorders or have acquired diseases, such as cancer or AIDS.

Despite many advances since the first patient study in 1990, production of effective gene vectors remains a major challenge. Gene vectors intended for clinical trials must be produced according to the strict "good manufacturing practices" (GMP) of the U.S. Food and Drug Administration (FDA). However, state-of-the-art facilities that can meet these requirements must be staffed by specially trained personnel and are usually too expensive for a single institution to build and maintain. In 1994, while a growing number of investigators were seeking access to vectors for clinical trials, only one major commercial contractor and one academic health center were generating suitable vectors within the United States.

Recognizing this problem, the National Institutes of Health (NIH) established the National Gene Vector Laboratories (NGVLs) in 1995 to provide centralized resources for the production and distribution of clinical-grade gene vectors. Today, three institutions—Indiana University, Baylor College of Medicine, and City of Hope—house NGVL vector production facilities, each specializing in the development of different types of gene vectors. Two additional laboratories—located at the University of Florida in Gainesville and at the Southern Research Institute in Birmingham, Alabama—perform preclinical toxicology testing of vectors, a frequent prerequisite for human studies.

In October 1999, the NGVL program received a favorable review from an *ad hoc* panel that had been assembled to advise the National Advisory Research Resources Council and the NIH Director on the utility of the program. In November 2005, a Gene Therapy Working Group convened to discuss how best to continue the development of these resources. The latter, composed of eight experts in gene therapy and related subjects, heard presentations from current NGVL directors, describing past and proposed activities of their NGVLs.

In the deliberations that followed, the Working Group drafted a series of recommendations intended to enhance the utility of NGVL facilities. This report summarizes the Working Group's recommendations and the rationale behind each.

History of the National Gene Vector Laboratory Program

In 1995, NIH established the NGVLs as a centralized, cooperative program for the production, maintenance, and distribution of gene vectors for clinical protocols. This came only five years after NIH had launched the first Federally approved human gene therapy trial. The NGVLs were created amid growing concerns that limited access to clinical-grade vectors was hindering the development of gene-based therapeutics—especially for rare disorders that might be commercially unattractive to industry.

Each of the five NGVL facilities specializes in the production or evaluation of a particular type of gene vector. The NIH National Center for Research Resources (NCRR) supports the research infrastructure of these facilities, while eight additional NIH Institutes support efforts relevant to their missions. Three academic centers received the original five-year NGVL grants that began August 1, 1995. In 2001, a second cycle of five-year NGVL grants was awarded, based on the favorable comments received from an *ad hoc* advisory panel. As this second cycle of funding comes to a close, a Gene Therapy Working Group met on November 1, 2005 to consider the activities and plans of the NGVLs and to make recommendations for the future.

Executive Summary

The Working Group found that, in general, the NGVLs have successfully fulfilled their mission. When the laboratories were first established in 1995, only a handful of academic institutions nationwide had the facilities, equipment, and staff needed to produce clinical-grade vectors for gene therapy trials, and reliable methods for producing these vectors had not yet been validated.

Since then, the NGVLs have streamlined and improved processes for gene vector production and have helped researchers through the regulatory procedures and approval processes needed to move a vector to clinical trials. To date, 270 individuals have been treated with vectors produced by the NGVLs.

Toxicology centers were first funded in 2001 to develop and conduct preclinical toxicology studies in support of clinical trial design. In addition, the development of an online Pharmacology/Toxicology database has allowed investigators and the general public alike to share summaries of toxicology studies. Using the database, investigators can determine whether their planned clinical trial is similar to trials already performed, so they can avoid repetition of these expensive and time-consuming studies.

In the years since the NGVL program was launched, several additional GMP facilities for gene vector production have been built nationwide in both industrial and academic institutions. More than a dozen universities have now made major investments in their own gene vector facilities, and NIH—through a variety of funding mechanisms—has also supported these state-of-the-art facilities. The Working Group believes that this GMP infrastructure represents an exceptional opportunity to build a strong national network that will promote scientific sharing and widespread use of these valuable production facilities to advance gene therapy.

After a thorough discussion, the Working Group generated a series of recommendations that could further strengthen the already successful NGVL program:

- **Produce New Types of Vectors:** Steering Committee consideration of such requests would ensure that new vector production is investigator driven and supported appropriately by the NGVLs.

- **Share Technologies and Common Procedures:** The NGVLs should continue to develop and share both unique and common Standard Operating Procedures.

- **Continue the Movement Toward Increased Production of Adeno-Associated Virus (AAV) and Lentivirus:** Significant progress in basic and pre-clinical research on lentivirus and AAV of various pseudotypes suggests that requests for clinical-grade materials will also be increasing.

- **Involve NGVLs in Improving Vectors and Processes:** NGVLs should continue to improve their products and the means by which they are produced.

- **Produce Vectors Primarily for Clinical Use:** Because of limited resources, effort should be directed toward clinical protocols rather than basic science studies.

- **Develop Therapeutic Vaccines Rather than Prophylactic Vaccines:** Because it would be difficult for the NGVL program to absorb the added demands associated with developing prophylactic vaccines, any vaccine efforts should focus on those of a therapeutic nature.

- **Require Further Vector Details from Investigators Prior to Review and Production:** This would help to reduce errors and speed the review/production process.

- **Allow NGVLs to Develop New Technologies for Clinical Vector Production:** Process development should ensue during times that are uncommitted to vector generation or testing.

- **Continue Industry Collaboration:** NGVLs should continue to work with industry as they have done in the past.

- **Increase Advertising of Toxicology Services:** Toxicology, clonality, repository, and production services should be advertised more heavily to reach a wider audience of investigators. It would be helpful to make investigators aware of the National Primate Research Centers (NPRCs) as a potential resource for pre-clinical evaluation of vectors.

- **Make the Pharmacology/Toxicology Database More Widely Known:** Additional hands-on demonstrations at meetings would familiarize users with the database and its uses.

- **Encourage Development of Local Regulatory Cores:** While a central NGVL regulatory core would provide help to investigators who lack such expertise, such cores would best be located within investigators' own institutions. The NGVLs should, however, continue to provide—and even increase—regulatory guidance to investigators.

- **Speed the NGVL Review Process:** Encourage the development of an NIH-wide procedure that would streamline the review process and still provide the Steering Committee with sufficient information and authority to evaluate and prioritize the scientific value and appropriateness of each application.

- **Encourage the NIH to Include Milestones in All Future Grants That Involve Clinical Gene Transfer:** This should facilitate a more rapid completion of these complex and expensive studies.

- **Continue Cooperation with Other NIH Gene Therapy Resources:** An effort should be made to avoid duplication of efforts as well as confusion among applicants.

These recommendations, along with presentations given at the meeting, are described in further detail in the following pages.

Welcome and Charge to the Gene Therapy Working Group

Barbara Alving, M.D., Acting Director of NCRR, welcomed all participants. She noted that the NGVL is a vibrant program that is due for recompetition soon. Dr. Alving asked the Working Group to assess the program's accomplishments and to recommend future directions for the NGVLs.

Richard Knazek, M.D., who assists in oversight of the NGVL program for the NCRR Division for Clinical Research Resources, reviewed the agenda with the group and suggested discussion topics. He explained that the session would begin with a series of presentations from the NGVL Coordinating Center, the vector production facilities, the toxicology centers, and representatives of the NHLBI and NCI gene therapy programs. These presentations were to be followed by a group discussion that would culminate with a set of recommendations for NCRR. Diane Wara, M.D., chair of the Gene Therapy Working Group, introduced Ken Cornetta, M.D., of the NGVL Coordinating Center.

I. NGVL Presentations

NGVL Coordinating Center

Dr. Cornetta, director of the NGVL Coordinating Center, noted that the program began in 1995, when Indiana University, the University of Michigan, and the University of Pennsylvania were selected to participate in five-year cooperative agreements. The vector production program was re-competed for a second five-year cycle beginning in 2001. Indiana University continued in its capacity as Coordinating Center and retroviral production facility, while the City of Hope/Beckman Research Institute and Baylor University were installed as non-viral and adenoviral vector production facilities, respectively. In that same year, toxicology centers at the University of Florida and Southern Research Institute were added in response to the scientific community's observation that such services were needed to advance the field.

The NGVLs provide the following services: generation of clinical-grade vectors, toxicology studies, development and maintenance of a toxicology database, archiving, clonality testing, and scientific and regulatory counseling.

To date, 270 individuals have received vectors or vector-treated cells produced by the NGVLs as follows: adenovirus – 127, retrovirus – 74, plasmid – 67, AAV-2 to address cancer – 179, AIDS – 21, monogenic diseases – 42, and other diseases – 28.

The Coordinating Center supports the five NGVLs. Its responsibilities include implementing logistical details for the NGVL Steering Committee meetings, advertising the services of the NGVLs in trade magazines and at meetings, receiving applications for vector production or toxicology studies, coordinating the review process, and maintaining the Pharmacology/toxicology database. The Coordinating Center also assists investigators

in addressing regulatory issues, maintains a Drug Master File in support of applicant IND submissions, and performs extensive post-distribution monitoring of vectors.

Requests for NGVL resources are first reviewed by *ad hoc* outside members of the Scientific Review Board. These written commentaries are discussed further by a 10-member Prioritization Subcommittee which makes the final scientific recommendations to the Steering Committee. The Steering Committee, which oversees all NGVL activities, is comprised of the directors of the 5 NGVLs; 2 voting and 8 non-voting representatives of the 10 participating NIH Institutes, Centers and Offices; and non-voting representatives from FDA and the NIH Office of Biotechnology Activities. Funding decisions are made by the participating Institutes and Centers.

Domestic, nonprofit organizations or government agencies having both the scientific capacity and financial resources to perform the proposed clinical studies can apply for NGVL support by completing an application available online at www.ngvl.org. There are two deadlines for applications during the year (March 1 and September 15), but expedited reviews are available for applicants that already have preliminary approval from an Institutional Review Board. The review process requires approximately three months. Investigators can expect to receive qualified vector within one year after meeting the regulatory requirements and completing the administrative processes.

The three-level review process, then, includes evaluation by the Scientific Review Board, the Prioritization Sub-Committee, and the Steering Committee. The Scientific Review Board is composed of approximately 30 individuals having expertise in gene therapy, toxicology, clinical medicine, and/or ethics. Their initial comments and inquiries are shared with the applicants whose replies—and the subsequent reviewers' comments—are provided to the Prioritization Sub-Committee. After discussion of the application's scientific merit, a recommendation is made to the Steering Committee.

The Prioritization Sub-Committee places the applications in one of five categories:

- Recommended for either vector production or toxicology studies without modification
- Recommended for either vector production or toxicology studies with modification
- Alternate status - high scientific merit, but limited NGVL resources prevent initiation of either vector production or toxicology studies
- Deferral - proposal felt to be of high scientific merit, but additional information is needed
- Not recommended for support by NGVL resources

The relevant NGVL facility then determines the resources that would be required to generate the vector or perform the toxicology study. Individual institutes consider whether the proposal falls within their mission and if funds are available. Once approved by the NIH, funds are dispersed to the appropriate NGVL facility for production of the specific vector or toxicology study. Frequently, the Coordinating Center will counsel applicants on protocol or vector design as well as discussions with the FDA.

Since 1995, 119 applications have been submitted for review. Of these, 21 were not recommended or withdrawn; 80 were recommended for production, and 18 are still pending. Although there have been peaks and valleys in the number of applications submitted, there was an increase in applications in 2004-05. Most studies have requested production of adenovirus vectors (37) and retroviruses (36), followed by plasmids (26), adeno-associated virus or AAV (10), herpes simplex viruses (6), and lentiviruses (4). Requests for AAV, lentiviruses, and herpesviruses are increasing, although they are still relatively low in number. Requests for plasmids remain stable. The NGVL will perform sequencing studies on the vector if necessary. The vectors have become significantly more complex in the past few years, requiring innovative approaches and modification of manufacturing and testing procedures.

Material Transfer Agreements that may be required are obtained by the Coordinating Center before vector production is initiated. The NGVLs are indemnified by each institution in which the clinical study is to be performed.

Investigators are required to inform the NGVL of Serious Adverse Events (SAEs) that occur in their clinical protocols to allow the manufacturing facility to determine whether failures in the production processes are causal or related. This is accomplished by requiring that investigators send copies of FDA or NIH Office of Biotechnology Activities SAE reports to the Coordinating Center.

Investigators are required to acknowledge the NGVL in publications in the same manner that funding support is acknowledged. Dr. Cornetta pointed out that the approximate time from the request of a vector to the publication of a scientific article is at least five years.

Dr. Cornetta described the Pharmacology/Toxicology database. Investigators who receive NGVL support for either vector production or toxicology studies are now required to enter a summary of their toxicology data into the database. Thirteen studies have been entered to date. Using the database, other investigators and the public at large can determine whether previously completed studies are relevant to a planned clinical trial. Although these data are neither detailed nor verified by the Coordinating Center, the inquiring investigator can use this information to determine if he/she should contact the original investigator to obtain a letter of cross-reference. Each original investigator has agreed during his/her application process to provide such a letter for subsequent submission to the FDA. This allows the inquiring investigator to avoid the needless repetition of toxicology studies when applying for an investigational new drug (IND).

Indiana University Vector Production Facility

Dr. Cornetta provided an overview of the Indiana University Vector Production Facility (VPF). The focus of the center is to develop novel improvements in integrating vectors.

The Indiana University VPF maintains 81 Standard Operating Procedures (SOPs) related to vector production, certification, and facility organization. Dr. Cornetta emphasized that approximately 80 percent of the costs for providing a retroviral vector for a clinical protocol are attributable to testing for vector certification prior to release. Dr. Cornetta reviewed the organization of the production center and its configuration, the Master Cell Banks and the vectors produced, and the status of the trials. During the first five years of funding, 7 of 14 producer cell lines submitted to the laboratory by outside investigators failed to generate clinical-grade material. Investigators are now encouraged to send plasmids rather than producer cells so that the NGVL can use its own defined cell line for vector production.

Between 1995 and 2000, vector requests for 18 clinical protocols were approved. While 13 accrued patients, 5 did not because of low-titered supernatant, mutations, or regulatory issues. From 2001 to date, 14 requests for vector have been approved. Six of these vectors are currently in production, and one of the associated protocols has accrued patients. Four investigators have either cancelled their requests or are undecided about initiating their clinical study; two investigators have left their institutions, and one institution withdrew support from the study. The laboratory capacity for vector production has not been exceeded. However, when not actively engaged in producing vector, the staff is involved in optimizing relevant laboratory techniques. The non-NGVL portion of the Indiana University Vector Production Facility will generate product on a fee-for-service basis for individuals who choose not to apply through the NGVL. The monies obtained in this manner decrease the short-fall in funding required to maintain adequate staffing.

Dr. Cornetta said that the duration of an investigator's clinical study frequently exceeds the duration of their R01 grants, which are usually awarded for five years. A five-year funding period is usually inadequate to perform the necessary basic research and preclinical studies, produce the vectors, address the regulatory requirements, and accrue patients for the proposed study. In addition, occurrence of an SAE can stop a trial for a year or more, further impeding completion of the study. As a result, progress is slow and publications lag far behind the date on which an R01 or other grant is awarded to a gene therapy investigator.

The development of vectors has changed significantly over the years. During the early years of the Indiana University NGVL, requests for vectors were similar. The first 22 vectors included similar murine packaging cells. Today, requested vectors are markedly different and exceedingly complex. Of the last four retrovirus vectors requested, three had unique envelopes. This adds significant challenges to the production and certification processes. Often, there is no existing guidance for the production of such vectors or even for replication-competent retroviruses (RCR) testing of the variants. Consequently, new

tests must be developed to appropriately certify the vectors. This has slowed the development of these new vectors.

The FDA requires some clinical trials to follow patients for 15 years. Investigators having limited funding may be unable to fulfill this guidance. To address this requirement, NCRR worked with the NGVL to arrange for patients who have participated in academic health center gene therapy trials to be seen at any General Clinical Research Center for follow-up that includes phlebotomy, clonality testing, and archiving of samples. In 2004, the Indiana University VPF launched repository and clonality-testing initiatives supported by NCRR. These allow the NGVL to promote investigator compliance.

Recently, the NGVL has received requests for batches of vector to support studies involving pre-clinical studies in animal models. While it would be an efficient use of NIH funding, and possible to do so should the NGVL policies be modified, the cost of providing this service to investigators should be carefully evaluated.

Dr. Cornetta concluded his presentation by reviewing current short- and intermediate-term goals for the Indiana University NGVL. In the short term, the laboratory plans to complete production of retrovirus vectors that have been requested by investigators. The process for generating lentiviral vectors, now in production, was described. The laboratory has GMP real-time PCR available for retroviruses, lentiviruses, and general virus screening. This allows the laboratory to perform these tests in-house and to realize savings in excess of $100,000 per year. In addition, the laboratory has developed clinical grade RD114 retroviral packaging lines and is testing the stability of lentiviral vectors. Intermediate-term goals include refinement of lentivirus production, working with foamy viruses, and developing RCR assays for self-inactivating retroviruses. Dr. Cornetta described construction plans for a new facility which will contain five vector production rooms funded, in part, by an NCRR C06 construction grant.

City of Hope Center for Biomedicine and Genetics

Larry Couture, Ph.D., Senior Vice President of the Center for Biomedicine and Genetics (CBG) at the Beckman Research Institute/City of Hope, described their 20,000-sq. ft. facility—housing 12 production rooms—that was completed in January 2000. The facility has a full-time staff of 28 people (including 6 Ph.D.s) and an annual operating budget of more than $1.7 million. Almost all of the research staff has a pharmaceutical industry background. On September 2001, the facility was designated as an NGVL.

The Beckman Research Institute produces vectors, vaccines, proteins, plasmid DNA (pDNA), pancreatic islets, monoclonal antibodies, and other cell products. Dr. Couture described the organization and physical configuration of the Institute. It includes quality assurance/quality control branches that support this multi-product program, as well as a recently developed regulatory affairs office. The latter helps academic investigators in dealing with the regulatory process, developing INDs, and supporting on-campus clinical trials. The City of Hope/Beckman Research Institute requires indemnification from the requesting investigator.

This NGVL has provided DNA plasmids for approximately 40 patients in 6 cancer-related clinical trials. Initially, the NGVL proposed to develop a 100 mg-scale pDNA manufacturing capability and to provide 12 pDNAs per year. Today, it can produce over 500 mg pDNA per run and 25 different plasmids per year. Most of these achievements have been attained by adopting the modular processes of the biopharmaceutical industry. Over the past five years, bringing assays in-house has reduced costs by 80 percent and shortened production time. This has led to developing more robust, quantitative, reproducible technologies such as Q-PCR to detect and quantify specific nucleic acids, and other PCR-based assays for detection of prior product contamination. Plasmids can be provided for clinical use within three months if the submitted construct was correct; six to nine months if the construct was incorrect.

The Institute has also developed a quality assurance (QA) program that uses a propriety bar-coding system for quarantine and release of all vectors. The bar-coding system uses a streamlined shipping and receiving database that tracks all inventories via a paperless batch record system. The program also includes QA check of equipment calibration/maintenance, environmental monitoring, regulatory assets, project management, Master Files, and development of cell bank resources.

Other programmatic achievements include a new fill-and-finish unit that can provide 1,000 vials of product, as well as the development of a recombinant DNA technology service that allows for plasmid vector reconstruction, backbone optimization, and gene modification by site-specific mutation. Recombinant DNA services will also help to create accurate constructs for investigators. This is important since few investigators submit accurate plasmids. The Institute has also developed programs in recombinant AAV, adenovirus, vaccinia virus, and embryonic stem cell banking.

In the future, investigators will need increased pDNA scale to meet increasing numbers of complex distribution and preclinical toxicology studies and recent requests for therapeutic DNA vaccines. To meet the projected requirements, the laboratory will increase its batch production capacity to 5 gram pDNA. Three or four additional current GMP and Good Tissue Practice (GTP) manufacturing facilities will be developed within the City of Hope CBG.

Center for Cell and Gene Therapy, Baylor College of Medicine

Malcolm Brenner, M.D., director of the Center for Cell and Gene Therapy at Baylor College of Medicine, detailed NGVL and other activities at the Center.

While the vector development lab has experience in adenoviral, AAV, lentiviral, HIV, and non-viral vectors, the NGVL is charged with the production of adenoviral vectors The laboratory has developed scale-up processes, optimized transfection and validation procedures, and established release assays. Dr. Brenner described the GMP facilities, which include the vector production facilities, a QC laboratory, and administrative offices. Five production suites cover approximately 2,400 square feet. The facility uses extensive bar coding for inventory tracking, an environmental database, validated cleaning methods,

electronic and visual pressure monitoring, and extensive changeover procedures. The Center has significant QC and QA programs. The QC laboratory is approximately 600 square feet and provides product testing, environmental monitoring, GMP/GTP compliance oversight, product management, and audits. The laboratory also maintains a drug master file for adenoviral vectors.

Adenoviral production has been standardized at the Baylor College of Medicine NGVL through the development of a master virus bank that creates viruses for archiving and stability testing, amplification for development of additional lots, and clinical product development. Dr. Brenner noted that, since 2001, nine clinical-grade vectors have been made by the NGVL, the majority being directed at various malignancies. He provided the details for each of the NGVL phase I studies, several of which have demonstrated clinical responses in the early phase of the protocols. The Baylor NGVL also has developed helper-dependent Adenoviral vectors for non-malignant diseases. These vectors have been "sprayed" into the lungs of nonhuman primates, resulting in wide distribution and high levels of expression. Currently, the Center is developing vectors for factor VIII deficiency and cystic fibrosis.

As investigators complete successful Phase I trials, they are initiating Phase II trials that require larger quantities of viral particles. The "Wave Bioreactor" is being employed for this purpose with the resultant product being purified by high-performance liquid chromatography.

In addition to its ability to manufacture and test clinical-grade vectors in a cost-effective manner, CAGT serves as a model for other academic centers. In the past few years, 31 investigator groups from the United States and throughout the world visited CAGT to observe the vector manufacturing process.

NGVL National Pharmacology and Toxicology Center—Southern Research Institute

John Page, Ph.D., Distinguished Scientist, Southern Research Institute (SRI), presented an overview of its NGVL Toxicology Center. SRI has over 40 years of experience in performing toxicology studies and was designated as an NGVL facility in 2001. Most of the work since then has been directed toward validating equipment and procedures, and developing standard operating procedures (SOPs). The Center has the capacity to perform biodistribution, pre-clinical toxicology, and animal imaging studies.

The Center can perform the testing necessary to obtain INDs and new drug applications, using the same strains of animals that were employed in the proof of concept work to ensure safety and efficacy. The Center has prepared protocols for testing in mice, rats, hamsters, and monkeys. Dose schedules are administered as single doses, daily for three consecutive days, or cyclically. The animal dosing schedules are the same as the schedules planned for human studies and can be administered interperitoneally, intravenously, or intracranially.

The facilities are certified by the Association for Assessment and Accreditation of Laboratory Animal Care and have biosafety level-2 (BSL-2) and BSL-3 animal rooms. Many of the vectors are handled at the BSL-2 level. Clinical pathology laboratories in the toxicology center are capable to determine chemistry, hematology, and coagulation parameters. The Center also has dose formulation laboratories for the dilution of virus prior to use and bioanalytical and PCR facilities to determine tissue levels, plasma levels, and pharmacokinetics studies. Post-life support facilities include necropsy facilities for both small and large animals and histology laboratories tissue studies.

An Institutional Animal Care and Use Committee at the Center reviews every protocol prior to its initiation. The Institutional Biosafety Committee ensures proper safeguards for the staff conducting biological studies. An archival facility holds raw data (e.g., tissue samples) from the studies performed. The laboratory is one of the few in the country that is fully good laboratory practice (GLP) certified.

Dr. Page stated that the Center was underutilized with only two toxicology studies of one vector having been performed thus far. Activity is about to increase significantly with several studies poised to be initiated at the Center during the next six months.

NGVL Toxicology Center—University of Florida

Barry Byrne, M.D., Ph.D., Director of the NGVL Toxicology Core Center at the Powell Gene Therapy Center (PGTC) of the University of Florida, presented an overview of their efforts.

The PGCT has evolved from a few investigators recruited to work on AAV vectors. Today, it hosts faculty from the Colleges of Medicine, Veterinary Medicine, Pharmacy, Health Professions, and Engineering—focusing on treatments for genetic diseases.

The goal of the NGVL Toxicology Center (a 1900-ft^2 cGMP-compliant facility within the PGTC) is to develop and conduct preclinical and toxicology studies in support of proposed clinical trials. The Center prepares vectors for biodistribution and toxicology studies. It also facilitates process development for AAV production. A reference standard for AAV is being developed under the auspices of the NGVL to establish consistency in AAV toxicology and clinical studies.

When compared to a similar commercial facility, the NGVL is efficient, costing approximately 20 percent of comparable commercial productions—while toxicology studies cost half that of commercial entities.

University of Florida has been a pioneer in developing AAV vector technology. The AAV vector was invented and first used for human gene therapy at the Powell Gene Therapy Center. The Center has also established methods for GLP and GMP manufacturing of AAV gene therapy products, developed assays for the characterization of AAV vectors used in preclinical and clinical studies, and validated the assays for quantification of vector biodistribution.

Currently, the PGTC is conducting studies in gene therapy using viral vectors for lung and cardiovascular diseases, retinal and central nervous system diseases, cystic fibrosis, diabetes, Leber's congenital amaurosis, and muscular dystrophy. The timeline from proof-of-concept to final product is between five and eight years.

The NGVL portion of the PGTC performs small animal toxicology studies. Acute and chronic toxicity studies include biodistribution studies, germline gene transfer and tumor formation, and immunogenicity. The NGVL works closely with investigators to establish the appropriate route and dosing schedules of animals that would be relevant to the anticipated IND submission. The material is evaluated for safety and its potential for vertical transmission. Four AAV toxicology studies involving mice, rabbits, dogs and monkeys have been completed. An additional four studies are to begin within the next few months.

The Toxicology Center offers support for Phase I clinical trials by evaluating vector safety, dose evaluation, and the potential for vertical transmission. Assays for quantification of AAV biodistribution have been validated. To date, a total of 23,204 GLP and non-GLP PCR reactions have been performed at the University of Florida NGVL, along with 4,122 GLP serum chemistries and 556 GLP complete blood counts. Assays of various AAV serotypes have been developed for pre-clinical and clinical studies.

II. NIH Presentations

National Heart, Lung, and Blood Institute (NHLBI) Resources in Gene Therapy

Sonia Skarlatos, Ph.D., NHLBI Deputy Division Director and Gene Therapy Coordinator, provided an update on the following four current programs and resources.

The aim of Programs of Excellence in Gene Therapy (PEGT) within the NHLBI is to facilitate clinical gene therapy studies in cardiovascular, pulmonary, and hematological diseases through nationally-shared cores and training programs for M.D.s and Ph.D.s. Four sites were funded in 2000: Stanford University, University of Pittsburgh, University of Washington, and Cornell University. Cornell was also funded as the data and coordinating center.

These PEGTs provide a cell morphology core, a preclinical vector production core (adenovirus, AAV, retrovirus), a clinical vector production core (adenovirus, herpes, AAV, pDNA), a hematopoietic cell processing core, and a primate stem cell transplantation core. Funding for these programs ends in August 2006.

The Center for Fetal Monkey Gene Transfer for Heart, Lung, and Blood Diseases program, based at the University of California-Davis, establishes monkey models to explore approaches for gene transfer in the fetus for heart, lung, and blood diseases. The program also evaluates the safety and efficiency of emerging gene transfer strategies. In the past 4 years, the program has completed 18 studies. The program will now be performing 10 studies annually.

The new Gene Therapy Resource Program (GTRP) will begin in FY '06 with a mission of translating basic gene therapy research studies into clinical applications for diseases of the heart, lung, and blood. It will provide resources for preclinical/clinical studies and support for gene therapy trials. Funds will support GTRP core laboratories and up to four gene therapy trials, and assist investigators in responding to questions raised by the Recombinant DNA Advisory Committee and FDA.

Beginning in 2004, NHLBI committed $5.4 million to the NGVL initiative in support of up to nine toxicology studies and production of up to nine AAV vectors for protocols related to heart, lung, and blood diseases.

National Cancer Institute (NCI) Biological Resources Branch

Rosemarie Aurigemma, Ph.D., Program Director of the Biological Resources Branch at NCI, discussed the biologic development programs (BDPs) as they relate to NGVL-related clinical trials. A cGMP facility, located in Frederick, Maryland, has 125 staff members and supports Phase I and II clinical trials, some of which are relevant to gene transfer.

The BDP performs process development, manufacturing, QA/QC, and regulatory affairs and has been used to develop vaccines for the Army. In addition, the facility develops mammalian cell production and possesses GMP-qualified Master Cell Banks for vector production. Development of viral vectors is an activity begun only in the past three years. The facility has an $18 million annual budget.

One BDP program is Rapid Access to Intervention Development (RAID). Its goal is to help academic investigators move their products into Phase I and II trials. While the technology can be licensed to a small business, only academic investigators are eligible to apply for RAID support. The 59 RAID projects that have been approved to date include DNA vaccines, genetically modified cell lines, recombinant proteins, peptides, viruses, humanized monoclonal antibodies, immune response modulators, targeted liposomes, and microspheres—some of which were destined for pre-clinical and clinical use. There are 34 active projects.

RAID applications undergo scientific review by a panel of extramural investigators. Top applications then proceed to a second-level technical feasibility review, which approves projects for support up to certain "milestones." Following approval, starting materials received from the principal investigator are reviewed for GMP suitability (e.g. sequence analysis and cell line history). This is followed by process development and assay development for QC product release prior to GMP manufacturing. The toxicology and pharmacology branch conducts IND-directed toxicology. Once the product is placed into its repository, the regulatory affairs group provides support to the principal investigator for any pre-IND meetings and IND application filing.

Dr. Aurigemma concluded by noting that she looks forward to determining how NGVL and the RAID program can support each other and adding that BDP processes for herpes, adenovirus, or chromatography are available for sharing with NGVL.

III. Working Group Recommendations

Following the presentations, the NGVL Directors recused themselves to allow the Working Group to initiate discussion and identify points that required clarification.

The group agreed that the NGVL program has played a positive, consistent, and important role in responding to the needs of the clinical gene therapy community by providing high-quality vectors and advice to investigators. The reduction of unnecessary repetition of toxicology studies and the production of master files for cross-referencing were considered to be significant contributions to the field of gene therapy. The cooperative spirit of the NGVL advances the translation of basic research in gene transfer into the clinical arena.

All parties then reconvened to develop a series of recommendations.

1. **Continue to Discuss Production of New Types of Vectors at Steering Committee Meetings**

   The NGVL production facilities currently generate retrovirus, lentivirus, adenovirus, and DNA plasmids with production of other vectors being considered by the NGVL Steering Committee upon receipt of requests. The NGVL directors can propose modification of their SOPs to make such vectors and then compete with proposals from non-NGVL GMP-compliant facilities. Vector production by the latter groups has been performed under contract through the Coordinating Center. The products have included adenovirus, plasmids, herpesvirus and AAV. Such Steering Committee discussions and consequent outsourcings should continue, based on new vector requests and inquiries by investigators. New vector production should be investigator-driven and supported by the NGVLs. The Steering committee should try to anticipate future community needs based on presentations and discussions at national meetings and newly published data. It is important for the NGVL program to be sufficiently versatile to provide these various types of vectors.

2. **Share Technologies and Common Procedures**

   NGVLs at various locations should continue to share technology and background documents so as to not "reinvent the wheel" in the production of a vector. The practice of sharing SOPs and performing commonly required complex tests in a central NGVL laboratory should continue. Centralizing PCR or sequencing might be considered.

3. **Continue Moving Toward Increased AAV and Lentivirus Production**

   The NGVLs should try to remain ahead of the state-of-the-art in gene therapy, pushing the frontier without over-reaching. Anticipating the increasing need for lentivirus vectors and various pseudotypes of AAV is deemed appropriate.

4. Involve NGVLs in Improving Vectors and Processes

Although large portions of their funding should not be redirected for this purpose, the Working Group agreed that the mission of the labs is to produce vectors and, consequently, they should also be involved with improving their products and processes. All of the NGVLs should continue to collaborate toward this goal, sharing technology and information. In addition, investigators requesting vector should be encouraged, perhaps even incentivized, to share these vectors with other investigators.

If a particular person at an NGVL holds intellectual property with respect to a particular vector design, he or she should disclose this to avoid conflict of interest. Vectors chosen for development and improvement should be chosen solely for the purpose of furthering the field of gene therapy as a whole. The NGVL should continue to facilitate Material Transfer Agreements between the centers, requesters and other holders of relevant intellectual property rights. In addition, all applications submitted to the NGVL should include the investigator's plan to share vectors with other investigators.

5. Produce Vectors Primarily for Clinical Use

Because of limited resources, it is best to generate vectors for clinical use rather than for basic laboratory studies. Producing vectors for use in an experiment involving a few small animals, for example, would not be a wise use of the NGVL resources. However, generation of vectors for large-animal pre-clinical studies may be justified.

6. Therapeutic Vaccines but Not Prophylactic Vaccines Should be Developed

The NGVLs currently produce some plasmids for use as anti-cancer therapeutic vaccines. Clinical trials of prophylactic vaccines involve very large numbers of participants and have different regulatory requirements than therapeutic vaccine trials. Furthermore, prophylactic vaccine manufacture requires specialized facilities, some of which would require containment. Following this route would take resources away from the NGVL program's fundamental work in gene therapy. Consequently, the Working Group recommended that only therapeutic vaccine development be supported within the NGVL program.

Furthermore, NGVL should continue to provide only core support for vector development, and investigators should provide the additional funding to support the conduct of clinical trials.

7. **Continue to Work With Investigators Throughout the Review And Production Process**

Investigators should be asked detailed questions about the requested vectors early in the application process and, certainly, prior to initiating production. This is intended

to prevent submission and subsequent manufacture of flawed vectors and, thereby, speed the review/production process. Materials submitted to the NGVL should continue to be evaluated by the NGVL prior to embarking on manufacture. Although the responsibility for correcting a flawed vector rests with the investigator, should a flawed vector be submitted, the NGVL should continue to work with the investigator to make the appropriate corrections.

8. Allow NGVLs to Develop New Technologies

The NGVL Steering Committee should play a proactive role in suggesting research topics that could be undertaken by the NGVLs during their "down" time. The Steering Committee could hold interactive sessions with outside investigators to make recommendations on new technologies to be pursued by the NGVLs. NGVLs might rebudget funds, with prior Steering Committee approval, to develop these new technologies.

9. Continue Industry Collaboration

NGVLs have a history of collaboration with industry. For example, an NGVL has developed products by using industry-donated packaging cell lines. Such interactions could help to make new methodologies available to academicians and accelerate the development of technologies needed for treatment of monogenic diseases in which there is no commercial interest. NGVLs should continue to work with industry as they have done in the past, but they should not contract with commercial organizations to produce vectors since this approach is much more expensive and unlikely to lead to innovations that could be shared with the research community**.**

10. Increase Advertising of Toxicology Services

The Working Group agreed that the Toxicology Centers have fewer requests for services than anticipated and have been underutilized. The group suggested continued advertising of the services available through the Toxicology Centers in an effort to reach a wider audience of investigators. It would be helpful if NIH Institutes with similar programs would inform their grantees about the services provided by the Toxicology Centers.

It would also be helpful to educate investigators about the toxicology studies that are required for FDA approval. This would reduce the frequency of performing redundant toxicology studies. The NGVL should continue to hold meetings with investigators prior to their pre-IND meetings and should continue to participate in certain pre-IND meetings prior to initiating any toxicology study.

Pharmacology/Toxicology services should continue to be provided for vectors that are poised to enter clinical studies. These services might be expanded to studies involving large animals but not to basic research or proof of concept studies.

11. Promote the NPRCs as a Resource

The NCRR-funded NPRCs are available for studies that involve non-human primates. The NGVL should make investigators aware of the availability of this resource for potential vector studies.

12. Make the Pharmacology/Toxicology Database More Widely Known

Currently, the Pharmacology/Toxicology database is available to investigators and the general public through www.NGVL.org. Investigators, both domestic and foreign, have cross-referenced NGVL studies through the database. The Working Group agreed that although the Pharmacology/Toxicology database is a powerful resource, it could be used more widely by informing more investigators about its availability with additional hands-on demonstrations at National meetings. The Working Group felt that the database should continue to be limited to NGVL-supported vector studies.

13. Support the Development of Local Regulatory Cores

Members agreed that a regulatory core would provide much-needed assistance to investigators who lack expertise in regulatory affairs. Working Group members noted while NCI and NHLBI require that their vector programs have a regulatory core, independent of where vectors are produced, this approach within the NGVL program would be too costly to employ, drawing funds from current activities. The Working Group suggested that regulatory cores preferably be established at institutions where the clinical studies are to be performed, perhaps through the new NCRR Clinical and Translational Science Awards. The Coordinating Center and relevant production center should continue to assist investigators' interactions with the FDA when appropriate.

14. Streamline the Review Process

Working Group members discussed the existence of a two-tiered NGVL review process. Investigators have usually received initial grant funding from an NIH institute. They then undergo review by the NGVL. Some members pointed out that such review is duplicative and wasteful of time and money. Members suggested having an NIH-wide procedure that would streamline the review process so that a proposed project that had previously been reviewed favorably by an NIH study section only receive a cursory administrative review by the NGVL. It was, however, also pointed out that an investigator's vector and clinical plan have usually changed significantly from the time that the original grant was submitted for NIH review. This argues strongly for continuing the detailed scientific and administrative review by the NGVL.

15. Include Milestones in All Future Grants

The Working Group continued to discuss the current grant review process. Participants suggested that the NIH consider introduction of awards whose funds would be delivered to R01 gene therapy grantees upon achieving pre-established milestones. This would be a two-step process: Basic research and pre-clinical studies could be funded for the first few years based on their scientific merit but support for the clinical aspects proposed for the latter part of the award would be dependent upon the results of those initial data. This strategy would justify moving complex and costly translational research protocols forward in an expeditious manner, if warranted, and avoid funding the final portions of those applications that fail to achieve the initially-promised scientific goals. The NGVL must be certain that investigators have documented the availability of financial support of the clinical trial before the NGVL embarks on vector generation or toxicology studies.

16. Continue Cooperation With Other NIH Gene Therapy Resources

The Working Group agreed that discussions between NCRR, NHLBI, and NCI continue in order to avoid duplication of their gene therapy programs and services. The NCI-RAID facility in Frederick, Maryland should be considered as a potential production site for vectors that are not usually made by the NGVLs.

Dr. Wara, chair of the Gene Therapy Working Group Committee, thanked all participants for their outstanding presentations and comments. Dr. Knazek also thanked participants for their contributions and wished them a safe trip home.

Appendices

Gene Therapy Working Group Members

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Meeting Agenda

Meeting of the Gene Therapy Working Group

Date:	November 1, 2005
Location:	National Center for Research Resources One Democracy Plaza Bethesda, Maryland
8:30 a.m.	Introduction and Charge to the Working Group
8:45 a.m.	NGVL Director Presentations Coordinating Center at Indiana University Indiana University City of Hope Baylor University Southern Research Institute University of Florida
11:00 a.m.	Discussion among Panelists, NGVL Directors, and Participants
12:00 Noon	Working lunch and discussion among Panelists and Participants
1:00 p.m.	NGVL Directors rejoin discussion
3:00 p.m.	Summary and development of recommendations
4:00 p.m.	Adjourn