FORUM 1996: GENE THERAPY

►*DEVELOPMENT AND EVALUATION OF PHASE I PRODUCTS*

►*VECTOR DEVELOPMENT*

SPONSORED BY

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION

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JULY 11-12, 1996
NATCHER CONFERENCE CENTER
NIH CAMPUS
BETHESDA, MD

GENE THERAPY CONFERENCE
SCIENTIFIC STEERING COMMITTEE

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Note from the Organizers:

This conference originated from Pre-IND meetings which suggested that some sponsors had misunderstandings of how the FDA went about the business of evaluating gene therapy products. We first started formulating ideas of a training session describing the FDA review process in September of 1995. Coincident with this were discussions with NCI concerning its new biotherapuetic production facility and their interest in producing gene therapy vectors. Shortly thereafter, the National Gene Vector Laboratories (NGVL) program was announced, and suddenly a two day conference emerged out of the mist. We never anticipated that this conference would turn out to be so large, but are delighted nonetheless. Lessons learned from this year's conference will be used to make next year's conference even more useful and productive.

OVERVIEW OF THE CONFERENCE AND GOALS

Entry of gene therapy products into the clinic requires overcoming many barriers. Foremost among them are funding and regulatory concerns. One of the most useful aspects of gene therapy product development to date has been the free exchange of information common to specific products and product areas. This open environment has been critical for keeping gene therapy regulation in pace with developments in the field. Moreover this information exchange has been instrumental in facilitating public acceptance and support of this rapidly developing area.

FDA and NIH share a common goal of promoting the development of useful, safe, and effective therapies for human disease. This conference was designed with two primary goals in mind. The first was to bring together scientists developing gene therapy products with the intention of explaining to them what kinds of information are appropriate and necessary for entry into Phase I clinical trials and what sources of funding the NIH has made available to facilitate early product development and evaluation. The second goal of the conference was to provide a forum for the open exchange of scientific, manufacturing, and clinical experiences as they relate to FDA regulatory requirements.

►ANNOUNCEMENT OF SECOND ANNUAL GENE THERAPY CONFERENCE

This conference is the first of what is hoped to be an annual event. The Second Annual FDA/NIH International Gene Therapy Conference is scheduled for July 15-18, 1997 at the Natcher Conference Center on the NIH campus in Bethesda, MD. Watch the FDA-CBER website for updates: http://www.FDA.GOV/CBER/CBERftp.html

Topics for discussion at the conference are solicited. Please send suggestions to our gene therapy conference e-mail account: GTINFO@A1.cber.fda.gov or call the Division of Cellular and Gene Therapies/FDA at 301-827-0681.

Watch the CBER web site for updates.

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Goals: To acquaint investigators with the necessary information required by CBER for evaluation of products intended for phase I clinical trials and the resources currently available to investigators through the NIH.

8:00 Welcome - Kathryn C. Zoon, Ph.D. - Director, CBER

8:05 Opening Remarks - Jay P. Siegel, M.D. - Director, Office of Therapeutics, CBER/FDA

8:10 RAC Update - Lana R. Skirboll, Ph.D. - Associate Director for Science Policy, NIH

CHAIR FOR THE MORNING SESSION: PHILIP NOGUCHI, M.D., FDA

8:20 Introduction - Philip Noguchi, M.D. - Director, Division of Cellular and Gene Therapies, CBER/FDA

8:30 Overview of the FDA IND review process. Andra E. Miller, Ph.D., CBER

9:00 Recommendations for products intended for Phase I clinical trials. Robert Anderson, Ph.D., CBER

9:30 Specific considerations for the manufacture of gene therapy products. Ancillary products, non-licensed products used in the manufacture of a therapeutic. Joyce L. Frey-Vasconcells, Ph.D., CBER

10:00 Break

10:30 The role of pre-clinical studies in support of phase I clinical trials: Proof of concept and safety studies. Anne M. Pilaro, Ph.D., CBER

11:00 Considerations in clinical trial design for gene therapy phase I trials. David. Wilde, M.D., CBER

11:30 International regulatory considerations, export and import issues. Elaine C. Esber, M.D., CBER

CHAIR FOR THE AFTERNOON SESSION, CRAIG REYNOLDS, Ph.D., NCI

1:30 Overview of NIH resources available for gene therapy product development. Craig Reynolds, Ph.D., NCI

1:50 National Cancer Institute, Diane Bronzert

2:00 National Institute of Allergy and Infectious Diseases, Nava Sarver, M.D.

2:10 National Heart, Lung, Blood Institute, Sonia Skarlatos, Ph.D.

2:20 National Institute of Diabetes and Digestive and Kidney Diseases, Catherine McKeon, Ph.D.

2:30 Discussion, Break

3:00 National Center for Research Resources - National Gene Vector Laboratories, Richard Knazek, M.D.

3:30 NCI-sponsored small scale manufacture of gene therapy products, George A. Robertson, Ph.D.

4:15 NCI-Office of Technology Development, Elizabeth C. Lovoy, J.D., M.P.H.

4:40 Electronic Activities & Registries under the FDA SMART Program, Mary A. Buesing, M.D., CBER

5:00 Closing Remarks

GENE THERAPY CONFERENCE - INTRODUCTION

Kathryn C. Zoon, Ph.D.
Director
Center for Biologics Evaluation and Research
Food & Drug Administration

There has been tremendous growth in investigational new drug (IND) applications for gene therapy. One IND was submitted in 1989 whereas in 1996, 41 were submitted. The current number of gene therapy INDs through June 30, 1996 is 142.

►THREE PRINCIPLES UNDERLIE THIS EMERGING FIELD:

- ◆ Sound science is the foundation for decision making.
- ◆ The development and regulation of gene therapy products are collaborative and iterative processes among government, industry, public, and academia.
- ◆ Development of gene therapy policy and regulation needs to be a public process which allows ethical, social, scientific, and other issues to be debated and discussed in an open and public forum.

►THREE CURRENT AREAS OF CONCERN IN GENE THERAPY

- ◆ Replication competent retrovirus (RCR) testing
  - ◇ Original policy was implemented because of Nienhuis (NIH) experiment in monkeys
  - ◇ Now that sponsors are increasing the size of production lots, this testing policy is becoming logistically burdensome.
  - ◇ Solution:
    - ✣ Data should be (and has been) publicly discussed;
    - ✣ Statistical models developed with preliminary testing validation;
    - ✣ Standards should be made available and
    - ✣ Further testing and results should be shared.
- ◆ Source and supply of "clinical" grade vector
  - ◇ With the growth in gene therapy products, the supply of vector suitable for clinical use is becoming a limiting factor.
  - ◇ Solutions:
    - ✣ The NIH National Gene Vector Laboratories (NGVL), with FDA as a liaison, are providing financial support for the production and distribution of vectors;
    - ✣ FDA meets with corporate and academic sponsors to help plan small scale production facilities;
    - ✣ NIH/NCI opens a GMP production facility that includes vectors (with the assistance of FDA scientists).
    - ✣ Education of new investigators:
- ◆ As the field expands, issues of how to effectively help new investigators through the IND process arise.
  - ◇ Solutions:
    - ✣ FDA/NIH sponsored workshops on the nuts and bolts of gene therapy regulation;
    - ✣ The RAC process allows publication of clinical protocols;
    - ✣ Increased emphasis by FDA on guidance documents & their availability (FDA-CBER & NIH Guidance documents are available on the World Wide Web);
    - ✣ FDA will be implementing electronic IND submissions.

►FUTURE ISSUES AND NEW CHALLENGES IN THE FIELD

- ◆ Development of new vector systems
  - ◇ These will require novel safety testing & pharmacology/toxicology studies.
  - ◇ There is a desire/need to coordinate studies to reduce unnecessary duplication of testing, and to be able to collect and correlate data from multiple sponsors for a given product area.
  - ◇ Some vectors present new challenges such as herpes and lentivirus vectors.
  - ◇ A major challenge will be how to deal collectively with the issues prospectively, rather than on a case by case basis.
- ◆ NEW APPROACHES AND NEW INDICATIONS FOR GENE THERAPY
  - ◇ In utero gene therapy.

- ◇ Germ line gene therapy.
- ◇ Enhancement gene therapy.
- ◇ Ethical and societal issues need to be discussed openly and decisions made.
- ◇ Establish a forum for public discussion to help guide FDA decisions.

◆ License approval of gene therapy products

- ◇ Develop a scientifically sound approach to regulate these novel products.
- ◇ Gene therapies are complex products and often use multiple facilities to support their production.

RAC UPDATE

Lana Skirboll, Ph.D.
Associate Director for Science Policy, NIH

ENHANCED NIH OVERSIGHT OF GENE THERAPY ACTIVITIES
Notice of Intent
To Propose Amendments to the NIH Guidelines
Regarding Enhanced Mechanisms for
NIH Oversight of Recombinant DNA Activities
Federal Register, July 8, 1996 (61 FR 35774)

STRUCTURAL COMPONENTS OF NIH PROPOSAL

►ORDA ADVISORY COMMITTEE (OAC)

- ◆Public forum
- ◆Chartered advisory committee
- ◆Notice of scheduled meetings will published in the Federal Register and convened 4 times/year
- ◆Standing membership of 6 to 10 individuals representing the scientific, legal, ethical, and public advocacy communities.
- ◆Maintain public accountability for gene therapy research
- ◆In-depth gene therapy data analysis
- ◆Through OAC, the NIH Director will continue to promulgate necessary amendments to the NIH Guidelines
- ◆Facilitate timely public reporting of significant clinical events
- ◆Identify and prioritize proposed GTPC topics

►GENE THERAPY POLICY CONFERENCES (GTPC)

- ◆Public forum
- ◆Numerous participants who possess significant scientific, ethical, and legal expertise and/or interest that is directly applicable to a specific recombinant DNA research issue.
- ◆Notice of scheduled meetings will be published in the Federal Register and convened 4 times/year
- ◆Discussion topics may include: (1) basic research on novel gene delivery vehicles, (2) novel applications of gene transfer, and (3) relevant ethical and societal implications of a particular application of gene transfer technology
- ◆Discussion topics do not preclude the use of novel protocols as a focus for conference discussion
- ◆Topics will be identified through OAC recommendations, interagency
- ◆communications, ORDA, the scientific community, patient advocates, ethicists, public health professionals, and the public
- ◆Where appropriate, findings and recommendations will be submitted to the
- ◆NIH Director for dissemination to DHHS components and the public

►OFFICE OF RECOMBINANT DNA ACTIVITIES (ORDA)

- ◆Continue to provide administrative support relevant to recombinant DNA activities
- ◆Enhance public awareness of human gene therapy research through data management activities
- ◆Identify, coordinate, and disseminate relevant information as novel technologies emerge
- ◆Provide administrative support to both OAC and GTPC
- ◆Optimize interagency communication and collaborative efforts to streamline procedures and facilitate the flow of relevant information

FUNCTIONAL ANALYSIS OF NIH PROPOSAL

	RAC	OAC	GTPC
Public Forum	Yes	Yes	Yes
Standing Members	Yes	Yes	No
Chartered	Yes	Yes	No
Broad Membership Representing Scientific, Ethical, Legal, and Public Health Concerns	Yes	Yes	Yes - But ad hoc expertise as required by discussion topic
Terms	4 years	3-4 years	No terms
Number of Meetings per Year			3-4
Policy Recommendations	Yes	Yes	Yes
Protocol Review and Approval/Disapproval	Yes	*No	*No
Minutes	Yes	Yes	Yes
Data Management and Adverse Event Reporting	Yes	Yes	Yes
Review Individual Protocol Informed Consent Documents	Yes	No - But may propose Informed consent issues for GTPC	No - But can provide forum for public discussion of informed consent issues

*Does not preclude a specific protocol used as topic for OAC or GTPC discussion.

►RECOMMENDATIONS OF THE RAC AD HOC REVIEW COMMITTEE (VERMA COMMITTEE)

- ◆ The RAC should no longer carry out case-by-case review of every clinical gene transfer protocol.
- ◆ Review of protocols by the RAC in an open public forum should continue in several areas of concern in which a particular protocol or new technology represents a significant degree of departure from familiar practices.
- ◆ The RAC should define the criteria and work out procedures for identifying specific protocols requiring public review.
- ◆ The RAC should continue to provide advice on policy matters revolving around gene therapy and other recombinant DNA issues to the NIH Director, individual members of the research community, institutional review boards, and the public.
  - ◇ This critical function should be extended, enabling RAC explicitly to provide advice and recommendations on policy matters to the Food and Drug Administration (FDA).
  - ◇ Hence, the committee recommends that the NIH Director urge the Commissioner of the FDA to exempt the broad area of gene therapy from many of the proprietary restraints reserved for ordinary therapeutic drug products and biologics that come under FDA review.

►NIH IMPLEMENTATION

- ◆ RAC is eliminated and Consolidated Review will not be maintained
- ◆ GTPC preserves such a forum and provides for more in-depth discussion of both the science and ethical issues related to a specific gene therapy issue
- ◆ Not applicable to the proposed new structure, since this proposal cedes review and approval to the FDA
- ◆ Fully met by GTPC. Each GTPC will focus on a single issue; therefore, policy advice will be substantially augmented under this new mechanism
- ◆ Fully responds to the recommendation regarding the continued need for data monitoring and adverse event reporting

INTRODUCTION TO THE FDA AND NIH ROLES IN GENE THERAPY CLINICAL TRIALS

Philip D. Noguchi, M.D.
Director,
Division of Cellular and Gene Therapies
OTRR, FDA

This morning's session will be devoted to those who would like to know how to conduct a gene therapy trial. Human gene therapy represents the publicly visible portion of a rapidly evolving medical revolution. Gene therapies could not be contemplated without the ready availability of purified cytokines to allow expansion and differentiation of cells. Likewise, monoclonal antibody technology has allowed the purification of a variety of cells from a number of tissues that can then be transduced with vectors. The investment by NIH in basic biomedical research studies has created the ability to isolate medically relevant genes and to create the vectors used in gene therapies.

The Food and Drug Administration (FDA) derives its authority from the Food, Drug and Cosmetic Act (revised) (FD&C Act) and Section 351 of the Public Health Service Act (PHS Act). Both Acts allow regulations to be promulgated that require federal oversight over clinical trials using experimental products. The authority used for the regulation of gene therapy had its origin in 1902 as the Biologics Control Act, which mandated that things biologic, such as viruses and serums, could not be shipped, sold or bartered unless it was prepared in a licensed and inspected establishment. In 1986, FDA announced in a policy statement that gene therapies would be considered biological products subject to licensure by FDA. The Center for Biologics Evaluation and Research (CBER) of the FDA is currently responsible for the regulation of the manufacturing and labeling of biologic products.

Investigational clinical trials in humans with gene therapy products are subject to the general requirements for drugs and biologics in Title 21 of the Code of Federal Regulations (CFR). This includes 21 CFR Part 312, the Investigational New Drug Application (IND) which has a number of requirements, including extensive documentation of methods of production and preclinical testing. In general, as products move from phase 1 through phase II and phase III, other portions of the 21 series pertain, including the 200 series on Current Good Manufacturing Practices (GMPS), the 300 series on new drug applications, the 600 series on biologics establishments and licensing. This level of regulation is the same for all biological products that are considered to be drugs, and focus on safety, purity, potency and efficacy considerations.

In 1975, the Recombinant DNA Advisory Committee (RAC) was formed to create guidelines that would allow recombinant DNA experiments to be conducted in laboratories under safe conditions. Over the past two decades, the RAC has evolved its guidelines and oversight to where only clinical protocols involving gene transfer are reviewed in public. FDA and NIH have interacted on an increasingly frequent basis during the past four years. The RAC meetings allow for public discussion of both accomplishments and adverse findings associated with gene therapy. Because these meetings are public, the discussions of safety concerns can be immediately communicated to the entire industry and a consensus to resolve the concerns can be reached with industry that would otherwise be restricted by the trade-secret regulations.

An instructive example occurred in June, 1993. The RAC and FDA received notice of an adverse reaction in the third patient to participate in a gene therapy protocol for cystic fibrosis using an adenoviral vector. Because of the public nature of gene therapy protocols, the RAC and FDA were able to communicate the details of this adverse event to other investigators. This allowed for appropriate modification of the protocol that allowed not only the original trial to continue with close FDA oversight, but also allowed four other protocols to be approved with modified dosing.

OVERVIEW OF THE FDA IND REVIEW PROCESS

Andra Miller, Ph.D.
Division of Application Review and Policy
CBER, FDA

- **OVERVIEW**
  - INVESTIGATIONAL NEW DRUG (IND) APPLICATION
    - Regulatory Approach
    - Pre-IND stage
    - When to file
    - Content of the IND submission
    - Review and notification processes
    - Types of submissions to the IND
- **SOMATIC CELL THERAPY**
  - DEFINITION
    - The prevention, treatment, cure, diagnosis or mitigation of disease or injuries in humans by the administration of autologous, allogeneic or xenogeneic cells that have been manipulated or altered ex vivo.
    - Includes cells that have been:
      - Propagated
      - Expanded
      - Selected
      - Pharmacologically treated
      - Altered in biological characteristics
- **GENE THERAPY**
  - DEFINITION
    - A medical intervention based on modification of the genetic material of living cells. Cells may be modified ex vivo for subsequent administration or altered in vivo by gene therapy products given directly to the subject.
    - Ex vivo manipulation of cells is also a type of somatic cell therapy.
- **REGULATORY AUTHORITY**
  - Federal Register Notice of Oct. 14, 1993 (Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products)
  - Compliance with part 312 of the Code of Federal Regulations at the investigational stage.
    - Clinical Trials conducted under IND
    - Subject to establishment and product licensure
    - PHS Act Points to Consider documents
- **REGULATORY STRATEGIES**
  - TRADITIONAL APPROACH
    - Evaluate and identify safety concerns
    - Quality Control
    - Sound Scientific Principles
    - Case by Case approach
  - NEW APPROACH: PUBLIC PROCESS
- **PHASES OF INVESTIGATION (21 CFR 312.21)**
  - Phase I Investigational Studies
    - Designed to evaluate safety and side effects
  - Phase II Investigational Studies
    - Designed to evaluate efficacy and dose ranging
  - Phase III Investigational Studies
    - Expanded study, additional information on efficacy and safety

► FDA'S OBJECTIVES

- ◇ To assure safety and rights of subjects in all phases of investigation
- ◇ To assure in phase II and III, that scientific quality of the investigations will support licensure
- ◇ Safeguard the public health while promoting development of new products

► BEFORE FILING AN IND

- ◆ Sponsor may request a pre-IND meeting or teleconference in writing
- ◆ Request should include:
  - ◇ Description of the product
  - ◇ Proposed development plan
  - ◇ Outline of specific issues to be addressed
  - ◇ Agenda/attendees list
- ◆ Written requests submitted to Chief of Cytokine and Gene Therapy Branch, DARP:
  - ◇ Dr. Joyce Frey
    Acting Chief
    Cytokine and Gene Therapy Branch
    Division of Application Review and Policy
    OTRR/CBER
    HFM-591
    1401 Rockville Pike
    Rockville, MD 20852
    301-594-0830

► PRE-IND MEETING REQUEST IS EVALUATED AND MEETING DATE SCHEDULED

- ◆ Meeting package due 2 weeks before meeting, including:
  - ◇ Preclinical data
  - ◇ Manufacturing summary
  - ◇ Clinical protocol
  - ◇ Issues to be discussed

► PRE-IND MEETING

- ◆ The sponsor should present:
  - ◇ Product manufacturing scheme
  - ◇ Data regarding the product characterization
  - ◇ Proposed specifications for lot release data or actual lot release data
  - ◇ Pre-clinical in vitro and in vivo data identifying the product's activity, efficacy and toxicity
  - ◇ Data supporting the product's clinical use
  - ◇ Proposed phase 1 clinical protocol

► IND SUBMISSION

- ◆ Sponsor must generate enough preclinical data to:
  - ◇ Ensure the safety of clinical trials
  - ◇ Determine the parameters for human studies
  - ◇ Establish the scientific rationale
- ◆ Request CBER IND Packet:
  - ◇ Office of Communication, Training and Manufacturers
    Assistance (OCTMA)
    HFM-40
    301-827-1800
- ◆ Submit INDs to:
  - ◇ Director, DARP
    HFM-99
    1401 Rockville Pike
    Rockville, MD 20852-1448
    FDA/CBER/OTRR

- **CONTENT AND FORMAT OF THE IND (21 CFR 312.23)**
  - Cover Sheet - Form FDA-1571
    - Provides basic information to FDA
      - Identifies sponsor and investigational drug
      - Identifies phase of clinical investigation
      - Identifies parties responsible for monitoring conduct of the trial
  - Table of contents
  - Introductory Statement and General Investigational Plan
    - Description of the drug
    - Summary of previous human experience
    - Overall plan for investigating the drug
  - Investigator's Brochure
    - Required if product is supplied to clinical investigators other than the sponsor
      - Description of the drug
      - Summary of pharm/tox effects
      - Pharmacokinetic and biological disposition
      - Summary of safety and effectiveness in humans from prior studies
      - Description of possible risks and precautions
  - Protocols
    - Protocol for each planned study including:
      - Statement of objectives and purpose of study
      - Inclusion & exclusion criteria
      - Doses to be administered
      - Description of clinical measurements to be used to monitor effects
      - Investigator data (Form FDA 1572)
  - Chemistry, Manufacturing and Control Information
    - Description of composition, manufacture and control of the investigational product
      - Physical, chemical or biological characteristics
      - Method of manufacture
      - Analytical methods used
      - Stability data/ Initial specifications
    - Description of placebo
    - Labeling
  - Pharmacology and Toxicology Information
    - Adequate information to support the safe conduct of the proposed clinical study
  - IRB approved Consent Form
  - Previous Human Experience with the Investigational Drug
  - Additional Information

- **MASTER FILE SUBMISSION (VS IND)**
  - CFR 314.420)
    - Alternative mechanism for submission of product and manufacturing information
    - Does not include clinical protocol
    - Permits holder to incorporate the information by reference when submitting an IND or
    - To authorize other persons to rely on the information, without disclosure
    - Five types I-V
      - Type II most useful for biological product development

- **MASTER FILE SUBMISSION**
  - Often contains:
    - Product manufacturing and purification schemes
    - Lot release protocols
    - Tissue culture media
    - Other proprietary information needed to support IND application
  - Example: retroviral vector supernatants

- ◆ FDA staff access to MF via cross reference letter
  - ◇ Filed to MF and the new IND
- ◆ CBER staff review and comment on contents of MF
  - ◇ Neither approved or disapproved

► IND REVIEW PROCESS

- ◆ Upon receipt of IND or MF, FDA issues:
  - ◇ Acknowledgment letter
  - ◇ IND or MF number
- ◆ IND application reviewed within 30 days
  - ◇ Review Team:
    - ✣ Product reviewer
    - ✣ Pharmacology/Toxicology reviewer
    - ✣ Clinical reviewer
    - ✣ Consult reviewer, as needed
- ◆ Emphasis of review is on data to support:
  - ◇ Manufacturing and quality control issues
  - ◇ Product characterization
  - ◇ Rationale
- ◆ Sound Scientific Principles
  - ◇ Pre-clinical studies
  - ◇ Product development
  - ◇ Clinical protocol
- ◆ Communication of Review Decision
  - ◇ Within 30 days (proceed or hold)
  - ◇ Clinical Hold
    - ✣ Unreasonable risk to the patient
    - ✣ Insufficient information provided to assess risk
    - ✣ Inadequate Investigator's Brochure
    - ✣ Clinical investigator not qualified
  - ◇ Letter issued detailing hold issues, comments and requests
- ◆ In order to proceed with clinical study:
  - ◇ Correct deficiencies
  - ◇ Submit additional information as amendment to IND
  - ◇ Receive notification to proceed by FDA

► SUBMISSIONS TO THE IND

- ◆ Amendments (21 CFR 312.30)
  - ◇ Protocol
    - ✣ New protocol
    - ✣ Change in protocol
    - ✣ New investigator
  - ◇ Information
    - ✣ New toxicology , product or other technical information
    - ✣ Discontinuance of a clinical study
- ◆ IND Safety Reports
  - ◇ Written reports of ADRs within 10 days
  - ◇ Telephone reports of fatalities within 3 days
- ◆ Annual Reports
  - ◇ Within 60 days of IND anniversary
  - ◇ Status of studies in progress
- ◆ Summary information
- ◆ General investigational plan for next year

REGULATORY PRINCIPLES FOR SOMATIC CELL AND GENE THERAPY
U.S. FDA PERSPECTIVE

Dr. Robert W. Anderson
Cytokine and Gene Therapy Branch
Division of Application Review and Policy
Center for Biologics Evaluation and Research
Food and Drug Administration

►REGULATORY CONCERNS COMMON TO ALL BIOLOGICALS

- ◆ Safety, identity, purity, potency
- ◆ Regulation of both the final product and the manufacturing, process
- ◆ Reproducibility/consistency of product lots

►CONCERNS RELEVANT FOR SOMATIC CELL AND GENE THERAPY PRODUCTS

- ◆ Product issues
  - ◇ Characterization of cells/cell lines
  - ◇ Adventitious agent testing
  - ◇ Replication competent virus
- ◆ Pre-clinical/clinical issues
  - ◇ Toxicity secondary to exogenous gene expression
  - ◇ Insertional mutagenesis
  - ◇ Effects on germ cells
  - ◇ In vivo recombination and pseudotyping

►CONTROL OF PRODUCTION PROCESS

- ◇ Vector development
- ◇ Cell and virus bank establishment and characterization
- ◇ Final product characterization and lot release
- ◇ Products used in manufacture (Ancillary Products)

►VECTOR DEVELOPMENT AND CHARACTERIZATION

- ◆ Derivation of vector
  - ◇ Source, modifications and function of component parts
  - ◇ Description of regulatory elements
- ◆ Molecular characterization of the final vector
  - ◇ All components of the vector required for its biological function should be verified by molecular methods of analysis
  - ◇ Sequence analysis may be limited to the insert, flanking regions and any regions of the vector which are modified is acceptable for early phases of clinical development

►MASTER VIRUS BANK CHARACTERIZATION

- ◆ History-generated from molecularly cloned and characterized constructs
  - ◇ Identity
    - ✣ Genetic stability and integrity
  - ◇ Potency
    - ✣ Expression of gene product
    - ✣ Biological activity
  - ◇ Safety-freedom from adventitious agents
    - ✣ Bacteria
    - ✣ Fungi
    - ✣ Mycoplasma
    - ✣ Virus, including RCV (Replication Competent Virus)

►VECTOR MODIFICATIONS

- ◆ Previously
  - ◇ Change in vector = New Product = New IND

- ◆ Presently
  - ◇ Related vectors ≠ New Product ≠ New IND
- ◆ Minor Modifications
  - ◇ IND amendment
- ◆ Abbreviated testing-focusing on specific safety concerns
- ◆ Case by case

► MASTER CELL BANK CHARACTERIZATION

- ◆ History
- ◆ Culture and storage conditions
- ◆ Identity testing
  - ◇ Genetic stability and integrity
  - ◇ Protein product
  - ◇ Cellular Morphology/Phenotype
  - ◇ Cellular Isoenzymes
- ◆ Safety testing-freedom from adventitious agents
  - ◇ Bacteria
  - ◇ Fungi
  - ◇ Mycoplasma
  - ◇ Virus, including RCV

► WORKING CELL BANK CHARACTERIZATION

- ◆ Freedom from adventitious agents
- ◆ Limited testing for RCV
- ◆ Limited routine identity testing
- ◆ Validation that aliquots can consistently be used for final product production

► VECTOR-CONTAINING SUPERNATANTS

- ◆ Purity
  - ◇ Residual cellular DNA, RNA, protein
  - ◇ Non-infectious virus (particle/PFU [IU])
  - ◇ Production materials
  - ◇ Endotoxin
- ◆ Potency
  - ◇ Biological activity
  - ◇ Rate and regulation of gene expression
- ◆ Identity
  - ◇ Genetic stability and integrity
- ◆ Safety
  - ◇ Sterility (bacterial and fungal)
  - ◇ Mycoplasma
  - ◇ General Safety
  - ◇ Tests for Adventitious Virus (vector system dependent)

► EX VIVO TRANSDUCED CELLS

- ◆ Description of cell source, isolation and culture conditions
- ◆ Purity
  - ◇ Viability
  - ◇ Endotoxin
- ◆ Potency
  - ◇ Biological activity
  - ◇ Rate and regulation of gene expression
- ◆ Identity
  - ◇ Patient's identity label

- ◇ Phenotypic and genetic markers
- ◆ Safety
  - ◇ Sterility (bacterial and fungal)
  - ◇ Mycoplasma
  - ◇ General Safety
  - ◇ Tests for Production Organisms

► PRODUCT-SPECIFIC SAFETY CONSIDERATIONS

- ◆ Retroviral vectors
  - ◇ Primarily ex vivo gene transfer
- ◆ Adenoviral vectors
  - ◇ Primarily in vivo gene transfer
- ◆ Plasmid vectors
  - ◇ Both ex vivo and in vivo
  - ◇ Often in the presence of liposomes

► RETROVIRAL VECTOR SAFETY CONSIDERATIONS

- ◆ Replication competent retrovirus (RCR)
  - ◇ Can arise from recombination events in retroviral vector packaging cell lines used during production
- ◆ Presence of RCR is a safety concern:
  - ◇ Retroviruses integrate into the genome
  - ◇ Murine retroviruses can pseudotype HIV, resulting in expanded host range
  - ◇ Immunosuppressed monkeys exposed to RCR develop lymphomas within 200 days

► RCR TESTING

- ◆ Testing performed during production and for each lot:
  - ◇ Analysis of cells and supernatant
    - ✣ Master Cell Bank
      - Cells (1% or 108) and supernatant (5%)
    - ✣ Production Lot
    - ✣ Ex vivo transduced cells (preferable to have results before patient administration)
  - ◇ Analysis of cells or supernatant
    - ✣ Working Cell Bank

► RCR ASSAY DESIGN

- ◆ STEP 1: Amplification
  - ◇ Culture cells or supernatant with permissive cell line, e.g., Mus dunni cells for 18-20 days
- ◆ STEP 2: Viral detection
  - ◇ PG4 S+L- focus forming assay
- ◆ Validated alternative assays are acceptable (marker rescue)

► PATIENT MONITORING

- ◆ Perform periodic monitoring for evidence of RCR infection
  - ◇ Assays
    - ✣ Serological assays for evidence of antibody to retroviral envelope protein
    - ✣ Direct assays for viral nucleic acid in peripheral blood leukocytes using PCR
    - ✣ Assays for reverse transcriptase
  - ◇ Frequency
    - ✣ Monthly during treatment
    - ✣ Monthly for the first three months following completion of treatment
    - ✣ Every three months for the remainder of the year following treatment
    - ✣ Yearly thereafter
- ◆ Identification of RCR via direct culture of patient PBL should be attempted
- ◆ Submit written IND safety report
- ◆ Submit results of monitoring in annual progress report

►ADENOVIRAL VECTOR SAFETY CONSIDERATIONS

- ◆ Administration of high doses of adenoviral vector to humans can result in both an acute viral toxicity and a vigorous immune response resulting in inflammation.
- ◆ It has been suggested that viral toxicity may be due to induction of a cytokine cascade. Animal testing of adenoviral vectors has shown that the immune response is CD8+ T cell mediated (CTL response).
- ◆ For some applications, target cells may have a rapid turnover requiring repeat administrations, resulting in potentially greater immune response each time.

►ADENOVIRAL VECTOR SAFETY CONSIDERATIONS

- ◆ Patient dose based on particle to infectious unit ratio
  - ◇ Specification < 100:1 particle: PFU (IU)
- ◆ Detection of replication competent adenovirus
  - ◇ Specification < 1 RCA/ patient dose
- ◆ Demonstration of prior immunity to adenoviruses
- ◆ Consent form should reflect risk of adenovirus infection
- ◆ Shedding of RCA as well as vector should be determined
- ◆ If RCA present, culture and characterize
- ◆ Risks of adenoviral vector administration may differ for different populations (e.g. cystic fibrosis vs cancer patients)

►PLASMID VECTOR SAFETY CONSIDERATIONS

- ◆ Removal of potential contaminants:
  - ◇ Bacterial RNA, Protein and DNA
- ◆ Avoid use of CsCl and EtBr
- ◆ Liposome preparations
  - ◇ Residual solvents used in production
  - ◇ Potential toxicity of liposome preparation

►PRODUCT MANUFACTURE AND CHARACTERIZATION AN EVOLVING PROCESS

- ◆ The method of product manufacture should be appropriate for the investigational phase of the study
  - ◇ cGMP continuum
  - ◇ Consult DEL (301-827-3031) concerning facilities issues
- ◆ The degree of product characterization should be appropriate for the investigational phase of the study
  - ◇ Safety, identity, purity and potency
  - ◇ Phase I
    - ✣ Safety (Product Specific)
  - ◇ Consult Product Reviewer

►COMMENTS AND QUESTIONS

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ANCILLARY PRODUCTS

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- **ANCILLARY PRODUCTS**
  - Definition
  - Regulatory Framework
  - FDA's Concerns and Recommendations
  - Qualification Program
- **DEFINITION**
  - Components used during manufacturing that should not be present in the final product
  - Examples
    - Growth Factors
    - Cytokines
    - Monoclonal Antibodies
    - Cell Separation Devices
    - Media and Media Components
- **REGULATORY FRAMEWORK**
  - As critical components with defined set of specifications
  - As a drug or biologic
  - As a device
- **REGULATORY FRAMEWORK: FDA'S CONCERN**
  - Ancillary products can affect
    - Safety
    - Potency
    - Purity
  - of the final therapeutic product
- **FDA RECOMMENDATIONS**
  - Clinical Grade Material
  - Licensed Product
  - Establish qualification programs and set Specifications
  - Adequate characterization
  - Adherence to GMPs
- **QUALIFICATION PROGRAM**
  - What is an adequate qualification program?
  - Questions to consider
    - What's the ancillary product?
      - animal derived ?
      - purified protein ?
      - cell supernatant ?
    - What's the process used to produce the ancillary product?
- **GENERIC QUALIFICATION PROGRAM**
  - Safety testing
    - adventitious agents
    - sterility
    - endotoxin
    - mycoplasma

- ◆ Activity (functional)
- ◆ Purity (consistency)
- ◆ Verification of residual levels in the final therapeutic product

► ANCILLARY PRODUCTS: PROBLEM

- ◆ Ancillary Product: Growth Factor called "ZIP"
- ◆ Problem
  - ◇ Clinical grade form of ZIP is no longer available
- ◆ FDA recommendation
  - ◇ ?

► CERTIFICATION OF ANCILLARY PRODUCT - ZIP

- ◆ sterility
- ◆ adventitious agents
- ◆ activity (functional)
- ◆ purity (consistency)
- ◆ endotoxin

► MECHANISM FOR CERTIFICATION

- ◆ Obtain a Certificate of Analysis from the manufacturer
- ◆ Sponsor can initiate testing
- ◆ Manufacturer can supply information in a Masterfile which would be cross referenced
- ◆ Combination of the above

► POTENTIAL SOLUTION TO THE PROBLEM

- ◆ Step 1: Set minimum specifications
- ◆ Step 2: Identify suppliers
- ◆ Step 3: Purchase samples
- ◆ Step 4: Verify specifications and initiate additional tests
- ◆ Step 5: Purchase quantities for the trial
- ◆ Step 6: If revial ancillary product, the sponsor should certify the sterility, activity, and purity

► SOURCES OF INFORMATION

- ◆ Federal Register, Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products, October 14, 1993, Vol 58, No. 197, page 53248-53251
- ◆ Joyce L. Frey, Cytokine and Gene Therapy Branch, Division of Application Review and Policy, (301)594-0830

PRECLINICAL SAFETY AND ACTIVITY TESTING OF CELLULAR AND GENE THERAPIES

Anne M. Pilaro, Ph.D.
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FDA/CBER

►DEFINITION OF GENE THERAPY

- ◆Introduction into the human body of genes or cells containing genes foreign to the body for the purposes of prevention, treatment, diagnosis or curing disease

►CANDIDATE DISEASES FOR GENE REPLACEMENT THERAPY

Disease	Target Cells	Gene Defect
thallassemia	erythrocytes	B-hemoglobin
SCID	lymphocytes	ADA deficiency
CGD	monos/neutrophils	cytochrome b
LAD	monos/neutrophils	CD18 B-subunit
Gaucher's	macrophages	glucocerebrocidase
Hunter's	macrophages	iduronate desulfase
cystic fibrosis	respiratory epi	CFTR

►GENE THERAPY PROTOCOLS REVIEWED BY FDA IN 1995

Indication or Disease State	Submissions
cancer	27
cystic fibrosis	3
bone marrow marking	5
AIDS	3
inborn errors of metabolism	5
infectious diseases	2

►TYPES OF VECTORS FOR GENE TRANSDUCTION

- ◆plasmid DNA
- ◆retroviruses
- ◆adenoviruses
- ◆adeno-associated viruses
- ◆others

►METHODS OF GENE INTRODUCTION

- ◆direct administration into host (e.g. tumor, s/c depot)
- ◆transduced somatic cells
- ◆transduced hematopoietic cells

►CONCERNS RELEVANT TO PRECLINICAL SAFETY TESTING OF VECTOR-BASED THERAPIES

- ◆aberrant localization or trafficking
- ◆level and persistence of viral gene expression
- ◆inappropriate expression of gene product
- ◆replication of viral vector
- ◆germ-line transfer of vector gene
- ◆insertional mutagenesis

►

►POTENTIAL SAFETY CONCERNS FOR GENE-MODIFIED SOMATIC CELL THERAPIES

- ◆phenotype/activation state of effector cell population
- ◆aberrant localization or trafficking
- ◆level and persistence of viral gene expression
- ◆inappropriate expression of gene product
- ◆inappropriate immune activation

- ◆ germ-line transfer of vector gene
- ◆ insertional mutagenesis

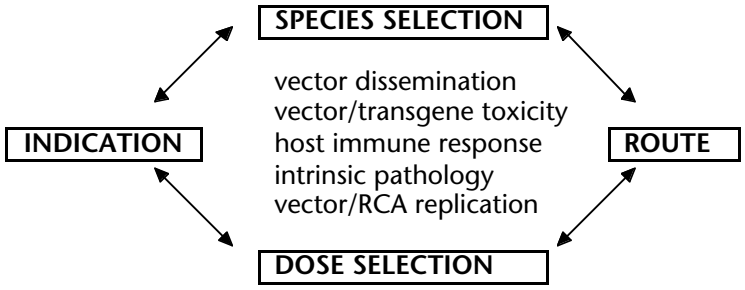
►GOALS OF PRECLINICAL SAFETY EVALUATION

- ◆ For All Therapeutic Products:
- ◆ Recommend initial safe starting dose and safe dose-escalation scheme in humans
- ◆ Identify potential target organ(s) of toxicity
- ◆ Identify appropriate parameters for clinical monitoring
- ◆ Identify "at risk" patient populations (inclusion/exclusion)

►APPROACHES TO PRECLINICAL SAFETY/ACTIVITY STUDIES

- ◆ creative, problem-solving
- ◆ data-driven
- ◆ no one "right" way to conduct studies
- ◆ should be based on best available science, technology to date
- ◆ careful design and judicious use of animals
  - ◇ should allow for early initiation of clinical studies
  - ◇ should allow for uninterrupted clinical development

►CRITICAL PARAMETERS IN PRECLINICAL STUDIES OF GENE THERAPY



►WHEN ARE SPECIFIC IN VIVO SAFETY STUDIES NEEDED?

- ◆ novel therapies, not amenable to in vitro models
- ◆ novel route, schedule of administration
- ◆ potential for aberrant host response, not evaluable in efficacy model

►CASE STUDY- PLASMID VECTOR ENCODING HLA/B7 AND B2MG

- ◆ How can route affect activity/toxicity of gene therapy products?
- ◆ introduction of foreign gene expression activates host immune response
- ◆ intra-tumor expression leads to expression in tumor, other organs but no toxicity
  - ◇ Nabel et al., Human Gene Ther., 3:649-656, 1992
  - ◇ Stewart et al., Human Gene Ther., 3:267-275, 1992
- ◆ "worst-case" scenario - iv injection - uptake/expression in all highly perfused tissues in the absence of organ pathology
  - ◇ Stewart et al., Human Gene Ther., 3:267-275, 1992
- ◆ human data show tumor uptake, expression only, no ADE's reported
  - ◇ Nabel et al., Proc. Natl. Acad. Sci. USA, 90:11307-11311, 1993

►SELECTION OF SPECIES FOR IN VIVO TESTING

- ◆ relevant to biology/mechanism of action
- ◆ relevant to route and method of delivery
- ◆ animal models of disease may provide useful safety information
- ◆ non-human primates are not required for all studies

- alternative models (transgenics, homologous tumors/species, SCID)

►CASE STUDY - REV M10-TRANSDUCED CD4 T CELLS IN HIV INFECTION

- Using alternative animal models to answer specific questions
- In vitro data demonstrate inhibition of HIV replication, strong "proof of concept"
  - Malim et al., J. Exp. Med., 176:1197-1201, 1992.
- question of whether transduction alters tumorigenicity, safety, of CD4 cells in vivo?
  - SCID/hu xenograft with RevM10 cells, no organ pathology, tumors
  - no changes serum chemistries, clinical picture

►CASE STUDY - CUSTOM-DESIGNED RAS OR P53 PEPTIDES

- Using a murine homologue to determine the safety of a therapy
  - no suitable animal model for human mutated ras or p53
  - no safety data on repeated administration of vaccine, vector distribution, or potential for autoimmune response
  - recommended use of mouse homologue prototypes to test safety of:
    - multi-dose administrations
    - trafficking of peptide-pulsed MNC,
    - induction of inappropriate immune activation
    - potential for autoimmune response to irradiated autologous cells
    - histopathology of target organs

►EXAMPLES OF ANIMAL MODELS OF DISEASE USED IN PRECLINICAL EFFICACY/SAFETY EVALUATION

Animal Model	Disease
MRL9/16/96/lpr mice	Lupus
cotton rats , +/- infection	RSV; cystic fibrosis
W/Wv mice	Fanconi's anemia
Wobbler mice	ALS
db/db mice	Diabetes
Streptozocin [mice/rats]	Diabetes
SIV [primates]	HIV
Canine E. coli fibrin clot	Sepsis
Galactosamine [dogs]	Hepatic failure
Woodchuck , chimp hepatitis	HBV infection
MPTP [primate] , 6-OHDA [rat]	Parkinson's disease
Aged primates	Alzheimer's disease
EAE [primates, rodents]	Multiple Sclerosis

►WHEN CAN PRECLINICAL SAFETY STUDIES BE MINIMIZED?

- strong efficacy model
  - rationally designed to answer specific questions
  - optimizing schedule and conditions
- previous human experience
  - similar product, dose, regimen

►CASE STUDY - PBL TRANSDUCTION WITH HAIRPIN RIBOZYME GENE

- How can in vitro data satisfy safety and efficacy requirements?
- In vitro "proof of concept", safety studies
  - in vitro evidence of positive gene transduction, resistance to HIV-1 infection in HeLa, Jurkat, Molt-4 cells
    - Ojwang et al., Proc. Natl. Acad. Sci. USA, 89:10802-10806, 1992
    - Yamada et al., Gene Ther., 1:38-45, 1994
  - in vitro evidence of transduction, resistance in primary PBL
  - no inhibition of cell proliferation, altered phenotype
  - no detection of viral "escape" or resistant clone development in long-term culture
    - Leavitt et al., Human Gene Ther., 5:1115-1120, 1994

►CASE STUDY - RETROVIRAL TRANSDUCTION OF HEMATOPOIETIC STEM CELLS WITH MDR-1 GENE FOR CANCER CHEMOTHERAPY

- ◆How can "efficacy data" satisfy safety requirements?
- ◆In vitro "proof of concept" studies
  - ◇in vitro evidence of positive gene transduction, resistance to taxol in both human, murine stem cells
    - ✣ Hegewisch-Beckeret al., Brit. J. Haematol., 90:1876-883, 1995
    - ✣ Hanania et al., Gene Ther., 2:285-294, 1995
  - ◇no inhibition of cell proliferation, altered phenotype by FACS
  - ◇to 11% increase in Rh123 efflux in transduced human cells by FACS (index of MDR-1 function)

►CASE STUDY - RETROVIRAL TRANSDUCTION OF HEMATOPOIETIC STEM CELLS WITH MDR-1 GENE FOR CANCER CHEMOTHERAPY

- ◆How can "efficacy data" satisfy safety requirements?
- ◆In vivo "efficacy" model
  - ◇murine transplant system ; serial transplants + taxol, 10 mg/kg
  - ◇no formal toxicology testing, HOWEVER, monitored mice:
    - ✣ no adverse effects on engraftment, reconstitution
    - ✣ beneficial effect on survival
    - ✣ conferred resistance to escalating doses of taxol
    - ✣ support from literature that overexpression of MDR-1 had no adverse effect on engraftment, differentiation
      - Mickisch et al., Cancer Res., 51:5417-5424, 1991
      - Mickisch et al., Proc. Natl. Acad. Sci. USA, 88:547-551, 1991

►CASE STUDY - IDS-TRANSDUCED PBL FOR HUNTER'S SYNDROME

- ◆Consideration of available information (e.g. SCID-ADA gene therapy)
  - ◇no relevant animal model
  - ◇strong in vitro "proof of concept" data
    - ✣ Braun et al., Proc. Natl. Acad. Sci. USA., 90:11830-11834, 1993
  - ◇methodology, vector, dosing regimen well characterized
  - ◇little associated toxicities, ADE in humans treated to date
    - ✣ Blaese et al., Human Gene Ther., 4:512-527, 1993

►THE NEXT GENERATION-WHAT DO WE AS REGULATORS NEED TO SEE?

- ◆ first generation vectors/new molecular entities, change in route of administration
  - ◇pharmacologic profiles, dose/response relationship, proof of concept
  - ◇full toxicology profiles, vector distribution
  - ◇focus is on SAFETY; rationale is secondary, but still important
- ◆ vectors - the next generation(s), minor changes in non-control regions
  - ◇abbreviated toxicology; bridging studies
  - ◇abbreviated pharmacology - does it work better? the same?

►CASE STUDY - ADENOVIRAL VECTOR FOR CYSTIC FIBROSIS

- ◆Comparison of first and second generation vectors in a "bridging" study
  - ◇question of biology, immune/inflammatory response to different constructs
  - ◇baboons; first/second generation vectors instilled into contralateral lung lobes
    - ✣ Goldman et al., Human Gene Ther., 6:1839-851, 1995
  - ◇methodology, vector, dosing regimen well characterized gene stability prolonged, inflammation decreased with second generation vector marked decrease in hexon protein levels in tissues receiving second generation vector

►THE NEXT GENERATION-WHAT DO WE AS REGULATORS NEED TO SEE?

- ◆ key points to define
  - ◇NOAEL, toxic doses; target organs, key pathology
  - ◇shape/steepness of the dose/response curve (both pharm & tox)
  - ◇ED, EC50 - may be in vivo or in vitro

- ◇ points to monitor in the clinic

► THE CHALLENGE OF ANIMAL STUDIES

- ◆ How have data from preclinical studies facilitated clinical development of gene therapy?
  - ◇ Example: Using preclinical data to recommend dose escalations for adenoviral vectors for CF dose-escalation studies of CFTR vectors in cotton rats show a 2 to 10-fold difference between NOAEL and threshold doses for toxicity
    - ✣ consistent between 2 different vectors, 2 serotype backbones
    - ✣ similar studies in Rhesus monkeys, baboons show 10-fold difference in dose between NOAEL and threshold toxic doses
    - ✣ clinical data demonstrated first ADR after 100-fold escalation; hypoxia, fever, pneumonia - some evidence of these effects in primate models at high doses
    - ✣ Based upon these data, recommendation to sponsors was to consider dose-escalations in 1/2 log (3-fold) increments between cohorts, and to tighten up stopping rules, patient monitoring to include these events.

► QUESTIONS TO BE ANSWERED BY PRECLINICAL STUDIES

- ◆ What is the relationship of the dose to the biologic activity?
- ◆ What is the relationship of the dose to the toxicity?
- ◆ Does the route and/or schedule affect activity/toxicity?
- ◆ What risks can be identified for the clinical trial?

► THE DILEMMA OF PRECLINICAL STUDIES FOR GENE THERAPIES

- ◆ Reversibility of the toxicity may be missed in in vitro models
- ◆ Adverse events in animals:
  - ◇ may not be relevant to clinical trials
  - ◇ may be acceptable in more seriously ill populations
- ◆ Long-term toxicities or events:
  - ◇ may not be determined in preclinical animal studies
  - ◇ may be missed in phase 1 trials;
  - ◇ may require long-term follow-up

► SUMMARY

- ◆ Preclinical studies for gene therapy should be rational, scientifically-designed, based on best available technology, methods to date
- ◆ Efficacy studies for gene therapy can also provide rationale, safety data for phase 1 trial entry
- ◆ Animal studies are dependent on body of information available, question being asked
- ◆ Maximal information may be obtained in alternative animal models; primates are not a priori necessary
- ◆ Sponsors are encouraged to conduct, publish experiments for further advancement of the field

► FURTHER QUESTIONS? WHEN IN DOUBT, TALK TO US!

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CONSIDERATIONS IN CLINICAL TRIAL DESIGN FOR GENE THERAPY PHASE I TRIALS

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►CLINICAL TRIAL DESIGN

- ◆Scientific rationale
- ◆Objectives (endpoints))
- ◆Experimental design
- ◆Selection of subjects
- ◆Informed consent
- ◆Compliance with therapy
- ◆Assessment of trial results
- ◆Tolerability of therapy
- ◆Data collection
- ◆Statistical analysis
- ◆Monitoring of study

►SPECIFIC ISSUES OF GENE THERAPY TRIALS

- ◆Size of trial (phase 1 OR 1/2)
- ◆Novel product
- ◆Unknown adverse reaction profile
- ◆Unpredicted host response
- ◆Functional lifespan of product
- ◆Informed consent
- ◆Access to therapy

►SIZE OF GENE THERAPY TRIAL

- ◆Safety concerns are paramount
  - ◇preclinical data using animal models or in vitro test data to support safety or efficacy
  - ◇expose fewest number of people to risk
    - ✣ Exploratory concepts
      - limits the scope of hypothesis testing
      - limits allocation of resources
    - ✣ Need for rapid feedback of information
      - technical performance of gene product in vivo
      - if in vivo results do not match expectations, need flexibility to alter vector production or experimental design

►USE OF A NOVEL PRODUCT

- ◆Vectors or gene products may change
- ◆Commonly used vectors contain a variety of different gene products
  - ◇Vectors "evolve" due to ongoing technical progress
  - ◇Natural gene product may be modified as a fusion protein or may contain point mutations
  - ◇Gene products using consensus sequences reduce genetic polymorphism, but do not represent naturally occurring products

►UNKNOWN ADVERSE REACTION PROFILE

- ◆Special concerns about adverse reactions
- ◆Lack of long-term safety data from historical controls
  - ◇Potential for atypical presentation of adverse reaction prior to its recognition
  - ◇Unknown frequency of adverse reaction secondary to genetic manipulation (RCR, insertional mutagenesis, gene migration, etc.)
  - ◇Do some vectors have a higher incidence of adverse reactions?
  - ◇Unknown reversibility of adverse reaction (unlike drug toxicity)

- ◇ Interaction of patient medications with vector (e.g., retroviral vectors)
- ◇ Duration of safety monitoring required, due to chronic nature of genetic defect
- ◇ Cost of monitoring for adverse reactions

► UNPREDICTABLE HOST RESPONSE

- ◆ How effective is the route of vector delivery (direct versus indirect inoculation)?
- ◆ After delivery, does the vector localize and remain in the desired site?
- ◆ Does gene transcription occur (use of vector-only control population)?
- ◆ Does the gene product have the expected functional activity?
- ◆ What is the host immune response to gene therapy?
  - ◇ autologous cells manipulated ex vivo
  - ◇ expression of viral products on cells
  - ◇ reactivity to antigen depots (plasmid DNA)

► FUNCTIONAL LIFESPAN OF PRODUCT

- ◆ Sparse data on kinetics of clearance or marked cells
  - ◇ Immune clearance
  - ◇ ADCC
  - ◇ antigen-specific cytolysis
- ◆ Sequestration (deficient homing)
- ◆ Cell types (e.g., lymphocyte, fibroblast, stem cell)
- ◆ Host resistance
- ◆ Ablation (suicide gene)

► INFORMED CONSENT

- ◆ Intent of trial
- ◆ Risks (known or potential)
- ◆ Proposed benefit
- ◆ Duration and methodology of therapy
- ◆ Duration and methodology of follow-up
- ◆ Provision for autopsy
- ◆ "Recycling" of study subjects

► ACCESS TO THERAPY

- ◆ Need well-defined patient population to offset small sample size
- ◆ Gender balance
- ◆ Patient drop-out and cross-over
- ◆ Commitment to long-term monitoring

INTERNATIONAL REGULATORY CONSIDERATIONS, EXPORT AND IMPORT ISSUES

Elaine C. Esber, M.D.

► REGULATORY CONSIDERATIONS

- ◆ Export of Gene Therapy Products
- ◆ Import of Gene Therapy Products
- ◆ Communication with Foreign Government Officials
- ◆ Memoranda of Understanding (MOU)/Memoranda of Agreement (MRA)
  - ◇ MOUs enlist aid of counterpart governments in assuring imports meet our requirements, e.g., GMPs, GLPs
  - ◇ MRAs generally mean either reliance upon one another's conformity assessment system or, where such reliance is not practicable, exchange of the results of conformity assessments

► EXPORT OF GENE THERAPY PRODUCTS

- ◆ Products approved (licensed) in the US fully meeting FDA requirements
  - ◇ Must meet requirements of importing country
  - ◇ No restrictions or controls to exportation
- ◆ Products that are unapproved in the US
  - ◇ Can be shipped under IND regulations (21 CFR 312), or
  - ◇ Subject to the Drug Export Act Amendments of 1986 and 1996

► DRUG EXPORT ACT AMENDMENTS OF 1986 AND 1996

- ◆ Drug Export Act Amendments of 1986
  - ◇ Pub L. 99-660; November 14, 1986
  - ◇ Created New Section 802; three tracks
- ◆ FDA Export Reform and Enhancement Act of 1996
  - ◇ Pub L. 104-134; signed by President Clinton April 26, 1996
  - ◇ Revises Sec. 801 and 802 of FD&C Act and Subsection (h) of Sec. 351 of PHS Act

► FDA EXPORT REFORM AND ENHANCEMENT ACT OF 1996

- ◆ Exports of products for investigational use in importing country
  - ◇ Exempted from regulation by FDA if shipped to one of 25 countries listed [§ 802 ©]
    - ✣ Australia, Canada, Israel, Japan, New Zealand, Switzerland, and South Africa, or any country "in the European Union or a country in the European Economic Area"
  - ◇ FDA approval of export required for shipment of investigational product to unlisted countries (21 CFR 312.110)
  - ◇ Review includes scientific review of protocol, safety data and a letter from importing country approving import
- ◆ Export of products for commercial use in importing country
  - ◇ Authorizes shipment to "listed country"
  - ◇ Authorizes exportation to "unlisted country" if approved by "listed country"
  - ◇ Eliminates need for FDA approval for export; replaces with FDA notification
  - ◇ Eliminates need for investigations in US
  - ◇ Others
- ◆ FDA may restrict exports, if:
  - ◇ Not manufactured in GMP facility
  - ◇ Adulterated
  - ◇ Not meeting basic export requirements
  - ◇ Imminent hazard
  - ◇ Inadequate labeling
- ◆ Imports for Export [§ 801 (d)(3)]
  - ◇ Statutory requirement is recordkeeping
- ◆ Stages in Manufacture
  - ◇ Final Drug Product

- ◇ Bulk - Drug Substance
- ◇ Mixture]
- ◇ Partially Processed
- ◇ Raw Material
- ◇ Partially processed biologics [§351(h)] may be exported if:
  - ✣ not in a form applicable to prevent or treat disease
  - ✣ not intended for sale in the US
  - ✣ is intended for further manufacture into final dosage form outside the US
  - ✣ must be manufactured using GMPs and meet basic export requirements

◆ Still undergoing interpretation

◆ Regulations are being considered for clarification

◆ Agency encourages questions if unclear

►IMPORT OF GENE THERAPY PRODUCTS INTO US FOR HUMAN USE

◆ Investigational New Drug Regulations apply [21 CFR 312.1] if a biologic/drug

◆ Investigational Device Exemption Regulations apply to device components [21 CFR 812]

◆ Marketing applications will depend on product - BLA, NDA, or PMA

►COMMUNICATIONS WITH FOREIGN GOVERNMENT OFFICIALS

◆ Formal relationships with governments:
- ◇ ICH with EU and Japan
- ◇ Bilateral relations, e.g., Tripartite, Trilateral
- ◇ WHO

◆ Formal communication with government officials, e.g.
- ◇ Information exchange of public information
- ◇ Harmonization

►JOINT REVIEWS

◆ Regulations codified in 1993 and 1996 to enable information exchange while maintaining confidentiality [21 CFR 20.89]

◆ Applicable to exchange of any document , e.g., draft proposed rules and predecisional documents; excludes trade secret information concerning manufacturing methods and controls

◆ Foreign government agency signs statement that:
- ◇ affirms its ability to maintain confidentiality
- ◇ commits not to disclose

◆ FDA determines either:
- ◇ sponsor of product has provided written authorization, or
- ◇ disclosure would be in the interest of public health, or

◆ disclosure is to a foreign visiting scientist who must sign confidentiality commitment

OVERVIEW OF FUNDING MECHANISMS AVAILABLE FROM THE NIH FOR THE SUPPORT GENE THERAPY PROJECTS

Craig Reynolds, Ph. D.
NCI/FCRDC

►MECHANISMS AVAILABLE FROM THE NIH FOR THE SUPPORT OF GENE THERAPY PROJECTS

- ◆Preclinical Basic Research
- ◆Clinical Trials
- ◆Production
- ◆Other

►PRECLINICAL BASIC RESEARCH SUPPORT

- ◆R01 - research grants
- ◆R03 - small research grants (RFA only)
- ◆R41/R42 - Small business technology transfer (STTR) grants
- ◆R43/R44 - small business innovation research (SBIR) grants
- ◆P01 - program project grant
- ◆P30 - core center grant (NIDDK)
- ◆P50 - specialized centers of research
- ◆U19 - research program cooperative agreement (RFA) only
- ◆U43/U44 - SBIR cooperative agreement

►CLINICAL TRIALS SUPPORT

- ◆R01 - research grant
- ◆R03 - small research grant (RFA only)
- ◆R21 - exploratory/developmental grant
- ◆P01 - program project grant
- ◆U01-U10 - cooperative agreement clinical research

►PRODUCTION SUPPORT

- ◆NGVL - National Gene Vector Laboratories (NCRR)
- ◆MARP - MoAb/Rec. Protein Production Facility (NCI)

►OTHER SUPPORT

- ◆Toxicology (nonprimate/primate)
- ◆Pharmacology and formulation
- ◆Preclinical animal models

TECHNOLOGY TRANSFER (OTD/OTT)FUNDING MECHANISMS FOR NCI SPONSORED GENE THERAPY RESEARCH

Diane Bronzert
Cancer Therapy Evaluation Program, Division of Cancer Treatment Diagnosis and Centers, National Cancer Institute, NIH, Bethesda, MD (Tel: 301-496-8866)

The National Cancer Institute (NCI) has a diverse program in gene therapy with approximately $28 million in extramural research project grants (RPG) awards for fiscal year 1995. The principal mechanisms used for funding gene therapy throughout NIH are investigator initiated research grants (RO1, R29), Small Business Innovation and Technology Transfer grants (SBIR, STTR), and program project grants (P01). Program project grants have proven to be a very effective mechanism to fund the multi-disciplinary efforts that are necessary to translate basic research discoveries to the clinic. Areas of special interest include the development of new delivery systems, animal models, and new therapeutic approaches. In addition , NCI has published Program Announcements using the small grant (R03) and exploratory grant (R21) mechanisms for the support of therapeutic clinical trials. The primary categories for oncology gene therapy studies are the following: (1) new vaccines or studies to increase/create immunity to cancer cells (e.g., production of cytokines); (2) studies to specifically kill or prevent proliferation of cancer (e.g., suicide genes, prodrugs, antisense); or (3) studies to increase the dose of chemotherapy agents administered to patients by protecting the hematopoietic system.

NIAID SUPPORT FOR HUMAN GENE THERAPY

Nava Sarver, Ph.D.
Division of AIDS, NIAID

For HIV induced disease and immune dysregulation, gene-based therapies can potentially be used with other current therapies to further enhance the therapeutic benefit to patients. Gene therapy approaches for treating HIV are designed to enhance immune function or to block specific functions critical to viral replication, thereby limiting or curtailing virus dissemination. HIV gene therapy approaches involve the introduction of therapeutic or protective genes into lymphocytes (CD4; CD8 CTL) or their precursor stem cells (CD34; others?), including those mobilized to the peripheral blood and those derived from cord blood. Antiviral genes include those encoding for (1) proteins (transdominant mutants, single-chain antibodies, toxin genes); (2) RNAs (ribozymes; RNA decoys; antisense); and (3) cytokines or regulatory factors (IFN; eIF-5A). For a therapeutic outcome, these strategies require efficient delivery of genes to target cells, homing to target cells, long term expression, localization to the proper cellular compartment, and fulfillment of other parameters common to most other human gene therapies.

The NIAID supports studies in gene-based therapies to address identified problem areas and to improve efficiency and predictable use in clinical setting. NIAID's Division of AIDS (DAIDS) evaluates gene therapy as a molecular-based approach to add to the existing arsenal of therapies to treat HIV disease antiviral drugs, adoptive immune therapies, cytokine therapy). Among these support programs are: (I) investigator initiated R01-type awards (R01, R29, R37, others); (2) the National Cooperative Drug Discovery groups for the Treatment of HIV Infection (NCDDG-HIV) which focuses on preclinical studies); (3) the Strategic Program for Innovative research on AIDS (SPIRAT) which focuses on translational research from advanced preclinical to pilot clinical studies; (4) Small Business Innovative Research grants (SBIR) and Small Technology Transfer Research grants (STTR). Research directions encouraged under these programs, eligibility, and scope will be discussed.

Nava Sarver, Ph.D.
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Division of AIDS, NIAID

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NATIONAL HEART, LUNG, AND BLOOD INSTITUTE: SUPPORT FOR GENE THERAPY RESEARCH

Sonia I. Skarlatos, Ph.D.
NIH, NHLBI

In 1992, the National Heart, Lung, and Blood Institute (NHLBI) convened a "Working Group on Gene Therapy Approaches and Resources for Heart, Lung and Blood Research" to provide a framework for the scientific evolution of human gene therapy in a suitable and systematic manner. The Working Group articulated scientific needs and identified broad avenues for the NHLBI to foster gene therapy. Strategies to facilitate the development of human gene therapy included: (1) encouragement of research efforts for the basic tasks involved in gene therapy; (2) development of animal models; (3) stimulation of collaboration among scientists from different disciplines; (4) establishment of shared support facilities; and (5) recruitment of established investigators and training of new investigators. Using the Working Group report as a guide, the NHLBI presently supports and will continue to support a diverse array of research activities to advance gene therapy of heart, lung and blood diseases.

The NHLBI currently supports six clinical studies. Three are for cystic fibrosis, which will test the clinical efficacy and safety of adenoviral vectors, adeno-associated vectors and liposomes. The other three are for the treatment of ADA deficiency with ADA-transformed peripheral blood stem cells, for gene therapy of Gaucher's disease with hematopoietic stem cells transformed to express glucocerebrosidase, and for gene therapy of Fanconi's anemia type C.

The NHLBI will be releasing, shortly, a request for applications (RFA) on "Fundamental Biological Principles for Gene Transfer for Cardiovascular, Pulmonary and Hematologic Diseases". This RFA invites Program Project grant applications for support of research efforts to advance gene transfer technology and its potential application to cardiovascular, pulmonary and hematologic diseases.

In addition, the NHLBI, through a program announcement on "Gene Therapy Vector Design and Development", will be encouraging collaboration between academia and industry to design and develop vectors and delivery systems for cardiovascular, pulmonary and hematologic diseases. Support for this program will be provided by the Small Business Technology Transfer Program.

For further information on any of these programs, please contact:

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NIH, NHLBI
Division of Heart and Vascular Diseases
Two Rockledge Center, Suite 10186
6701 Rockledge Drive, MSC 7956
Bethesda, MD 20892-7956
Phone: 301-435-0550
FAX: 301-480-2858
E-Mail: skarlats@gwgate.nhlbi.nih.gov

NIDDK SUPPORT FOR GENE THERAPY RESEARCH

Catherine McKeon, Ph.D.
Metabolic Diseases and Gene Therapy Research Program, National Institutes of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892 (Tel: 301-594-8810)

The NIDDK supports research in diabetes, endocrinology, metabolic diseases, nutrition, kidney, digestive, urologic and hematologic diseases. NIDDK support for gene therapy research has been predominantly to develop treatments for genetic metabolic and hematologic diseases, however, other applications of interest to NIDDK can be envisioned as the technology develops. Most of our support for gene therapy research is through Investigator Initiated research grants (RO1). In addition, the NIDDK supports program projects (P01), Core Centers for Gene Therapy Research on Cystic Fibrosis and Other genetic Disease (P30), Centers of Excellence in Molecular Hematology (P50), and co-sponsors the National Gene Vector Laboratories. Approximately $40 million was spent on basic and applied gene therapy research in 1995.

Other mechanisms for funding of gene therapy research are the Small Business Innovation Research Grant (SBIR) and the Small Business Technology Transfer Grant (STTR). The NIH is Congressionally mandated to expend 2.5% of it's budget on the STTR program. These programs are designed to promote collaborations between academic scientists and small business concerns to develop and market new technologies. Because of the unique collaboration between academic researchers and industry in the field of gene therapy, this mechanism should be considered. One example of a commercial product developed with NIDDK funds provided through the SBIR program is the liposome component, Lipofectamine. Special emphasis topics published by NIDDK include improving vector production methods; development of new and improved vectors; development of packaging lines with new or altered viral envelope proteins; and engineering tissue specific promoters which provide stable expression.

THE NATIONAL GENE VECTOR LABORATORIES

Richard A. Knazek, M.D., Ph.D.
Clinical Research, NCRR, NIH

ABSTRACT

The National Gene Vector Laboratories (NGVL) provide shared resources to facilitate the production of clinical grade adenoviral, DNA plasmid and retroviral vectors for use by eligible U.S. investigators in Phase I or II experimental human gene therapy protocols. This is an NIH resource, with laboratories located at Indiana University, University of Michigan and University of Pennsylvania, that produces vectors under GMP conditions and provides them, at no cost, to the requesting investigator. The NGVL is supported by cooperative agreement awards from the National Center for Research Resources, co-sponsored by the National Cancer Institute, National Institutes of Diabetes and Digestive and Kidney Diseases, and National Heart, Lung and Blood Institute, and with co-funding from the Office of AIDS Research.

Applications received by the coordinating NGVL must contain detailed descriptions of both the requested vector and the clinical protocol in which it is to be used. Such vectors must have completed pre-clinical testing. Proposals will be evaluated by an external Scientific Review Board and the Steering Committee of the NGVL with the ultimate selection being based upon scientific merit, feasibility and availability of NGVL resources. Priority for vector production will be given to protocols that have received peer-reviewed grant support. Preference will be given to NIH-sponsored research.

Application packets can be obtained from:

Kenneth Cornetta, M.D.
NGVL Coordinating Center
Indiana University
IB442, 975 West Walnut Street
Indianapolis, Indiana 46202-5121
Tel: 317-274-0843
Fax: 317-274-0396
e-mail: Ken_cornetta@iucc.iupui.edu

Richard A. Knazek, M.D.
Clinical Research
National Center for Research Resources/NIH
6705 Rockledge Drive, Room 6030
Bethesda, Maryland 20892-7965
Tel: 301-435-0790
Fax: 301-480-3661
e-mail: Richardk@ep.ncrr.nih.gov

The next deadline for submission of applications is September 3, 1996.

►NATIONAL GENE VECTOR LABORATORIES
- ◆An interactive group of laboratories whose purpose is to provide clinical grade adenoviral, DNA plasmid, and retroviral vectors to eligible clinical investigators for gene therapy protocols.

►DISEASES TARGETED BY GENE THERAPY PROTOCOLS

►APPROVED BY THE RAC
- ◆Rheumatoid arthritis
- ◆HIV
- ◆Storage Diseases
- ◆Cystic Fibrosis
- ◆Monogenic Diseases
- ◆Arterial Diseases
- ◆Cancer

►FIRST ONE-FOURTH OF PROTOCOLS REVIEWED BY THE RAC
- ◆Adenovirus
- ◆Non-Virus
- ◆Retrovirus

►LAST ONE-FOURTH OF PROTOCOLS REVIEWED BY RAC
- ◆Adenovirus
- ◆AAV
- ◆Non-Virus
- ◆Retrovirus

►VECTOR MILESTONES
- ◆Basic Research
- ◆Initial Vector Generation
- ◆Preclinical Studies
- ◆Safety Studies
- ◆Large-Scale Vector Production
- ◆Pharmacology/Toxicology Testing
- ◆Patient Studies

►NATIONAL GENE VECTOR LABORATORIES
- ◆Indiana University-Coordinating Center Retroviral Vectors
- ◆University of Pennsylvania Adenoviral Vectors
- ◆University of Michigan Non-viral Vectors

►ELIGIBILITY REQUIREMENTS
- ◆Qualified Investigators
- ◆Qualified Domestic Institutions or Government Agencies

- ◆ Capability for Both Clinical Research and Scientific Support
- ◆ Submit Request to coordinating NGVL
- ◆ Follow Guideline of Oversight Committees
- ◆ IRB approval and initial discussions with FDA
- ◆ Agree to Post-distribution monitoring

► STEERING COMMITTEE

- ◆ Directors and Associate NGVL Directors
  - ◇ Indiana University
  - ◇ University of Pennsylvania
  - ◇ University of Michigan
- ◆ Outside Experts
  - ◇ University of Chicago
  - ◇ Salk Institute
  - ◇ St. Jude Children's Research Hospital
  - ◇ University of Pittsburgh
- ◆ NIH Representatives
  - ◇ NCRR
  - ◇ NHLBI
    NIDDK
    NCI

► SCIENTIFIC REVIEW BOARD

- ◆ Scientists with expertise in gene therapy and relevant fields

► COMPETITIVE REVIEW

- ◆ Pre-clinical laboratory and animal data
- ◆ Current status of the proposed vector
- ◆ Suitability and technical feasibility of the proposed gene therapy
- ◆ Clinical Protocol Design
- ◆ Qualifications of the Investigator
- ◆ Qualifications of the Laboratory
- ◆ Qualifications of the Clinical Facility
- ◆ Ethical Implications
- ◆ Resources Required
- ◆ Likelihood of Success relative to other clinical trials and other gene therapy approaches

► APPLICATION

- ◆ Letter of Agreement
- ◆ Abstract
- ◆ Biographical Sketches of Investigators
- ◆ Resources
- ◆ Summary of Proposal
- ◆ Clinical Protocol with Consent Form
- ◆ IRB and IBC Approval Letters
- ◆ Sequence Data (if available)
- ◆ Toxicology Data (if available)
- ◆ Relevant Publications
- ◆ Letters of Collaboration
- ◆ FDA Contact Information
- ◆ Vector Distribution Agreement

► **"REQUEST FOR VECTOR PROCEDURE"**

- ◆ Submit application to coordinating NGVL
- ◆ Initial Review by 2 outside reviewers and 1 SC Member
- ◆ Reviewers request additional information through the coordinating NGVL
- ◆ Reviews returned to coordinating NGVL for distribution to the Steering Committee Members
- ◆ Availability of NGVL resources determined
- ◆ Notification of Applicant

►**UPON APPROVAL OF 'REQUEST FOR VECTOR'**

- ◆ Biological Materials are submitted to NGVL
- ◆ Batch of vector is generated at NGVL
- ◆ Samples of batch are returned to investigator
  - ◇ Safety Testing
  - ◇ Toxicology Studies
- ◆ upon final approvals from regulatory groups clinical vector is shipped to investigator

►**DEADLINES FOR SUBMISSION OF 'REQUESTS FOR VECTOR'**

- ◆ September 3, 1996
- ◆ April 7, 1997
- ◆ September 8, 1997

Kenneth Cornetta, M.D.
NGVL Coordinating Center
Indiana University School of Medicine
IB442, 975 West Walnut Street
Indianapolis, Indiana 46202
tel. (317) 274-0448

NCI SPONSORED SMALL SCALE MANUFACTURE OF GENE THERAPY PRODUCTS

George A. Robertson, Ph.D.

The Monoclonal Antibody/Recombinant Protein Production Facility (MARP) provides biopharmaceutical manufacturing support for NIH-supported investigators. Biologics are manufactured under FDA Good Manufacturing Practices (GMP) for Phase I/II human clinical trial, or advanced preclinical animal testing.

The MARP is operated under contract by Scientific Applications International Corporation (SAIC), which provides operations and technical support to the Frederick Cancer Research and Development Center for the NCI. The NCI Project Officer of the MARP is the Chief of the Biological Resources Branch of the National Cancer Institute.

A newly renovated production facility, located in building 459, has just been occupied by the MARP. This facility contains laboratories capable of producing cell banks, monoclonal antibodies using hollow-fiber bioreactors, complete biochemistry laboratories for product purification and characterization, aseptic areas for purification and vialing and a BL3-designed production suite equipped to support animal cell and microbial propagation.

Projects for gene therapy and/or DNA based therapeutics can be products in one of several areas, depending on the level of biocontainment required. Infectious agents and those requiring BL3 containment can be produced in the bldg. 459 containment laboratory, on a campaign basis. Packaging cell banks can be prepared in the animal cell culture laboratories and characterized in house or by utilizing contract testing laboratories. DNA can be fermented in one of several laboratories, depending on quantity and containment requirements. Aseptic purification and vialing can be conducted in the aseptic production suite in bldg. 459. Critical spaces include three class 100 rooms.

The MARP staff, in conjunction with the Quality Assurance group, is able to produce the Chemistry, Manufacturing and Control (CMC) section of INDs as a regular part of every project destined for the clinic.

For further information, contact Dr. Stephen Creekmore, Biological Resources Branch, DTP, DCTDC, NCI-FCRDC, Frederick, MD 21701-1201, (tel) 301-846-1098, (fax) 301-846-5429, (e-mail) Creekmor@ncifcrf.gov.

CURRENT AND SPECIAL NIH RESOURCES FOR THE DEVELOPMENT OF GENE THERAPY PRODUCTS

Elizabeth C. Lovoy, J. D., M. P. H.
Office of Technology Development, NCI
(301) 496-0477

ABSTRACT

Among the traditional sources of funding for NIH scientists are: funds appropriated by Congress; royalties received from the licensing of NIH patents (the use of which was previously restricted to "technology transfer-related" activities, and more recently made available for "mission-related" activities); gift funds received from the private sector; and funds received from biotechnology and pharmaceutical industry collaborators under Cooperative Research and Development Agreements (CRADAs). The NCI Office of Technology Development (OTD) provides an array of services to NCI scientists, helping them to combine and leverage the resources of both the NCI and the private sector to speed the development and commercialization of technology arising in NCI's laboratories for the overall benefit of the public health. One of the major services offered by the OTD is the negotiation of transactional agreements between the NCI and outside parties (including universities, research institutes, and pharmaceutical and biotechnology companies) which allow resources to flow both to and from the NCI. These transactional agreements enable the exchange of research materials under Material Transfer Agreements (MTAs); the provision of experimental drugs and biologics to the NCI under Clinical Trail Agreements (CTAs); and the contribution of staff, facilities, equipment, supplies and intellectual property (as well as funds to the NCI, but not to the collaborator) under CRADAs. In addition, there are an array of "hybrid" agreements (such as MTA-CRADAs) which can extend NCI scientists' access to various companies' propriety materials and other technology for use in gene therapy, as well as other research. The objective of these agreements and the manner in which they are negotiated by the NCI OTD are to foster partnerships between NCI scientists and outside institutions, and to do so as expeditiously and as easily as possible. Each of these agreements offers unique opportunities to NCI scientists to establish such partnerships and to broaden their access to necessary resources, including in some cases, funding. Any NCI scientist who wishes to explore these avenues should contact the NCI Office of Technology Development an (301) 496-0477. In addition, information on these opportunities may be obtained from our Website at http://www.nci.nih.gov/hpage/ttrans.htm

►TRADITIONAL SOURCES OF FUNDING FOR NIH SCIENTISTS

- ◆Funds appropriated by Congress
- ◆Royalties from the licensing of NIH patents
- ◆Gift funds from the private sector
  - ◇Funds received from biotechnology and pharmaceutical industry collaborators under a Cooperative Research and Development Agreement (CRADA)

►FORMAL COLLABORATION

- ◆Material Transfer Agreements (MTAs)
- ◆Clinical Trial Agreements (CTAs)

- ◆ Cooperative Research and Development Agreements (CRADAs)
- ◆ Confidentiality Agreements (CDAs)

► MATERIAL TRANSFER AGREEMENT (MTA)

- ◆ Provides for the transfer of research material
- ◆ Research purposes only—not for commercial purposes such as screening, production or sale
- ◆ Material may not be used in human subjects
- ◆ No option or grant of future intellectual property rights.
- ◆ No funds may be received by PHS
- ◆ UBMTA (Uniform Material Transfer Agreement) has been developed to expedite transfer of research materials among non-commercial entities

►

► CLINICAL TRIAL AGREEMENT (CTA)

- ◆ Similar to an MTA but provides for the transfer of material for use in human subjects under an approved clinical protocol
- ◆ No option or grant of future intellectual property rights
- ◆ No funds may be received by PHS
- ◆ Companies may provide a "Guest Researcher" to NCI. Guest Research is paid directly by the company.

► COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

- ◆ Agreement between one or more federal laboratories and one or more non-federal parties
- ◆ Federal laboratories may provide personnel, services, facilities, equipment (but not funds)
- ◆ Collaborator may provide funds, personnel, services, facilities, equipment
- ◆ Areas of research may include basic research, pre-clinical studies, clinical research, and/or any miscellaneous areas of interest to the NCI and the Collaborator

► CONFIDENTIALITY AGREEMENT

- ◆ Used to transfer information (data) between parties
- ◆ Restrict the disclosure of information to third parties
- ◆ Prohibit commercial use of any information shared
- ◆ Define the boundaries as to what is confidential information
- ◆ require that confidential information be clearly indicated "CONFIDENTIAL"
- ◆ Limited duration of confidentiality

► CRADA DEVELOPMENT

- ◆ CRADAs can develop from
  - ◇ A collaborative research project or
- ◆ Soliciting CRADA proposals in the Federal Register and followed up by marketing through trade journals or direct company contact

► CRADA

- ◆ For the NIH the CRADA:
  - ◇ Makes private sector resources (reagents or drugs, facilities, expertise, personnel and funds) available for research, development, or clinical testing
  - ◇ Acts as a Strategic Business Plan for product development or commercialization which adds value to NIH science, technology, or inventions
- ◆ For the private sector the CRADA:
  - ◇ Makes government resources (facilities, intellectual property and expertise) available for research, development or clinical testing
  - ◇ Addresses present and future intellectual property rights
  - ◇ Allows an option to negotiate and exercise non-exclusive or exclusive licensing rights

►CRADA

- ◆Both parties must make an intellectual contribution to the collaboration
- ◆Confidentiality obligations are provided
- ◆Exclusive access to NCI-generated data
- ◆An option to exclusively license NCI or joint inventions arising from the collaboration
- ◆NCI may receive funding from the collaborator to support CRADA research
- ◆Extensive review process

►CRADA LETTER OF INTENT (LOI)

- ◆Permits collaborative research to begin prior to the final approval and execution of the CRADA
- ◆When the CRADA is signed, the effective date of the CRADA is retroactive to the date of the LOI for purposes of confidentiality of information exchanged between the parties and for purposes of intellectual property developed between the date of execution of the LOI and the date of execution of the formal CRADA
- ◆Effective for one year—may be extended for an additional year if the collaboration is still active and if the CRADA is still under negotiation

►PHS CRADA POLICY

- ◆CRADAs are authorized when the collaborator will make significant intellectual contributions to the research project, or will contribute essential research materials or technical resources, not otherwise reasonably available
- ◆Outside organizations must have fair access to the collaborative opportunities. Special consideration is given to small business and preference to those that are located in the U.S. and agree to manufacture in the U.S. products developed under the CRADA
- ◆CRADAs are not intended to be a general funding mechanism to support research in a PHS laboratory

►CRADA FUNDS CAN BE USED FOR:

- ◆Supplies
- ◆Equipment
- ◆Travel Expenses
- ◆Salary for Non-FTE personnel
- ◆Contractor Services

►CRADA DEVELOPMENT AND APPROVAL PROCESS

- ◆A written CRADA should be developed as soon as the federal scientist and their counterpart(s) negotiate the research plan (written description of the research and development project, including each party's contribution to the planned research and development
- ◆The CRADA process consists of two stages. The first step is a "Drafting and Negotiation" stage where a "Draft Agreement" is created
- ◆The second stage of the CRADA process the "Approval" stage

►HYBRID AGREEMENTS AND OTHERS

- ◆MTA-CRADAs
- ◆CTA-CRADAs
- ◆Letter of Intent (CRADAs)

►MATERIAL TRANSFER CRADA (MTA-CRADA)

- ◆Form of CRADA in which PHS may obtain a unique research resource for use in the conduct of specified research which is consistent with the missions of the laboratory
- ◆Thirteen month term—includes a 30 day period for PHS to disapprove of the CRADA in which case it is returned for review by the NIH CRADA Subcommittee
- ◆No funds may be received by PHS under the MTA-CRADA
- ◆Streamlined approval process

► **CLINICAL TRIAL CRADA**

- ◆ CRADA with additional terms appropriate for collaboration involving human clinical studies
- ◆ As with any CRADA, the parties have certain rights to CRADA subject inventions. The federal laboratory may accept funds
- ◆ Exclusive access by the collaborator to the clinical data and to the Investigational New Drug Application may be granted

► **LETTER OF INTENT (LOI) FOR CRADA**

- ◆ Can be used to permit collaborative research to begin pending final approval and execution of the CRADA
- ◆ Patentable inventions may be made by NCI employees and employees of the collaborator in the course of this joint research and confidential information exchanged
- ◆ NCI agrees that should a CRADA be approved and executed, it will be retroactive to the date of the Letter of Intent
- ◆ The Letter of Intent is not an irrevocable commitment on the part of NCI to enter into a CRADA with the collaborator

► **SUMMARY**

- ◆ The NCI has a variety of tools available to foster collaboration and facilitate protection and transfer of information and technology

ELECTRONIC ACTIVITIES AND REGISTRIES UNDER THE FDA SMART PROGRAM

Mary A. Buesing M.D.
CBER SMART Project Officer

►AGENDA

- ◆ Two main focuses in 1996:
  - ◇ Workflow and document management
    - ✣ RMS IND Internal Regulatory Correspondence
    - ✣ Executive Document Exchange
  - ◇ Regulatory Management System: the targeted integrated information system (electronic database)
- ◆ Ongoing efforts in:
  - ◇ CHAPEL
  - ◇ Lot release
  - ◇ Gene Therapy Information network

►IND INTERNAL REGULATORY CORRESPONDENCE (ICOM)

- ◆ Scope
  - ◇ Generate, store and retrieve internally generated documents such as meeting minutes, telecons, review comments, letters to sponsors
- ◆ Link internally generated documents to specific submissions
- ◆ Phase 1: Provide ad hoc routing for some key documents

►ICOM - PURPOSE

- ◆ Enable CBER staff to perform better reviews
  - ◇ Manage documents critical to IND review process
    - ✣ Generate and retrieve internally generated documents
    - ✣ Link related documents
    - ✣ Query data pertinent to the review
- ◆ Enable CBER to perform faster reviews
  - ◇ Route documents quickly
  - ◇ Track review status
    User's In-Box Letter Generation

►ICOM - IMPLEMENTATION

- ◆ Conduct user acceptance testing with representative users
- ◆ Deliver to 75 users this year

►RMS: EXECUTIVE DOCUMENT EXCHANGE

- ◆ Scope
  - ◇ Route correspondence: response to congressional inguiries, points to consider, federal register notices, all guidelines, ICH/GRP docs
- ◆ Between the Center Director, Office Directors, and Division Directors
- ◆ Create, import, route, comment upon, and consolidate comments
- ◆ Champion : Mark Elengold, Dir. Office of Communication, Training and Manufacture's Assistance

►EDE - PURPOSE

- ◆ Enable CBER management to review and respond quickly to high priority requests
  - ◇ Replace "Pony Express" system
  - ◇ Route and comment upon documents
  - ◇ Improve current search capabilities
  - ◇ Utilize advanced tools to facilitate rapid response
- ◆ Conduct acceptance testing with 3 representative users
- ◆ Deliver to 35 users this year

► **COMMON CAPABILITIES FOR ICOM & EDE**
- ◆ Document Management
- ◆ Routing
- ◆ Organization and Navigation
- ◆ Viewing and Modifications

► **STATUS TRACKING**

► **RMS - ANNOTATIONS WITH RE:MARK LOT RELEASE IMAGING SYSTEM (LRIS) LRIS PURPOSE**
- ◆ Allow parallel reviews of Lot protocols
- ◆ Provide Guidance to Industry on submissions of Lot Release Data electronically

► **INTEGRATE COMMERCIALLY AVAILABLE SOFTWARE (ACROBAT) TO SUPPORT SUBMISSIONS AND REVIEW OF IMAGES**

► **LRIS - VALUE**
- ◆ Reduces review time; improves quality of review process
- ◆ Enables all manufacturers to submit electronic protocols using existing tools
- ◆ Helped pioneer use of Adobe Acrobat technology within CBER
  - ◇ Imaging technology can be used in many CBER initiatives (e.g., CAPLA), and is being used for RMS
  - ◇ Helped define resolution standards capturing images and graphics
- ◆ Facilitates archiving - photos deteriorate quickly

► **CAPLA - PURPOSE**
- ◆ Manage incoming CAPLAs
- ◆ Provide guidance to industry
- ◆ Develop procedures for handling CAPLAs
- ◆ Build system to track CAPLAs
- ◆ CAPLA - Guidance for Industry
  - ◇ Specifies preferred COTS software
  - ◇ Provides guidance on who to contact and when
  - ◇ Describes CBER technical environment
  - ◇ Supports move from sponsor-submitted hardware/software to CBER-owned equipment
  - ◇ Supports move to network CAPLAs
- ◆ CAPLA - Internal Procedures
  - ◇ Documents procedures for CBER to follow in advance of and upon receipt of CAPLA submission
  - ◇ Defines roles and responsibilities
- ◆ Establishes time-frames for key activities
  CAPLA Guidance Manual
  - ◇ Availability of the CAPLA Guidance Manual -FR 3/21/96
  - ◇ Internet email requests for the manual:
    - ✣ FTP : "FTP.FDA.GOV", or
    - ✣ "CDVS2.CDER.FDA.GOV"
  - ◇ Browsers: "HTTP:9/16/96/www.fda.gov/cber/cberftp.html"
- ◆ CAPLA Guidance Manual
  - ◇ For questions about CBER FTP
    - ✣ contact Mark Elengold at:
    - ✣ or
    - ✣ elengold@A1.cber.fda.gov

► **GENE THERAPY – BACKGROUND**
- ◆ NIH Recombinant DNA Advisory Committee (RAC)
  - ◇ Public forum for discussion of gene therapies

- ◇ Collected and published clinical and product data
- ◆ Congress
  - ◇ Mandated FDA build registry for longitudinal follow-up
- ◆ CBER
  - ◇ Currently 130+ INDs, over 600=0 patients
  - ◇ PLAs in the near future
- ◆ GTIN – Objectives
  - ◇ Subject registry to meet Congressional mandate
    - ✣ Permits longitudinal analysis to addresses concerns on long-term effects of gene therapy
    - ✣ Provides prototype of functional system to drive FDA policy on data collection requirements
    - ✣ Prototype populated with Cystic Fibrosis Foundation (CFF) data
- ◆ GTIN - Accomplishments
  - ◇ Delivered Registry (Alpha prototype) to OTRR/DCGT in Spring 1996
    - ✣ Laptop
    - ✣ PC
  - ◇ Delivered capabilities that provide information on gene therapy INDs
  - ◇ Delivered capability for NIH to generate reports to support RAC
- ◆ GTIN- Value
  - ◇ Satisfied Congressional mandate
  - ◇ Serves as a catalyst to establish policy necessary to implement Novel Therapy registries
    - ✣ Confidentiality
    - ✣ Who collects the data
    - ✣ Who enters the data
    - ✣ Length of follow-up
- ◆ Future Steps:
  - ◇ Address policy issues of data gathering from "womb to tomb"(e.g. CRF modification), linking to other registries such as the cystic fibrosis registry
  - ◇ Await new CBER database completion
  - ◇ Budgetary constraints re:longitudinal follow-up & data integrity (?PDUFA renewal)
  - ◇ Implementation of a xenotransplantation registry

► OTHER AUTOMATION EFFORTS

- ◆ CBER Electronic IND Pilot
  - ◇ CBER working group forming
    - ✣ chair: Fred Miller M.D.
    - ✣ every office represented
    - ✣ Pilot to begin within next few months to review Gene Therapy electronic INDs

► INTERCENTER EFFORTS

- ◆ CDER/CBER Electronic Submissions Committee
  - ◇ formed May 1996
  - ◇ charter: electronic format for CFRs and line listings (draft by fall 1996, industry workshop fall 1996, FR notice DEC 1996)
  - ◇ chair: Kaye Fendt (CDER)
- ◆ CARS (computer review of safety)
  - ◇ charter : data model for review of safety
  - ◇ chair: Kaye Fendt (CDER)

JULY 12
VECTOR DEVELOPMENT

Goals: To present and discuss issues concerning the development, production, and use of viral vectors for gene therapy.

8:00 Overview and Goals, Philip Noguchi, M.D., Director Division of Cellular and Gene Therapies, CBER

8:15 Plenary Talk, Alan E. Smith, Ph.D., Senior Vice President, Genzyme Corporation

9:00 Morning Breakout Sessions

1. Adenoviral Vectors
2. Ancillary Products
3. Facilities & Manufacturing
4. Getting Started in Gene Therapy Vector Development

1:30 Afternoon Breakout Sessions

5. Development of New Vector Systems
6. Retroviral Vectors
7. Pharmacology & Toxicology

5:00 Conclusions and Summaries of the Breakout Sessions

PLENARY TALK

Dr. Alan E. Smith
Senior Vice President
Genzyme Corporation

►GENE THERAPY-WHAT IS IT?

- ◆Direct use of nucleic acid vectors for any therapeutic purpose

►HOW IS IT DONE?

- ◆Many applications -each is different
  - ◇ADA, cancer, CF, HIV, etc.
- ◆In Vivo or Ex Vivo
- ◆Various vectors
  - ◇retrovirus, adenovirus, AAV, cationic lipids
- ◆Many genes
  - ◇wild type genes, cytotoxic genes, tumor suppresser genes, molecular decoys, ribozymes, etc.

►WHY ARE WE EXCITED BY IT?

- ◆Simple, rational theory
- ◆Broad application
- ◆Potentially very powerful
- ◆Initial, preliminary successes

►HOW IS IT GOING IN THE CLINIC?

- ◆Adenosine Deaminase/SCID
  - ◇Retrovirus mediated transfer to T-cells - Sept. 1990
  - ◇Long term improvement in patient immune function
  - ◇Selective advantage of transduced cells?
  - ◇Patients still on protein replacement therapy
  - ◇Very fortunate first choice
- ◆Cancer
  - ◇Many successful experiments in animal models
  - ◇Animal data often hard to reproduce in humans
  - ◇Various studies in progress
  - ◇Mobilization of immune system
  - ◇Cytotoxic drugs with bystander effect
  - ◇Direct injection
  - ◇Primarily safety studies to date
- ◆Cystic Fibrosis
  - ◇Adenovirus and lipid trials reported
  - ◇Aerosolization and repeat dosing in progress
  - ◇Safety data generally satisfactory but doses low
  - ◇Efficacy data modest

Although the basic principles of gene transfer and gene function are established in several model systems, there is no unequivocal case of efficacy yet established in humans

►WHAT ARE THE SCIENTIFIC ISSUES?

- ◆Identity of and access to target tissue and cells
- ◆Efficacy of gene transfer and expression
- ◆Inflammation associated with vector administration
- ◆Duration of expression
- ◆Efficacy of repeat dosing
- ◆Clear cut clinical end points

► **IDENTITY OF AND ACCESS TO TARGET TISSUE AND CELLS**

- ◆ Identity and isolation hematopoietic stem cells - CD34+?
- ◆ Accessibility of airway cells/submucosal glands, mucocillary clearance, mucus layer
- ◆ Systemic targeting of tumor cells, organs
- ◆ Diseased tissue

Knowledge of and access to target cells essential but often problematic

► **EFFICACY OF GENE TRANSFER AND EXPRESSION**

- ◆ Retrovirus vector transduction of human hemopoietic stem cells
- ◆ Adenovirus vector transduction of differentiated human airway epithelia
- ◆ Cationic lipid mediated gene transfer
- ◆ AAV transduction of most human cell types

The potency of vectors, in terms of i.u./cell or molecules/cell required, needs to be improved

► **INFLAMMATION**

- ◆ Associated with liver, muscle and lung administration adenovirus
- ◆ Mediated by inactivated vector
- ◆ Associated with lung administration of cationic lipids
- ◆ Involves innate immune system (macrophage, neutrophils, NK cells, cytokines)

Toxicity associated with repeated administration of both viral and non viral vectors may well become dose limiting

► **DURATION OF EXPRESSION**

- ◆ Retroviruses
  - ◇ transcriptional shut off
- ◆ Adenovirus
  - ◇ influenced by vector construct
  - ◇ CTL responses)beware of transgene)
- ◆ Cationic lipids
  - ◇ mechanism unknown
- ◆ Muscle, perhaps brain, appear exceptions

Transient gene expression is caused by a variety of different molecular mechanisms

► **REPEAT DOSING**

- ◆ Efficacy of adenovirus re-administration limited
- ◆ Probably mediated by neutralizing Ab
- ◆ Likely barrier for all proteinaceous vectors
- ◆ Is possible using cationic lipid vectors

Cationic Lipids and integrating vectors less problematic

► **CLINICAL END POINTS**

- ◆ CF - No obvious, readily measured clinical end point
- ◆ Cancer - Studies often involve terminal patients
- ◆ ADA - Additional ongoing therapies complicate interpretation

Urgent need for better clinical measures of success and meaningful surrogate markers

► **WHAT CAN BE DONE?**

- ◆ Establish whether these are barriers in humans
- ◆ Fine tune amount and frequency of dosing
- ◆ Improve vector
- ◆ Co-administer other agents

►WHAT NEXT?

- ◆Basic Science
- ◆Enhance our knowledge of virology, immunology, gene expression, cell biology, etc.
- ◆Novel vectors, cell culture and animal models
- ◆Clinical Studies
- ◆Animal data cannot predict human
- ◆Human clinical studies essential
- ◆Will drive formulation of future hypotheses

►WHAT ARE THE REGULATORY ISSUES?

- ◆RAC review of individual applications discontinued
- ◆FDA in pivotal role
- ◆Safety paramount
- ◆Facilitate collection of scientifically sound, interpretable human data

Crucially important role in ensuring safety and timely accumulation of human data to test and to formulate hypotheses leading to eventual clinical success

►HOW IS IT GOING AT FDA?

- ◆Regulations are rigorous, but not onerous
- ◆Many issues negotiable-driven by data
- ◆Review is timely

Approach agency in spirit of collaboration not confrontation, staff as colleagues not bureaucrats

►CF ADENOVIRUS GENE THERAPY: GENZYME CORPORATION'S EXPERIENCE

Vector/Trial	Type	Submitted	Time for "Approval"
Ad2/CFTR-1 (Nasal Trial)	IND	4/2/93	3.5 months
Ad2/CFTR-2 (Repeat Dose Nasal Trial)	IND	12/10/93	2.5 months
Ad2/CFTR (Lobar/Aerosol Trial)	Major Amendment	11/10/94	<30 days
Ad2/CFTR-8 (Vector Switch)	Major Amendment	7/26/95	2 weeks

►CF CATIONIC LIPID GENE THERAPY

Vector/Trial	Type	Submitted	Time for "Approval"
#67:pCF1	IND	12/5/95	<30 days

30 days included:

- ◇Christmas
- ◇New Year
- ◇Governmental shut down
- ◇Washington snow emergency

►GENE THERAPY - JULY 1996

- ◆Unusual case of drug development involving especially close collaboration between public and private sectors and between investigators and FDA
- ◆Many constituencies: patients, scientists, clinicians, regulatory bodies, private sector, investors, media, advocacy groups, politicians
- ◆Barriers to gene transfer identified in animals, more basic science and clinical studies essential
- ◆Despite hurdles, great promise with undoubted contributions to medicine in next century

Time frame of ultimate and widespread success hard to predict, important to manage expectation

BREAKOUT SESSION 1:
ADENOVIRAL VECTOR DEVELOPMENT

SUMMARY

Kathleen Hehir, Genzyme Corporation; Parris Burd, FDA/CBER; Robert Anderson/FDA/CBER

Part I. Determination of Adenovirus Particle Number and Infectivity

Bruce Trapnell discussed considerations for the determination of adenovirus vector particle concentration and infectivity. Enumeration of total virion concentration by optical absorbance was found to be very precise, but accuracy was dependent upon on physical disruption of the virion to eliminate artifacts of light scattering and was affected by the value used for the extinction coefficient. Biologic assays for enumerating infectious or functional virions, however, were highly dependent upon the conditions under which the assay was performed.

Paul Shabram discussed an alternate method for particle determination which does not require a highly purified test article. He also presented data from infectivity modeling experiments which suggest that the true rate of Adenovirus infectivity may approach unity.

This notion was reinforced further by data presented by Beth Hutchins who described a FACS-based infectious titer assay which showed that infectivity rates are consistently higher than conventional plaque assays would suggest.

John Spalatro brought us back to earth and reminded us that no matter what methods are used, one must be mindful of the statistical considerations and limitations of the assay employed.

Part II. Production Issues for Phase I and Beyond

Our ultimate goal is to bring adenoviral-based products to licensure. A great many issues remain to be solved for this to occur. This session presented three approaches towards bringing a product to market and attempted to provide concrete examples of what we mean by the GMP continuum.

Dominic Vacante presented an overview of the regulatory and manufacturing issues associated with developing Adenoviruses as products. These included selection and characterization of the cell substrate and seed virus, qualification of raw materials, methods of cell expansion, infection, harvest and purification as well as considerations for product formulation, filing, stability and final product characterization and lot release specifications. How far one must develop these concepts was then put into the context of different schemes of product development.

Estuardo Aguilar described the approach of the Baylor College of Medicine Gene Vector Lab has taken to develop products intended only for proof of concept/safety studies in Phase I clinical trials.

Jim Wilson of the University of Pennsylvania then described his approach for developing products through Phase II and early Phase III studies. A key component of this program is the emphasis upon the toxicology of vector development.

Kathy Hehir described Genzyme Corporation's single sponsor approach for bringing Adenovirus-based products to licensure.

The take home question is: WHEN THE TIME COMES WILL YOU BE READY FOR SUCCESS?

►AGENDA

The goals of the session are to explore the different approaches for quantitating virus particles and their infectivity and to explore sponsors's approaches to the production and scale-up issues associated with moving products towards phases ii and iii and licensure. Questions to be addressed include:

Is viral plaque formation the only way to evaluate virus infectivity?
How does production scale-up affect particle infectivity?
What are the hurdles to large scale Adenovirus production?

►PART I. DETERMINATION OF ADENOVIRUS PARTICLE NUMBER AND INFECTIVITY

- ◆Considerations for the Determination of Adenovirus Particles and Infectivity
  - ◇Bruce Trapnell
- ◆Rapid Identification of Encapsidated Viral DNA and Assessment of Viral Infectivity
  - ◇Paul Shabram
- ◆New, More Sensitive Method to Assess Adenoviral Vector Infectivity
  - ◇Beth Hutchins
- ◆Statistical Considerations for the Quantification of Adenovirus Infectivity
  - ◇John Spaltro
- ◆Discussion and Summary

►PART II. PRODUCTION ISSUES FOR PHASE I AND BEYOND

- ◆Manufacturing and Regulatory Issues for Developing Adenovirus Vectors as a Product
  - ◇Dominic Vacante
- ◆Small Scale Production of Adenovirus Vectors Intended for Phase I Studies
  - ◇Estuardo Aguilar-Cordova
- ◆Development of Adenovirus Vectors for PhaseII/III Studies in a University Setting
  - ◇James Wilson
- ◆Single-Sponsor Development of Adenovirus Vectors Intended for Product Licensure
  - ◇Kathy Hehir

Discussion Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:
GTINFO@A1.CBER.FDA.GOV

CONSIDERATIONS FOR THE DETERMINATION OF ADENOVIRUS VECTOR PARTICLE CONCENTRATION AND INFECTIVITY

Bruce C. Trapnell, M. D.
Genetic Therapy, Inc., Gaithersburg, Md.

Development of adenoviral vectors as therapeutic agents for a number of applications of in vivo human gene therapy has resulted in numerous preclinical and clinical studies. However, lack of standardization of the methods for quantifying the physical concentration and functionally active fraction of virions used in the various studies has often made comparison of data between studies difficult or impossible. In this context, several of the common procedures for quantifying adenoviral vectors have been extensively evaluated to define the variables which affect quantification of adenoviral vector concentration and bioactivity. The methods of evaluation of total virion (particle) concentration included electron microscopy and optical absorbance. The methods for evaluation of the concentration of functional (infectious) virions included detection of adenovirus-mediated gene transfer (transgene transfer and expression) and plaque assay on 293 cell monolayers. Enumeration of total virion concentration by optical absorbance was found to be very precise, but the accuracy was dependent on physical disruption of the virion to eliminate artifacts from light scattering as well as on the value used for the extinction coefficient. Both biological assays for enumerating functional or infectious virions were highly dependent on the conditions under which the assay was performed. Virion adsorption time and volume were particularly important; target cell density and amount of serum present during adsorption did not affect results significantly and multiplicity of infection was not important independent of its effect on vector concentration. Under optimal conditions, the bioactivity of the vector, defined as the fraction of total virions which leads to detected target cell infection, was determined to be 0.10 using the plaque assay and 0.29 using the gene transfer assay. This difference is most likely due to the fact that detection by gene transfer requires only measurement of levels of transgene expression in the infected cell while plaque formation is dependent on a series of biological events of much greater complexity. These results show that the exact conditions for determination of infectious virion concentration and bioactivity of recombinant adenoviral vectors are critical and must be standardized for comparability of data and for accurate assessment of bioactivity. These observations may be very useful in comparison of data from different preclinical and clinical studies, and may have important implications for how adenoviral vectors can optimally be used in human gene therapy.

RAPID IDENTIFICATION OF ENCAPSIDATED VIRAL DNA AND ASSESSMENT OF VIRAL INFECTIVITY

Paul Shabram
Canji, Inc.

Analytical Anion Exchange High Performance Liquid Chromotography of Recombinant Ad-5 Particles

The expanding use of adenoviral vectors for gene therapy has brought about the need for new analytical tools. We have developed an Anion Exchange High Performance Liquid Chromatography (AEHPLC) method to analyze recombinant adenovirus serotype 5 samples. Before this assay available analytical methods consisted of either long term biological assays or required highly purified test articles. These methods were inadequate for optimizing adenovirus production and purification. The AEHPLC assay can be used to enumerate virus particles in either crude lysates or highly pure samples because it selects for virus by virtue of its surface characteristics. It can be used to assess particles in both dilute and concentrated samples over a wide dynamic range. Moreover, the population of virus particles eluted in the peak contains most of the infectious virions. The AEHPLC assay is a sensitive technique that overcomes the

limitations of previous methods and provides an essential tool to accomplish process optimization. Since this assay can quantitate particles in a complex medium it can be used for studies in cell culture. In the infectivity study below AEHPLC was used to control for particle concentration.

Are Recombinant Ad-5 Particles Mostly Infective?

It is commonly held that the majority of adenovirus particles are non-infective. For gene therapy this belief has led to concerns that a dose of adenovirus would consist of mostly inactive and potentially toxic material. The industry standard is currently set to at least 1 active particle in 100 particles. Serial infection experiments, performed by others and ourselves, indicated that infectious titer assays used to calculate the "particle to infectious particle ratio" were misleading. Many events must occur before an infection can be detected. The first event, the collision of a particle with a cell, can be predicted on the basis of thermodynamic properties. Using Fick's Laws of diffusion a collision rate can be predicted; but the assay conditions that are typically used (plaque assay or TCID50 assay) do not allow for simple data analysis. Flow cytometry, however, is perfectly suited to allow experiments to be set up in such a manner that "semi-infinite solution" conditions apply. Under these conditions an equation can be derived that will predict the number of cells that have experienced a collision with an Ad-5 particle. The results we obtained using this scheme was surprising. Our data suggests that material possessing a particle to infectious unit ratio (TCID50) of ≅ 50:1 is no more than 2:1 after considering diffusion limitations. From this data we conclude that most particles are infective.

NEW. MORE SENSITIVE METHOD TO ASSESS ADENOVIRAL VECTOR INFECTIVITY

Beth Hutchins, Ph.D.
Canji, Inc.

A challenge in the delivery of a gene by an adenoviral vector is the preparation and accurate characterization of clinical dosage forms. Quality of dosage forms is evaluated using both biological and physical measurements. One critical biological property of a replication incompetent adenovirus is the infectious titer. This measure is used in combination with the particle number to assess the specific activity of an adenoviral preparation (particle number to infectious titer ratio). In addition, dosing has frequently been conducted using the infectious titer measurement.

Two types of infectivity assays are traditionally used to determine the infectious titer of adenovirus preparations. The plaque-forming unit assay (pfu) is a quantitative procedure that scores viral plaques (complete cytopathic effect) as a function of dilution. The tissue-culture infective dose procedure TCID50) is a quantal assay that assesses infectivity as a function of intracellular staining for a viral antigen by direct immunofluorescence. Both methods suffer from limitations including a high degree of inter-assay variability and are affected by factors such as virus replication status, vector characteristics, and virus-293 cell interactions.

We have recently developed a new, flow cytometry-based methods (FACS) to assess adenovirus infectious titer. This assay appears to be more sensitive and reproducible than the more traditional methods. The FACS method is a quantitative procedure that assesses infectivity as a function of the number of cells staining positively for a viral antigen. The FACS method is more sensitive to the events used to monitor evidence of infection than is either the pfu or TCID50 method. This results in infectious titers that are consistently higher than the titers determined using the pfu or TCID50 assays when assessing the same material.

Our experiences also indicate that none of the assays determines the total amount of infectious material present. We performed serial infection experiments during which sample was transferred to fresh 293 cells after 15 minute incubations. Each set of exposed cells was assessed for evidence of adenovirus infection 48 hours post infection, with infectious titers calculated for each well. When these individual well titers were summed, the total infectious titer was greater than would be predicted using any of the infective titer assays discussed previously. The subsequently calculated particle number to infectious titer ratio was lower than the previously calculated ratio using the TCID50 or pfu assay titers.

All of these results indicate that more particles are infectious than the traditional assays would suggest and that infectious titer should only be used as a secondary test for quality. Physical based methods, such as OD 260 nm in SDS, total DNA , or the Resource Q HPLC method, are powerful and reliable measures of virus concentration. Particle number measurements have low inter-assay variability and have been shown to be accurate through comparison with direct particle counts using electron microscopy. As a result, particle numbers to infectious titer ratio specifications are based on two methods with very different precision. The ability to set a specification for this ratio must be based on the reliability of the less precise method.

Because of the limitations of infectious titer assays, the measure of adenovirus infectivity is best used, not as an absolute indicator of active virus, but as a relative assessment of virus batch quality. Given that clinical dosage forms are characterized by a variety of means, including specific activity based on transgene function and/or expression, the use of infectivity as a relative assessment of batch quality makes sense. By applying this strategy and understanding the limitations of infectious titer assays, a consistent yet reliable quality dosage form can be produced using set specification ranges by which the product can be evaluated for release.

VIRAL SAFETY OF BIOTECHNOLOGY AND GENE THERAPY PRODUCTS—STATISTICAL REVIEW OF RCA TEST METHODS

John Spaltro, Ph.D.
Microbiological Associates, Inc.

Viral safety and the risk of viral contamination are major issues in the manufacturing and control of biotechnology and gene therapy products. The potential for the occurrence of replication competent adenovirus (RCA) in replication defective gene therapy products requires that RCA testing be uniform.

Statistical analysis of adenovirus titration and RCA test method data will be presented. Procedures required to determine the probability of detection (P. O. D.) for the RCA method, as well as, current limits of detection for the RCA methods will be described.

Findings will be discussed in terms of a new perspective, using the assay P. O. D., to identify and compare allowable limits to currently defined 1 RCA per dose specification.

MANUFACTURING AND REGULATORY ISSUES FOR DEVELOPING ADENOVIRUS VECTORS AS A PRODUCT

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Gene therapy clinical trials using adenoviral vectors were initiated in early 1993. Production involved the relatively straight-forward transfer of research processes to a GMP laboratory for small-scale manufacture of the vector. As adenovirus vectors move from phase I clinical trials to

phase III and licensure, the challenges for manufacturing and regulatory groups will increase dramatically. An overview of current methodologies and regulatory guidelines will be presented with a view to the future. Specific areas of discussion will include:

- Selection and characterization of the cell substrate and seed virus
- Raw materials
- Cell Expansion
- Infection/harvest
- Purification
- Formulation, filing, and stability
- Product characterization and release specifications

SMALL SCALE PRODUCTION OF ADENOVIRUS VECTORS INTENDED FOR PHASE I CLINICAL STUDIES

Cassandra Nyberg and Estuardo Aguilar-Cordova
Gene Vector laboratory, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX 77030

In biologics, lot-to-lot identity is essential since the final product is complex and may not be tested in its entirety. Therefore, when considering the production of viral vectors for clinical studies two predominate variables must be taken into account: where and how the vector will be produced. The first refers to the physical characteristics of the production facility. The facility must be of sufficient quality to minimize potential irregularities from equipment or environmental sources. The "how" addresses the technical and regulatory procedures of the process. The technical aspects include the biological and physical variables of production which might affect the uniformity of the final manufactured product. In the case of adenoviral vectors these variables include the amplification process (cells and seed vector utilized, multiplicity of infection, volume of transaction, time of harvest, etc.), the purification process (isolation from cellular components), the storage conditions (buffer and temperature), and the vector characterization (quantity and potency). Understanding the effects of these variables of production can lead to increased yields and more predictable end-product characteristics. The regulatory aspects of the "how" include the quality assurance and quality control procedures established to ensure process and product purity, potency, and identity. We will discuss the approach the Baylor College of Medicine Gene Vector Laboratory has taken to address the issues mentioned above towards production of adenoviral vectors utilized in Phase I clinical studies.

DEVELOPMENT OF ADENOVIRAL VECTORS FOR CLINICAL TRIALS AT THE UNIVERSITY OF PENNSYLVANIA

James M. Wilson, M.D., Ph.D.
University of Pennsylvania, Philadelphia, PA

A fully integrated program from basic discovery to clinical trials has been established within the Institute for Human Gene Therapy at the University of Pennsylvania. The scientific foundation for this Institute lies in its faculty who participate in basic research relevant to the ultimate development of effective gene therapies. Over 150 faculty participate in research programs focused on cystic fibrosis, genetic diseases, cancer, cardiovascular diseases, and infectious diseases. An independent program in translational and clinical research has been established to facilitate the translation of basic discovery to human pilot experiments. Within the translational research program is our vector manufacturing laboratory, called the Human Applications Laboratory, as well as a program in toxicology. Critical to the implementation of appropriate

preclinical studies in animals was the establishment of an animal service unit, which includes primate and rodent facilities, managed by the Institute staff. A clinical research program supports the investigator in the design and conduct of a clinical trial and interfaces with those support units necessary for the study to develop. Quality assurance and quality control for all translational and clinical trials is provided through the Regulatory Affairs, which is run by the Deputy Director of the Institute for Human Gene Therapy, Dr. Nelson Wives.

We have learned that the production of clinical grade vector is only one part of a successful program in the development of human gene therapy. The characterization of the product and development of the protocol is highly dependent on the performance of a diverse array of preclinical studies to assess safety. During the last two years we have received IND approval for five clinical trials. Our experience has reinforced the importance of toxicology, not only in the initial application process, but during the conduct of the trial, when adverse events emerge or modifications in the vector are made. Several programs are rapidly proceeding through Phase I clinical studies with plans for Phase II studies underway. In anticipating the eventual success of one or more of these programs, we have considered issues relevant to the eventual licensure of the resulting products, if improvements clearly need to be made in the manufacturing and purification process of the vector. Our current experience suggests an anticipated cost of approximately $1,000,000 for vector production and safety testing through the preclinical phase to the completion of a phase I trial. Over 50% of this cost is in the manufacturing of the vector which clearly can be reduced with improved technologies. We are also addressing the role of contaminating chromosomal DNA in the final prep and the impact this will have on the eventual safety of the product. Finally, we believe it is essential that issues of long-term safety and the reproductive toxicology be addressed at an early phase before we are confronted with the licensure of a product.

SINGLE SPONSOR DEVELOPMENT OF ADENOVIRUS VECTORS FOR PRODUCT LICENSURE

Kathy Hehir
Genzyme Corporation

What are the key issues for the production of Adenovirus vectors for Phase III clinical trials and toward product licensure? New production scale-up and purification methods must be coupled with assays that are able to assess product activity, function, purity and equivalence to CsCl centrifugation methods. Batch sizes may be limited by testing requirements or assay sensitivity. Facility design that accommodates single products must also allow flexibility during product development. Formulation must address shelf-life stability as well as modes of drug administration. Product release specifications that pose challenges include RCA and host cell protein and DNA contamination. RCA assays must be able to detect low levels of RCA in a background of high levels of vector and the assays must be able to be validated. RCA is a patient safety concern but product specific limitation or allowances can be addressed if appropriate pre-clinical and clinical data is available. Current production and assay methods as well as planned development will be discussed.

BREAKOUT SESSION 2: ANCILLARY PRODUCTS

SUMMARY

Joyce L. Frey-Vasconcells, FDA/CBER

The first half of the breakout session discussed the feasibility and the type of testing that constituted a good qualification program for ancillary products. Dr. Joyce Francis presented a qualification program had been established at Genetic Therapy, Inc. and the rational behind the program. The general consensus after much discussion was that qualification programs were a reasonable mechanism for ensuring product quality. However, many participants voiced concern over trying to acquire clinical grade material. Many times if a company finds out that the client is using their product to manufacture a biologic the company will refuse to supply the product and this impedes the progress of clinical trials.

The second half of the session discussed the regulatory framework for approving ancillary products and how such products could be labeled. Mr. Keith Gittermann of Life Technologies Inc. discussed issues related to media products as ancillary products. Under the current regulations, ancillary products would be regulated as a device but the device regulations for labeling require that if a manufacturer has knowledge that the product is being used "off label", it is the manufactures responsibility to provide adequate labeling to include the new use. This is a liability concern for many manufacturers. Mr. Gittermann presented the concept paper by the Association of Medical Diagnostics Manufacturers which has been submitted to the FDA. This involves a very generic labeling claim and groups related products into product families. A 510(k) would be submitted for the product family with no clinical data. In addition, the manufacturer would submit a master file to CBER for each product family. Mr. Mark Gauthier of Immunex Corporation discussed quality aspects that ancillary products should be analyzed for in order to maintain the quality of the final therapeutic product. In addition, he discussed several existing regulatory mechanisms which could potentially be employed to approve ancillary products. Finally, Mr. Gauthier presented proposed labeling for ancillary products used in the manufacture of ex vivo cell products. The last talk was given by Dr. Mark Hukkelhoven of Sandoz Inc. He discussed the regulatory framework to license cytokines for ex vivo use. He presented the advantages for sponsors to be able to obtain licensed ancillary products. Dr. Hukkelhoven suggested that device regulations could be used and that the labeling would be restricted to the ex vivo use of the product with no claims on the final cell product. The discussion focused on what data would be reasonable for approval of an ancillary product. This intertwined with discussions on how these products could be labeled to reduce liability for "off label" use. The final question presented to the group was whether the FDA should narrow its definition of ancillary products. This question was not discussed but participants were encouraged to submit responses to the e-mail address for the conference.

►AGENDA

The goals of the Ancillary Product Workshop will be to evaluate the usefulness of qualification programs for beginning studies and their effects upon the sponsor. The second half of the workshop examines industry's perspective on the regulatory framework for ancillary products.

►PART I. QUALIFICATION PROGRAMS

- ◆Introduction and Questions
  - ◇Joyce L. Frey, FDA/CBER
- ◆Setting up a Qualification Program, Things to Think About!
  - ◇Joyce Francis, Ph.D., Genetic Therapy Inc.

- ◆ Discussion

► PART II. REGULATORY FRAMEWORK AND LABELING

- ◆ Overview of Regulations
  - ◇ Joyce L. Frey, FDA/CBER
- ◆ Ancillary Product Manufacturer's Issues and Recommendations,
  - ◇ Keith D. Gittermann, Life Technologies Inc.
- ◆ Regulatory Framework for Ensuring Quality of Products and Potential Labeling
  - ◇ Mark W. Gauthier, Immunex Corp.
- ◆ Sandoz Inc., How to License an Ancillary Product?
  - ◇ Math Hukkelhoven, Ph.D.
- ◆ Discussion

Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

GTINFO@A1.CBER.FDA.GOV

INTRODUCTION TO THE BREAKOUT SESSION ON ANCILLARY PRODUCTS

Joyce L. Frey, Ph.D.
FDA/CBER

► **INTRODUCTORY QUESTIONS**
- ◆ Are qualification programs a reasonable mechanism to ensure the quality of an ancillary products?
  - ◇ Are there additional tests which need to be performed?
  - ◇ Are these programs reasonable for single sponsor investigations?
  - ◇ Are there other alternatives for ensuring the quality of ancillary products and ultimately of your final therapeutic product?
- ◆ REGULATORY FRAMEWORK
  - ◇ If regulated as a device, 21 CFR 801.4 discusses the labeling for devices and intended uses.
  - ◇ If regulated as a device, for most products there is no predicate so a PMA would have to be submitted.
  - ◇ If regulated as a biologic, a BLA or PLA and ELA would have to be submitted.
- ◆ QUESTIONS FOR THE SESSION
  - ◇ Are there other regulatory mechanisms for approval of ancillary products?
  - ◇ Could these products be classified as either a Class 1, 2, or 3 device? (Labeling regulation) What would be the major concerns?
  - ◇ How should an ancillary product be labeled?
  - ◇ What data would be reasonable for approval of an ancillary product?

A PLAN FOR CERTIFICATION OF GROWTH FACTORS FOR USE IN EX VIVO GENE THERAPY

Joyce D. Francis, Ph.D.
Genetic Therapy, Inc.
Gaithersburg, MD

► **USE OF CYTOKINES**
- ◆ retroviral vector-mediated gene transfer
- ◆ expansion of bone marrow cells

► **AVAILABILITY**
- ◆ Limited Access
- ◆ Length Negotiations
- ◆ Potentially Costly

► **CERTIFICATION PLAN**
- ◆ Facilitate initiation of clinical trials
- ◆ Protect proprietary products
- ◆ Less expensive

► **CERTIFICATION PLAN**
- ◆ Identification of Vendors
- ◆ Handling and Lot Number control
- ◆ Lot Release Testing
- ◆ Inventory Control

► **IDENTIFICATION OF VENDORS**
- ◆ Commercial supplier
- ◆ High purity
- ◆ Low endotoxin
- ◆ Custom production and filling

► **HANDLING**
- ◆ Assign Internal Lot Number
- ◆ Quarantine
- ◆ Lot Release Testing
- ◆ QA Audit and Approval
- ◆ COA Issued

► **LOT RELEASE TESTS**
- ◆ Identity
- ◆ Sterility
- ◆ Activity
- ◆ Purity
- ◆ Endotoxin
- ◆ Toxicity
- ◆ Residuals/Contaminants

► **INVENTORY CONTROL**
- ◆ Request for shipment approved by QA
- ◆ COA attached
- ◆ Complete traceability

► **SUMMARY**
- ◆ Plan is acceptable to FDA for Phase I/II Trials\Work on cytokine lots in progress
- ◆ Use in trials scheduled for 1997, if results acceptable

AN ANCILLARY PRODUCT MANUFACTURER'S ISSUES AND RECOMMENDATIONS

Keith D. Gittermann
Director of Regulatory Affairs, Life Technologies, Inc.

ABSTRACT

This talk will focus on the current regulatory issues facing today's manufacturers of ancillary products for use in various ex vivo clinical applications. Due diligence considerations restrict the ease of sales of products for ancillary use in the gene and cell therapy arena. When considered medical devices, these products cannot be knowingly sold for end uses for which the manufacturer has not qualified them. Gaining FDA approvals through the PMA process is not a reasonable option as there are a myriad of potential different end uses which may need to be qualified. Using cell culture medial as an example, we will discuss the various regulatory pathways available for a product to travel down, from research uses to in vivo uses. We will discuss a possible solution to the issue of off-label use which has been proposed by FDA and by the Association of Medical Diagnostics Manufacturers.

► **DEFINITION OF AN ANCILLARY PRODUCT**
- ◇ "Application of Current Statutory Authorities to human Somatic Cell Therapy Products and Gene Therapy Products"
- ◇ These products"...are intended to act on cells, rather than to have an independent effect on the patient. Additionally, the intended action of these products is not dependent upon incorporation into the somatic cell with maintenance of the products' structural or functional integrity."

► **THE ANCILLARY PRODUCT MANUFACTURER'S DILEMMA IS 21 CFR PART 801.4 "MEANING OF INTENDED USES"**

- ◆ "...If a manufacturer knows, or has knowledge of facts that would give him notice that a device introduced into interstate commerce by him is to be used for conditions, purposes, or uses other than the ones for which he offers it, he is required to provide adequate labeling for such a device which accords with such other uses to which the article is to be put."
  - ◇ In other words, this means a research use product or a simple 510(k) exempt IVD can suddenly become a medical device requiring a 510(k) or more likely a PMA.
  - ◇ This is both impractical and not economically feasible when one considers the large number of unrelated end uses in which ancillary products are used. Each use could require a new submission.
  - ◇ Cell Culture Products are good examples of a product group whose intended use is largely research, but for which numerous other uses have evolved; some of which don't meet the requirements of 801.4
- ◆ Products include:
  - ◇ Cell and Tissue Culture Media
  - ◇ Balanced Salt Solutions
  - ◇ Human and Animal Sera
  - ◇ Reagents
    - ✣ Essential for the growth, survival, and development of cells of human and animal origin. Some products are used as a wash or holding solution for cells and tissues, and some products are used as supplements to media. 50-80% of these product's uses are in research setting for nonmedical purposes which are not regulated by FDA.

► **THEY ARE USED IN CLINICAL DIAGNOSTIC PROCEDURES TO SUPPORT CELL CULTURES FOR VIRAL ISOLATION**

- ◆ Class 1 medical devices
- ◆ Exempt from premarket notification 510(k) procedures
- ◆ Device establishment registration and device listing are required
- ◆ Must comply with current Good Manufacturing Practices as described in 21 CFR Part 820
  - ◇ These are products specifically intended and labeled for sperm washing/handling procedures and specimen transport procedures.
  - ◇ These uses are subject to 510(k) clearances.
- ◆ Utilized as raw materials for the further manufacture of monoclonal antibodies, vaccines, and biologic therapeutics.
  - ◇ The cell culture media is not directly regulated by FDA.
  - ◇ Drug or device master files are, on occasion, submitted to FDA.

► **USED BY PHYSICIANS IN BIOMEDICAL APPLICATIONS REQUIRING INVESTIGATIONAL NEW DRUG (IND) OR INVESTIGATIONAL DEVICE EXEMPTION (IDE) SUBMISSIONS MADE BY THE END USERS**

- ◆ These applications include such procedures as ex vivo cell culture, tissue/organ suspension for transplantation, and hematopoietic stem cell manipulation.
- ◆ For some uses, drug or device master files have been submitted to FDA by the media manufacturer to support end user's submission.

► **USED BY PHYSICIANS ON OTHER BIOMEDICAL APPLICATIONS FOR WHICH THE END USER IS NOT REQUIRED TO SEEK FDA CLEARANCE**

- ◆ These applications include bone marrow transplants, in vitro fertilization and related procedures, artificial skin production, and some transplantation procedures.
- ◆ Per 21 CFR Part 801.4, these last two applications could be interpreted to require the manufacturer of the ancillary product to seek a Premarket Approval Application (PMA) for each end use oaf which they are aware.
  - ◇ Over the past few years, the number of new and different types of "off-label" uses have proliferated while other "off-label" uses have become the standard of care in some fields.

- ◇ Currently each manufacturer must develop its own individual policies for selling products for these end uses.
- ◇ To allow meaningful dialog with end users, to level the playing field between manufacturers with different policies, and to reduce regulatory and liability risks to the manufacturer, some sort of reasonable guidelines are needed.

► SOLUTION ZERO

- ◆ Consider cell culture media a raw material for use in further manufacture. It is the end user's responsibility to have appropriate specifications in place and to qualify the material for his end use. Sale for any application under current research use or in vitro diagnostic use labeling allowed.

► SOLUTION ONE

- ◆ Submit master files for product groupings with known off-label uses.
- ◆ Notify FDA when sales are made to end users without an IND/IDE
- ◆ 801.4 still a problem because product will not be labeled or qualified for the end user's intended application

► SOLUTION TWO

- ◆ Association of Medical Diagnostics Manufacturers (AMDM) concept paper for regulation of tissue culture media products
  - ◇ Tissue Culture Sub-committee comprised of:
    - ✣ BioWhittaker, Inc.
    - ✣ Hyclone Laboratories, Inc.
    - ✣ Irvine Scientific
    - ✣ JHR Biosciences
    - ✣ Life Technologies, Inc.
  - ◇ Provides for a generic label claim which we believe would meet the requirements of 21 CFR Part 801.4 "For cell and tissue culture processing"
    - ✣ Related products with known history of "off-label" use grouped into products families
    - ✣ Submit master file for each product family
    - ✣ Notify FDA of sales to known end users with no IND/IDE
    - ✣ For more specific label claims, submit 510(k) or PMA as appropriate

We are ready to move forward to resolve these issues which have been plaguing the industry since the mid 1980's. We are prepared to commit resources to work with FDA in designing a reasonable and equitable solution.

REGULATORY FRAMEWORK FOR ENSURING QUALITY OF PRODUCTS AND POTENTIAL LABELING

Mark W. Gauthier
Immunex Corp.

ABSTRACT

Immunex will present a framework for the regulation of ancillary products, for ensuring the quality of these products (including the applicability of cGMPs, safety from infectious agents, specifications, and stability testing), and their labeling to distinguish them from reagents or drug products that may already be approved or are under investigational use for in vivo treatment.

► REGULATORY FRAMEWORK FOR ANCILLARY PRODUCTS

- ◆ Quality Aspects
- ◆ Registration
- ◆ Conclusions
- ◆ Labeling

►ANCILLARY PRODUCTS FOR MANUFACTURE OF EX VIVO CELL PRODUCTS

►IMMUNEX CORPORATION

- ◆Develops cytokines/growth factors for in vivo therapeutic use
- ◆Provides cytokines/ growth factors for use as ancillary products in the manufacture of ex vivo cell products

►ANCILLARY PRODUCTS FOR USE IN THE MANUFACTURE OF EX VIVO CELL PRODUCTS *

- ◆Not part of the final stem cell product; intended to act solely on the cells and not on the patient
- ◆May have an impact on the safety, purity, or potency of the final stem cell product
- ◆Subject to either drug or device CGMP's
  - ◇*From "Draft Document Concerning the Regulation of Peripheral Blood Hematopoietic Stem Cell Products Intended For Transplantation or Further Manufacture Into Injectable Products", February 1996

►REGISTRATION PROCESS

- ◆Well defined for recombinant DNA products for in vivo therapeutic use
- ◆Not defined for cytokines/ growth factors used as ancillary products
- ◆Issues:
  - ◇Quality
  - ◇Drugs or device
  - ◇Registration process
  - ◇Labeling

►REGULATORY FRAMEWORK FOR ANCILLARY PRODUCTS

- ◆Quality:
  - ◇CGMP's should be applied
    - ✣ ie: drug vs. device
  - ◇Safety from infectious agents should be demonstrated
    - ✣ ie: viral inactivation/removal
  - ◇Specifications should be established
  - ◇Product should be stable
- ◆Registration
- ◆Labeling

►PRODUCT CONTROL STRATEGY

- ◆Material should be manufactured in compliance with GMPs and includes:
  - ◇Process validation
  - ◇Raw material controls
  - ◇In- process controls
  - ◇Bulk drug substance release testing
  - ◇Product release testing
  - ◇Stability evaluation

►SPECIFICATIONS

- ◆Appropriate release specifications should be established to ensure the safety and quality of the ancillary product. However, these may not need to be as rigorous as those for a licensed parenteral product
  - ◇Cell Culture Reagent ---> Ancillary Product --->Licensed Parenteral Product
- ◆Bovine Transferrin
  - ◇Quality
    - ✣ Appearance- off white lyophilized powder
    - ✣ Solubility- soluble at 1% in water
    - ✣ pH- 4.0 - 5.0
    - ✣ Residual moisture ≤ 5.0%
  - ◇Purity

- ✣ ≥ 98%
- ✣ Iron content

◇ Strength
- ✣ ≥ 95%

◇ Safety
- ✣ Endotoxin < 10 EU/mg
- ✣ Bioburden < 10 CFU/mg
- ✣ Adventitious viral agents - none detected
- ✣ Mycoplasma
- ✣ Cytotoxicity

◆ IGF-1

◇ Quality
- ✣ Appearance

◇ Identity
- ✣ MW 9110 daltons (by mass spectrometry)
- ✣ NTS > 95% single sequence

◇ Purity
- ✣ >70 %(by HPLC)

◇ Potency
- ✣ ng/ml (by L6 myoblast stimulation)

◇ Safety
- ✣ Endotoxin < 0.1 EU/μg

◆ Sargramostim (Leukine®)

◇ Quality
- ✣ Appearance
- ✣ pH
- ✣ Moisture
- ✣ Content uniformity
- ✣ Reconstitution Time

◇ Purity
- ✣ Protein content
- ✣ SDS-PAGE
- ✣ RP-HPLC
- ✣ SE-HPLC

◇ Identity
- ✣ Peptide map
- ✣ Western blot

◇ Strength
- ✣ BCA

◇ Potency
- ✣ TF-1 Bioassay

◇ Safety
- ✣ Endotoxin
- ✣ Sterility
- ✣ Particulate Matter

◇ Extensive testing conducted on the bulk drug substance

► PROPOSAL FOR ANCILLARY PRODUCT SPECIFICATIONS

◆ Identity - Single identity test

◆ Purity - Some product related impurities may be acceptable

◆ Strength/potency - in-vitro bioassay required

◆ Safety - product should not pose safety risk to process or patient

◇ (ie, sterility, endotoxins, viral contaminants)

◆ Stability testing: Perform specification tests at 0, 6, 12, 24 months at intended storage condition.

◆ Establish minimum testing requirements for release:

◆ Identity (One test sufficient)

- ◇ Peptide Map, Western Blot, HPLC
- ◇ Electrophoresis, N-Terminal Sequence
- ◇ Analysis, Mass Spectrometry

◆ Purity (One test sufficient)
- ◇ Chromatographic methods, SDS - PAGE

◆ Strength (One test sufficient)
- ◇ HPLC, UV Spectroscopy, Amino Acid Analysis
- ◇ Colorimetric redox methods

◆ Potency (one test sufficient
- ◇ In vitro Bioassay, Receptor Binding Assay

◆ Safety
- ◇ Bioburden
- ◇ Endotoxin
- ◇ Host cell DNA*
- ◇ Viral agents*
  - ✣ Demonstrate removal through process validation

► SAFETY

◆ Adventitious viral agents
- ◇ Viral contamination could ultimately compromise patient safety
- ◇ Viral inactivation/ removal should be demonstrated

◆ Host cell DNA
- ◇ The removal should be demonstrated through the validation of the manufacturing process

◆ Endotoxins

◆ Bioburden

► APPROACHES FOR REGULATION

◆ As in vitro reagents

◆ As recombinant DNA products for human use

◆ As "Manufacturing Materials" subject to class I, II, or III device designation

◆ As "Bulk Drug Substances" subject to drug regulation (21 CFR 314)

◆ As "Critical Reagent"

► MANUFACTURING MATERIALS - TWO OPTIONS

◆ CFR 864.2240 cell and tissue culture supplies and equipment
- ◇ Devices used to examine, propagate, nourish, or grow cells and tissue cultures - Class I devices

◆ CFR 820.60 (d) Manufacturing Material
- ◇ Any substance used to facilitate a manufacturing process and which is not intended to be included in the final device
- ◇ Must be removed from the final product or limited to a specified amount, with written procedures for use and removal

► DEVICE REGULATIONS

◆ Possible mechanisms for registration of ancillary products under the device regs exist
- ◇ GMP's less stringent than for drugs
- ◇ Labeling requirements may allow diversion
- ◇ May need additional safeguards to assure that patient safety and integrity of ex vivo cell product will not be compromised

► DRUG SUBSTANCES

◆ Master file required

◆ Manufacturer subject to FDA inspection

◆ Not formally subject to CGMP's, but the current trend is toward increasing applicability

◆ Change to alternate source of drug substance requires demonstration of equivalence & prior FDA approval

Master files are not officially "approved" but are reviewed and deficiencies must be corrected when referenced by an application

- **CRITICAL REAGENT**
  - Certificate of analysis needed
  - Reference to Master File or IND for method of manufacture
  - "Approved" in the context of the product of which the critical reagent is a component
- **RESPONSIBILITY OF EX VIVO CELL PRODUCT MANUFACTURER**
  - Change in ancillary product supplier would require
    - In vitro demonstration of equivalence in transduction efficiency
    - Prior FDA approval
  - Demonstration of removal of ancillary product from cell culture via highly sensitive assay (ie, ELISA, etc.)
- **CONCLUSION: PROPOSED REGULATORY FRAMEWORK**
- **FOR ANCILLARY PRODUCTS**
  - Recommend hybrid system
    - Submission of Master File/ IND for method of manufacture
    - Subject to drug CGMP's
    - Viral inactivation/ removal required
    - C of A required
    - Other safety tests (sterility, endotoxins)
-
- **LABELING**
  - We recommend use of the following statement for ancillary products:

"CAUTION: Ancillary Product for use in the manufacture of ex vivo cell products. Not for parenteral use."

- **ANCILLARY PRODUCTS CURRENTLY MANUFACTURED BY IMMUNEX**
  - GM-CSF (Sargramostim, LEUKINE®) - licensed product
    - stimulates macrophages and granulocytes
  - PIXY 321 (Milodistim, PIXYKINE) - IND
    - stimulates platelets, macrophages and granulocytes
  - Flt3 Ligand - IND
    - mobilizes peripheral blood progenitor cells.

LICENSURE OF CYTOKINES FOR EX VIVO USE

Mark Hukkelhoven, Ph. D.
Sandoz Research Institute
East Hanover, NJ

- **CYTOKINES ARE NECESSARY FOR THE EX VIVO**
  - Facilitation of retroviral vector-mediated gene transfer
  - Expansion of hematopoietic cells

As such they are used in most ex vivo gene therapy protocols and ex vivo expansion studies

Since they are intended to act on the cells and are not intended to have an independent effect on the patient, they are regarded as "ancillary products"

►HOW TO CURRENTLY OBTAIN AND USE THEM

- ◆Own manufacture (non-GMP) with release specifications
- ◆Buy as reagent, release them internally (develop certification plans)
- ◆If IND exists: obtain product and get cross-reference to IND from sponsor

►DISADVANTAGES OF CURRENT PRACTICE

- ◆Companies/investigators need to develop own certification plan
- ◆Access to some growth factors is difficult
- ◆Non-GMP grade material used (adventitious agents)

Potential solution for current practice would be to offer cytokines licensed for ex vivo use...

►ADVANTAGES

- ◆General access to an off-the-shelf product (both in clinical development and once a gene or cellular therapy product is licensed for therapeutic use)
- ◆no internal release testing
- ◆consistent GMP grade quality

►CONSIDERATIONS

- ◆If available, a licensed cytokine must be the preferred source for ex vivo protocols, especially in Phase III and certainly once a gene or cellular therapy product is licensed for therapeutic use
- ◆if not available FDA should allow an internal certification plan

►REGULATORY FRAMEWORK

- ◆Medical Device Regulations would be an appropriate, and the preferred, framework
- ◆If conceptually difficult, licensure as a "well characterized biotechnological product" could be considered

►LABELING

The indication for a licensed cytokine would be restricted to the ex vivo use of the product without claims on the reinfused cells:

- ◆Cytokine X is indicated for ex vivo use to facilitate:
  - ◇retroviral-mediated gene transfer into somatic cells (increase of transduction efficiency) prior to reinfusion of manipulated cells
  - ◇expansion of hematopoietic cells and their precursors prior to reinfusion of the expanded cell population
- ◆Cytokine X is not approved for direct in vivo use
- ◆The safety and efficacy of the generated gene therapy products or expanded cell population has not been established

Principle: Allow and promote the availability of licensed cytokines for ex vivo use without unnecessarily constraining the scientific field

►PROPOSED DATA REQUIREMENTS

- ◆CMC
- ◆Full CMC section with some flexibility to be allowed for:
  - ◇specs for release of drug substance and drug product (purity)
  - ◇stability program
  - ◇shorter description of manufacturing and test methods
- ◆In Vitro Pharmacology
  - ◇documenting the activity of the cytokine in the ex vivo process
- ◆Pre-clinical safety
  - ◇limited amount of pre-clinical tox data, e.g., acute tox study in appropriate species (also to cover overdoses)
  - ◇no clinical data (on cytokine and reinfused cell product

- No ELA

▶ CONCLUSION

Proposal provides easy access to consistently high quality cytokines without compromising developments in science and medicine in this area

BREAKOUT SESSION 3:
FACILITIES AND MANUFACTURING "THE GMP CONTINUUM"

SUMMARY

Jay Eltermann, R.Ph., M.S.

The breakout session on facilities and manufacturing was designed to be more educational rather than address specific problems. In this aspect we were somewhat different than most of the break-out sessions in this conference. We welcomed the opportunity to discuss facility and manufacturing issues, and receive comments to determine where additional guidance is still needed. The FDA presentations focused on several aspects of the design and operation of gene therapy facilities. We started with an overview of the regulatory aspects, what are GMPs and how they could be applied to manufacturing gene therapy products. We also went over some of the concerns we had for these facilities, with recommendations for design and operation of transduction and viral vector facilities. There were presentations regarding the specifics behind the design, operation and maintenance of a facility designed to manufacture gene therapy products. We understand that early clinical manufacturing will not be in compliance with full GMPs,. but attempts should be made to be in the spirit of GMPs. In keeping with the GMP continuum, we had presentations on academic and commercial scale facilities. They gave us an excellent overview of their facilities, important manufacturing issues, and some of the problem areas they have encountered. It was important to hear the industry's perspectives on these issues.

In summary, the following points were made during the breakout session.

- ◆We propose and support a sliding scale approach to meeting GMPs for the gene therapy facilities. We recognize that there are going to be differences in what a Phase I and commercial facility will be able to achieve with respect to process control and system validation.
- ◆We extended an invitation to manufacturers to discuss issues early in the process.
- ◆We request comments regarding the type and scope of guidance documents needed for gene therapy facilities.

►AGENDA

The goal of this breakout session is to provide an overview of the design and operational features that are recommended for facilities intending to manufacture gene therapy products. Specific topics will include an overview of the regulatory framework for the manufacture of these products, HVAC systems, environmental monitoring, equipment and validation issues, QC/QA oversight and appropriate documentation. Speakers from academia and industry will present the design, operation and manufacturing issues of these facilities, ranging from small Phase I facilities to commercial scale.

There will be two panel discussions to address questions that arise during the presentations.

►PART I. FDA PERSPECTIVE

- ◆Introductions
  - ◇Jay Eltermann, R.Ph., M.S.
- ◆Regulatory and GMP Considerations for Gene Therapy Facilities.
  - ◇Jay Eltermann, R.Ph., M.S., FDA/CBER, Division of Establishment Licensing
- ◆Facility Design Considerations.
  - ◇Robert Sausville, FDA/CBER, Division of Establishment Licensing
- ◆Operational Aspects.
  - ◇Mary Malarkey, FDA/CBER, Division of Establishment Licensing
- ◆Questions for the Panel.

► **PART II. ACADEMIC AND INDUSTRY PERSPECTIVES.**

- ◆ Gene Therapy Initial Manufacturing Facility.
  - ◇ William R. Tolbert, Ph.D., WR Tolbert & Associates
- ◆ University of Michigan Human Application Laboratory, Transition from GLP to GMP: An Academic Perspective.
  - ◇ Blake Roessler, M.D.
- ◆ Genetic Therapy Inc., Considerations and Strategies in the Planning and Design of a New Manufacturing Facility.
  - ◇ Victor Santamarina, Ph.D.
- ◆ Questions for the Panel.

Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

GTINFO@A1.CBER.FDA.GOV

REGULATORY AND CGMP CONSIDERATIONS FOR GENE THERAPY FACILITIES

Jay Eltermann, R.Ph., M.S.
Division of Establishment Licensing
CBER/FDA

ABSTRACT

Manufacture of products intended for human use, including products for clinical trials, should be performed according to current Good Manufacturing Practices (cGMP). This provides assurance that there is consistency between lots and that adequate controls are in place to assure the safety, efficacy, and potency of the product. The principles of cGMPs apply to the manufacturing process as well as the facilities and equipment in which products are product. This presentation will be an overview of the applicable regulations and guidance documents, with emphasis on those relating to equipment and facilities.

►REGULATORY AUTHORITY

- ◆Federal Register Notice of 10/14/93, Application of Current Statutory Authorities to Human and Somatic Cell Therapy and Gene Therapy Products"
  - ◇Regulated as biological products (CBER)
  - ◇Clinical trials conducted under IND
  - ◇Methods of manufacture and facilities conform with CGMP regulations
  - ◇Commercial ventures subject to licensure
- ◆RELEVANT GUIDANCE DOCUMENTS
  - ◇CFR 211 (facilities, records, personnel)
  - ◇CFR 600s (facilities, personnel, live viral vaccines)
  - ◇Guidelines (records, validation, testing)
    - ✣Aseptic processing
    - ✣Preparation of Investigational New Drug Products
  - ◇CDC/NIH booklet and RAC guidelines (facility design, control, special practices)

►CURRENT GOOD MANUFACTURING PRACTICES (CGMPS)

- ◆DEFINITION: "A set of current, scientifically sound methods, practices or principles that are implemented and documented during the development and production to ensure consistent manufacture of safe, pure and potent products."
- ◆Applies to the process as well as the facilities

►GMPS VS GLPS

- ◆Good Laboratory Practices (GLPs) are not Good Manufacturing Practices (GMPs)
- ◆GLPs (21 CFR 58) cover nonclinical facilities - testing, animal facilities
- ◆GMPs cover facilities manufacturing products for human use
- ◆Similar aspects covered by both sets of regulations (records, procedures, quality control)
- ◆Quality of materials, type of records, manufacturing controls are different

►PRINCIPLES ON WHICH CGMPS ARE BASED

- ◆Quality, safety, effectiveness must be designed and built into the product
- ◆Quality can't be inspected or tested into the product
- ◆Each step in the manufacturing process must be controlled to ensure final product meets specifications
- ◆Documentation provides evidence that compliance with cGMPs has been met

►CONTROL OF PRODUCTION STEPS FROM START TO FINISH

- ◆Vector development
- ◆ Cell bank characterization (also patient cells)
- ◆Final product characterization

- ◆ Production components (ancillary products)
- ◆ Testing (analytical, environmental, product)

►INFLUENCES ON FACILITY DESIGN AND OPERATION

- ◆ Production of vectors (single vs multiple)
- ◆ (viral vs non-viral)
- ◆ Transduction of patient cells
- ◆ Growth/harvest of transduced cells (Cell bank vs Patient cells)
- ◆ Multi-use scenarios
- ◆ Campaigning vs concurrent production

►CONCERNS COMMON TO ALL BIOLOGICALS

- ◆ Sterility, endotoxins, identity, purity, potency
- ◆ Regulation of both the product and the process
- ◆ Reproducibility/consistency between product lots

►PRODUCTION CONCERNS

- ◆ Characterization of final gene product, whether vector containing supernatant or transduced cells
- ◆ Appropriate test methods & validation
- ◆ QC/QA oversight
- ◆ Worker/environmental safety

►KEY CONCERNS WITH GENE THERAPY FACILITIES

- ◆ Cross contamination
  - ◇ Generation of aerosols
  - ◇ Spills
  - ◇ Campaigning within same area
  - ◇ Simultaneous production
- ◆ Potential for mix-ups
  - ◇ Segregation (clean vs dirty)
  - ◇ Labeling
    - ✣ at various production stages
    - ✣ (n different areas/rooms
- ◆ CHANGE OVER PROCEDURES
  - ◇ Cleaning/inactivation
  - ◇ Proper area clearance and storage
  - ◇ Regowning
- ◆ Control of the facility/process
  - ◇ Production area separate
  - ◇ Equipment dedicated for production
  - ◇ Restricted access
  - ◇ Proper maintenance/surveillance
  - ◇ Documented production/testing

►TRANSDUCTION AREAS: OVERVIEW

- ◆ Open manipulations performed in a biosafety cabinet (Class 100 conditions)
- ◆ HEPA-filtered air/Class 100,000 for manufacturing areas
- ◆ No containment features unless steps involve generation of aerosols/viral vectors
- ◆ Hoods cleaned between cell lines
- ◆ Personnel appropriately attired
- ◆ sterile gloves/mask with open steps

►VIRAL VECTOR PREPARATION AREAS: OVERVIEW

- ◆ HEPA-filtered air with containment features- modules or facility

- ◆ Double-door entrance to facility, usually and air-lock
- ◆ One vector per contained area at a time
- ◆ Open steps performed in biosafety cabinets
- ◆ Clean area between vectors
- ◆ Extra gowning in vector prep areas
- ◆ Change of gowning between vectors

► IN SUMMARY

- ◆ CBER has regulatory oversight for gene therapy products
- ◆ GMPs are applied, even for clinical trial materials
- ◆ Control process from start to finish
- ◆ Meet with FDA early to discuss facility, manufacturing, testing or clinical trials

FACILITY CONSIDERATIONS

Robert Sausville
Division of Establishment Licensing
CBER/FDA

ABSTRACT

The design of facilities should be based on their intended use. Materials of construction, Heating Ventilation and Air Conditioning (HVAC) systems; both for containment and product protection; gowning practices, multi-use issues and environmental monitoring programs will be discussed, with an emphasis on vector preparation and ex vivo transduction of patient's cells. Requirements for maintaining appropriate Biological Safety Level will also be addressed.

► GENERAL CONSIDERATIONS

- ◆ Designed for aseptic processing
  - ◇ Smooth surfaces, seamless tile, etc.
  - ◇ Easily cleanable and impervious finishes
- ◆ HEPA filtered air from separate air handling unit
  - ◇ separation of production from other areas of the facility (often a hospital)
  - ◇ Class 100,000: general manufacturing areas
  - ◇ Class 100: all open manipulations
- ◆ Separate entrance/gowning area
  - ◇ Air locks (containment)
- ◆ Room(s) for support areas (buffer, media prep.)

► STORAGE AREAS (RAW MATERIALS AND CELL BANKS)

- ◆ Material and personnel flows designed to maximize efficiency and minimize mix-ups
  - ◇ also controlled by procedures
  - ◇ unidirectional flows where possible
- ◆ Concurrent vs. campaigning
  - ◇ Segregation of:
    - ✣ HVAC and personnel
  - ◇ Changeover procedures in place

► APPROPRIATE BIOSAFETY LEVEL

- ◆ BioSafety Level 2
  - ◇ Transduction
- ◆ BioSafety Level 3
  - ◇ Vector Preparation

► **BIOSAFETY LEVEL 2**
- ◆ access limited
- ◆ personnel trained in handling pathogenic agents
- ◆ infectious wastes are decontaminated before disposal
- ◆ gowning required
  - ◇ gloves should also be worn if skin contact potential
- ◆ Class I or II BioSafety Cabinets to be used:
  - ◇ for procedures potentially creating aerosols
    - ✣ aerosol generation should be minimized
  - ◇ with high concentrations or large volumes of infectious agents

► **BIOSAFETY LEVEL 3**
- ◆ Negative pressure or "sink" for containment
- ◆ All activities with infectious materials are conducted in biological safety cabinets
  - ◇ Class I, II or III may be used
- ◆ Passage between two sets of doors is a basic requirement
- ◆ An autoclave for decontaminating waste is available
  - ◇ preferably in the laboratory
- ◆ Ducted exhaust provided
  - ◇ not recirculated
  - ◇ may be discharged to the outside without being filtered

► **BIOSAFETY LEVEL 4**
- ◆ Class III biosafety cabinets
  - ◇ or personnel in suits with life support systems
- ◆ All materials must be autoclaved before leaving BSL4 area
- ◆ Exhaust air HEPA filtered

► **AIR QUALITY**
- ◆ Recommend that production areas receive single pass air (no recirculation)
  - ◇ Dedicated air handler where possible
- ◆ HEPA-filtered air
  - ◇ In-line vs. terminal
  - ◇ Objective: to meet Class 100,000 specifications
- ◆ Air pressure differentials between areas
  - ◇ Positive (aseptic processing)
  - ◇ Negative (containment) for steps needing greater than BSL-2
- ◆ Open steps in biosafety cabinets (Class II)
- ◆ Monitored to assure facility is acceptable for production

► **ENVIRONMENTAL MONITORING**
- ◆ Demonstrate facility under control
  - ◇ as part of validation
- ◆ Evaluate periodically
  - ◇ routine monitoring program
- ◆ Viable/non-viable particulates and surfaces
  - ◇ specifications based on desired Class
- ◆ Viable particulates
  - ◇ active air monitoring devices
  - ◇ settling plates
- ◆ Non-viable particulates
  - ◇ particle counters
    - ✣ certification of biosafety cabinets and rooms

- ◆ Surface "contact" plates or swabs
  - ◇ monitor cleaning efficacy and personnel asepsis

► NASA STANDARDS: VIABLE AIR PARTICLES

- ◆ Class 100,000
  - ◇ 2.5 CFU/ ft2
- ◆ Class 10,000
  - ◇ 0.5CFU/ ft2
- ◆ Class 100
  - ◇ 0.1CFU/ ft2

► SETTLING PLATES: EXPOSURE TIMES

- ◆ Class 100,000
  - ◇ 1 CFU/ 9cm plate
    - ✣ 0.11 hours
  - ◇ 2 CFU/ 9cm plate
    - ✣ 0.21 hours
  - ◇ 1 CFU/ 14cm plate
    - ✣ 0.04 hours
  - ◇ 2 CFU/14 cm plate
  - ◇ 0.09 hours

OPERATIONAL ASPECTS

Mary Malarkey
Division of Establishment Licensing
CBER/FDA

ABSTRACT

The "sliding scale" approach to equipment and process validation, with respect to the GMP continuum, will be discussed. Other operational considerations including handling and testing of raw materials, personnel training and responsibilities, functions of the Quality Control/Quality Assurance unit (QC/QA), and the importance of proper documentation will also be presented.

► EQUIPMENT

- ◆ Certification
- ◆ Calibration
- ◆ Validation

► EQUIPMENT AND PROCESSES

- ◆ Program in place to demonstrate that equipment or process operates as expected
- ◆ Should include periodic monitoring
- ◆ Air handlers
- ◆ Biological safety cabinets
  - ◇ pressures
  - ◇ filter integrity
  - ◇ airflows; velocities
  - ◇ leak testing
- ◆ Incubators
  - ◇ uniform temperature
  - ◇ carbon dioxide
  - ◇ filters
- ◆ Centrifuges

- ◇ speed
- ◇ vacuum (ultras)
- ◇ temperature

◆ Autoclaves
- ◇ temperature
- ◇ pressure
- ◇ kill cycle

◆ Lyophilizers
- ◇ shelf temperature
- ◇ vacuum
- ◇ PROCESS

► "SLIDING SCALE " APPROACH: AN EXAMPLE

◆ Autoclave used to prepare sterile materials

◇ Early (Phase I/II)
- ✣ demonstrate proper cycle achieved
- ✣ monitor temperature, pressure, and time
- ✣ use of biological indicators for verification
- ✣ loads not well defined

◇ Middle (Phase II/III)
- ✣ temperature mapping done to determine cold/hot spots
- ✣ biological indicators placed to verify cycle at problems points
- ✣ loads are somewhat defined

◇ Late (Phase III+)
- ✣ lethality of cycle determined at monitored points
- ✣ loads are well defined and standardized
- ✣ each load configuration has been mapped or worst-case load has been validated

► PROCESS VALIDATION

◆ Sterilization

◆ Decontamination

◆ Aseptic Processing

◆ Cleaning

◆ Inactivation/removal of adventitious agents and other contaminants

► RAW MATERIALS

◆ Critical raw materials - established criteria for acceptance from vendors:
- ◇ sterility
- ◇ adventitious agents
- ◇ activity/purity

◆ Vendor's Certificate of Analysis
- ◇ identity test where possible

◆ Inventory Control
- ◇ proper storage
- ◇ FIFO

► WATER

◆ Should meet Water for Injection (WFI) specifications :
- ◇ microbials <10 CFU/100ml
- ◇ endotoxin <0.25 EU/ml
- ◇ chemical tests per USP

◆ WFI for all product contact surfaces and formulations

◆ May be purchased

► **PERSONNEL**
- ◆ Designated person in charge of facility
  - ◇ Responsible for:
  - ◇ limiting access
  - ◇ training
  - ◇ maintenance/safe operations
  - ◇ writing and enforcing procedures
- ◆ Production personnel are trained (periodic retraining)
- ◆ Appropriately gowned for production step

► **WRITTEN PROCEDURES**
- ◆ Develop and implement standard operating procedures (SOPs)
  - ◇ use of equipment
  - ◇ decontamination and cleaning
  - ◇ proper handling
  - ◇ Documentation in the form of
  - ◇ batch records
  - ◇ log books/recording charts
  - ◇ quality control test records

► **QUALITY ASSURANCE AND QUALITY CONTROL**
- ◆ QC "unit" to pass or reject:
  - ◇ raw materials
  - ◇ in-process materials
  - ◇ final "product"
- ◆ QA "unit" to review
  - ◇ batch records
  - ◇ test results
  - ◇ environmental results
  - ◇ evaluate effectiveness of current process/SOPs

► **FINAL THOUGHTS**
- ◆ Design facility for worst-case = maximum flexibility
- ◆ Consider filing a Master File for facilities handling several IND products

Meet with FDA to discuss your Phase I/II (or III!) facility.

GENE THERAPY INITIAL MANUFACTURING FACILITY

William R. Tolbert, Ph.D.
WR Tolbert & Associates

ABSTRACT

New gene therapy advances most often take place in academic institutions and early stage biotech companies. Transformation of these research break-throughs into approved new therapies for the treatment of human disease is a long an arduous task. One of the first road blocks is the bringing of research processes and facilities into compliance with Current Good Manufacturing Practices (CGMPs). Change, creativity and continual improvement are hallmarks of basic research, while safety, consistency, emphasis on process control, documentation and resistance to change are essential to ensure comparability of products from the clinic to commercial production. There is a real difference in philosophy that makes conversion of research process to biopharmaceutical manufacturing particularly difficult for research based institutions and scientists.

One of the first steps in the course of CGMP conversion is design of an initial manufacturing facility for preparation of clinical material to be used in human trials. This presentation will describe come of the basic facility requirements for production of viral vectors for either in vivo or ex vivo applications. Support facilities will also be addressed.

►GENE THERAPY FACILITY
- ◆Research vs. CGMP Requirements
- ◆Required Functions
- ◆Facility Planning
- ◆Aseptic and Contained Processing
- ◆Facility Design
- ◆Flow Patterns

►RESEARCH VS. CGMPS
- ◆Philosophical Approach
  - ◇Research - Change, Improved Methods, New Knowledge
  - ◇CGMPs - Purity, Safety, Efficacy, Consistency
- ◆Practical Considerations
  - ◇Research - Decision at Level of Researcher
  - ◇CGMPs - Regulation Compliance, Documentation, No Major Operator Decisions

►REQUIRED FUNCTIONS AND AREAS
- ◆Receiving / Quarantine Storage
- ◆Released Materials Storage
- ◆Quality Control Laboratory
- ◆Cell Bank
- ◆Aseptic / Contained Processing Laboratories
- ◆Mechanical Space
- ◆Quality Assurance / Documentation Area

►FACILITY PLANNING
- ◆Process Definition / Scale of Operations
  - ◇Vector Production - In vivo / Ex-vivo
  - ◇Patient Cell Transduction
  - ◇Number of Products / Patients
  - ◇Vector Type / Level of Containment
- ◆Clinical Phase I, II, III / Initial Commercial
- ◆Internal Institutional Applications or External Sale of Products
- ◆Limitations of Space, Personnel, Financial Resources - Maximize Flexibility
- ◆Renovation of Existing Laboratories Areas or New Construction
- ◆Autoclave and WFI or Pre-sterilized Single Use Disposables and Prepared Medium
- ◆Stick-Built or Modular Construction
- ◆Schedules for Construction/Validation/Use

►ASCEPTIC/CONTAINED PROCESSING AREAS
- ◆Class 100,000 HEPA Filtered, Single Pass Air - Recommend Low Filtered Air Returns
- ◆Defined Air Flow and Pressure Differentials
  - ◇Aseptic Areas - Positive Air Pressure
  - ◇Contained Areas - Negative Air Pressure
- ◆Class II, Type B Biological Safety Hoods
- ◆Seamless Welded Flooring with Covering
- ◆Solid Ceiling with Sealed Lighting
- ◆Epoxy Resin Wall and Ceiling Coating

- ◆All Penetrations Sealed (Silicone Caulk)
- ◆No Personnel Access Between Vector Production and Transduction Areas
- ◆Controlled Access - Recommend Card Sys
- ◆Emergency Power for Critical Systems
- ◆Recommend Interlocking Gowning Room Doors and Interlocking Pass Throughs
- ◆Recommend Stainless Steel Work Surfaces and No Cabinets, Drawers, Sinks or Drains
- ◆Recommend Use of Dedicated Rolling Carts for Material Transfer
- ◆Recommend Full Coverage Single Use Disposable Gowning

►FACILITY DESIGN EXAMPLE

- ◆Two Vector Production Laboratories
- ◆Two Transduction Laboratories
- ◆No Autoclave - Pre-Sterilized Disposables
- ◆Access from One Side - General Use Hall
- ◆Mechanical Space Available in Penthouse
- ◆Manufacturing Space - 1800 Sq. Ft.
- ◆Support Space - 700 Sq. Ft.

►SUMMARY

- ◆Conversion of Research Advances to CGMP Production is Challenging
- ◆A Well Designed Facility Provides a Controlled Environment for Manufacturing
- ◆Facility Design Should Enhance Process Consistency by Defining Flow Patterns
- ◆Facility - Procedures - Personnel Must All Be Combined for Successful Manufacturing

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TRANSITION FROM GLP TO GMP: AN ACADEMIC PERSPECTIVE

Blake Roessler, M.D.
University of Michigan Human Applications Laboratory

ABSTRACT

The purpose of The Human Applications Laboratory (H.A.L.) At the University of Michigan Hospital is to provide a physical plant for medium scale production of biological reagents and culture of human cells for autologous transplantation as required for Phase I/II investigator-initiated studies.

The University of Michigan H.A.L. is currently nearing completion of a major renovation project that will transform the H.A.L. from a single room facility into a multi-room, multi-use biological production facility. An overview of the transition process from a smaller to a medium sized facility will be discussed. Discussion will also include an overview of problems encountered in the design, construction, start up, and daily operation of a medium sized facility in an academic environment.

►LIMITATIONS:

- ◆Suboptimal GMP
- ◆One room, one time, one project
- ◆Materiel storage
- ◆Product storage

► **LABORATORY GOALS:**
- ◆ Performance of multiple projects
- ◆ Vector production
- ◆ Autologous cell culture
- ◆ 'Not too big, not too small'

► **LABORATORY REDESIGN:**
- ◆ Modular floorplan
- ◆ Efficient utilization of available space
- ◆ Worker safety and convenience
- ◆ Ease of cleaning
- ◆ Quality air

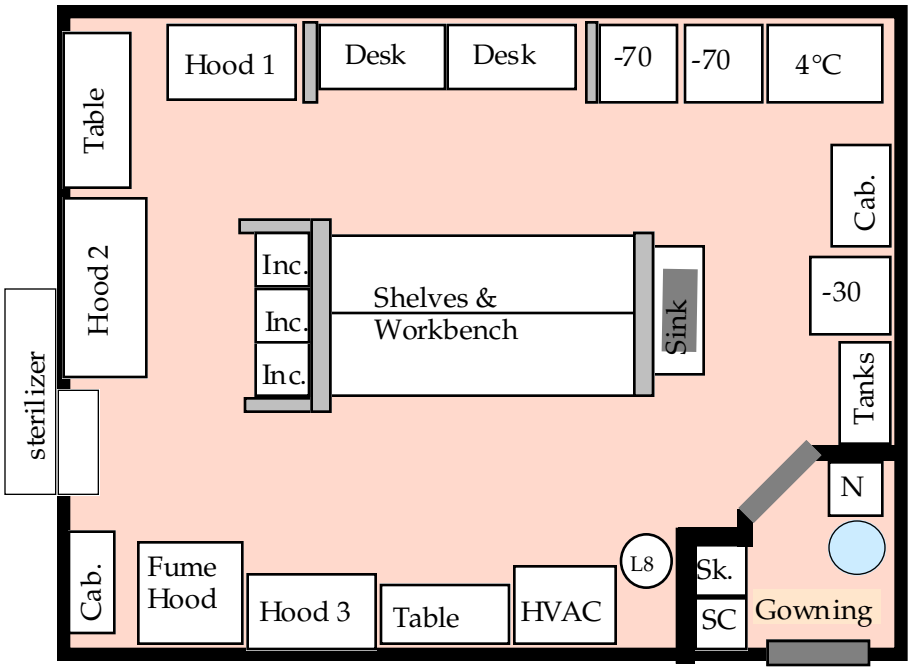
► **CONSTRAINTS:**
- ◆ Existing building
- ◆ Fixed floorspace
- ◆ Shared HVAC
- ◆ Total cost

► **SPECIFICATIONS:**
- ◆ 750 square feet
- ◆ 3 laminar flow hoods
- ◆ 3 pair stacking incubators
- ◆ Class 10,000 air
- ◆ One technician

► **PRODUCTION STATISTICS:**
- ◆ Retroviral supernatants
- ◆ Batch size 1-5 liters
- ◆ 5 batches per annum
- ◆ Contract validation

► **SPECIFICATIONS:**
- ◆ 1500 square feet
- ◆ 4 production rooms
- ◆ Class 100,000 air
- ◆ Class 100 hoods
- ◆ Two technicians

The Human Applications Laboratory (1992-1995)



The Human Applications Laboratory 1996-

CONSIDERATIONS AND STRATEGIES IN THE PLANNING AND DESIGN OF A NEW FACILITY

Victor Santamarina, Ph.D.
Genetic Therapy, Inc.

- **THE MOST CRUCIAL BUILDING COMPONENTS ARE:**
  - Physical isolation of steps
  - Cleaning and decontamination
  - HVAC
- **TIME TO MARKET MUST BE SHORTENED, SEVERAL**
- **CONSTRUCTION-RELATED OPTIONS EXIST:**
  - Initiate construction of a purpose built facility during phase II/III Clinical Trials
  - Enter into contract manufacturing agreement.
  - Construct an interim limited facility
- **CONSIDERATIONS AND STRATEGIES IN THE PLANNING & DESIGN OF A NEW MANUFACTURING FACILITY**
  - Issues
    - Timing - Schedule
    - Scope - Design Requirements
  - Scope
    - Process Definition
    - Defines process requirements
    - Process flow diagrams
    - Defined by Phase III
    - Marketing Requirements
    - Forecast determines production levels
    - Plant capacity and construction schedule
    - Plant Definition
    - Process
    - Capacity
    - Floor plan/Building components
  - Plant Definition
    - Process Flows
    - Material Flows
    - Equipment Flows
    - Personnel Flows
- **PLANT DEFINITION**
  - Market Assessment
    - Initial
    - Market Increase
  - Pre-launch Inventory
    - Initial Market Needs
    - Stability
  - Post Launch Production Requirements

▶ PLANT DEFINITION

Process Areas
Consistent with process steps
Unidirectional flows

Physical Isolation
Process Areas
Process - Non process areas

Support Activities
QC
Materials Handling
Final product storage & shipping

Utilities
Environment control - HVAC
Sanitary & non sanitary systems

- ◆ HVAC
  - ◇ Determined by floor plan and HVAC design
  - ◇ Room Classification
    - ✣ Processing Areas
    - ✣ Airlocks
    - ✣ Corridors
  - ◇ Pressure differences & flows
  - ◇ Multiple vs dedicated air handlers
  - ◇ Recycle vs one-pass-thru
- ◆ Isolation (Barrier) Technology

▶ SCOPE-DESIGN REQUIREMENTS

- ◆ Room Classification
  - ◇ Class 100,000
    - ✣ Corridors processing areas airlocks
  - ◇ Class 10,000
    - ✣ All processing areas & airlocks between processing areas
  - ◇ Class 1,000
    - ✣ Filling room if required by scope
  - ◇ Class 100
    - ✣ All open operations
  - ◇ Air Flows
    - ✣ Clean room vs containment issues compromise
    - ✣ Clean room positive toward access/exit airlocks
    - ✣ Airlocks slightly negative toward the outside or
    - ✣ access corridors to provide containment
  - ◇ Air Handlers
    - ✣ Preferably dedicated in critical areas
    - ✣ Required dedicated air handler for filling room
  - ◇ HVAC Design Options
    - ✣ One-pass-thru recommended as permitted by economy of design

▶ ENGINEERING

- ◆ Schematic design should start and be updated as process develops into clinical trials. Complete when Phase III starts
- ◆ Development design should parallel Phase III followed by detailed design
- ◆ Validation
  - ◇ The validation issues must be addressed in the schematic design and should parallel the design development

▶ CHARACTERISTICS OF HVAC OPTIONS

Single Pass
Less Mechanical Equipment
Expensive to Operate
Low risk of cross contamination of system

Recirculated
More Mechanical Equip (Blowers, Ductwork)
Cost Effective Operation
Potential for cross contamination (Exhaust air recycled)

Burnett, M.B., Santamarina, V.G., Omstead, D.R. (1991).Design of a Multiple Purpose Biotech Pilot Plant and Production Facility. Recombinant DNA Technology I. Annals of the New York Academy of the Sciences, Vol. 646, pp. 357-366. New York Academy of Sciences, New York.

► SUMMARY

- ◆ Timing
  - ◇ Conceptual design should start with clinical trials
  - ◇ Full process design and conceptual plant design should be in place by phase III
- ◆ Scope
  - ◇ Decision for plant size should be in place with market data by phase III.
  - ◇ Design should incorporate as much as possible the following:
    - ✣ Unidirectional flows
    - ✣ One-pass-thru and dedicated air handlers
    - ✣ Combination of positive and negative airflow to airlocks to accommodate clean room requirements and containment to the outside
  - ◇ Support activities such as QC labs, materials handling of raw materials and finished products and shipping should be included in the envelope of a manufacturing plant design

BREAKOUT SESSION 4:
GETTING STARTED IN GENE THERAPY VECTOR DEVELOPMENT

SUMMARY

Angus J. Grant, Ph.D. FDA/CBER

During this session, we heard a series of presentations that were intended to help investigators bring gene therapy ideas from the research bench to phase I clinical trials. This endeavor has challenges unique to gene therapy. To make matters more difficult, as in any emerging technology, the challenges keep shifting. For example, from a regulatory perspective, review by the NIH-ORDA-RAC has been changing and may soon be a thing of the past. Special considerations must still be taken into account for the societal and human subject issues involving the utilization of this technology. The IRBs, the proposed NIH-ORDA-GTPC specific subject conferences, and the FDA will oversee the development and use of gene therapy technologies. As an investigator confronts the challenges in bringing gene therapy protocols to the clinic, a great number of skills and resources will be tested. The presentations within this session were designed to provide insight and information to make these challenges more attainable.

The first set of presentations pointed out the importance of evaluating the strengths and weaknesses of ones particular gene therapy program and how to use this information in the early planning process. It was pointed out how good planning can avoid costly and time consuming mistakes. While experience in the strategic planning of gene therapy vector development can be of great utility, so can the experience of a variety of contract services. For investigators unfamiliar with the IND process, there are resources that can be turned to, some for a fee, that can reduce or eliminate fatal flaws in your program development. There are a variety of consulting and contracting companies that can cover the spectrum of tasks involved in gene therapy program and product development, including support for overseeing the legal ramifications in other corporate interactions. In addition, there are companies that in addition to providing specific services not immediately available in every institution, can provide valuable advice on a variety of related issues for the overall gene therapy program due to their experience with other programs. Outsourcing support for this fast moving technology can provide established experience and cutting edge technologies, that are in place, that may be especially important for the smaller institutions.

The second set of presentations centered more on the production of the gene therapy vectors. The GLP and GMP continuum was discussed for investigators planning on producing the product themselves or for investigators that need to understand what is required or necessary when they discuss outsourcing the production. Finally, we heard from a company that, for a fee, can produce the entire gene therapy vector, ready for use in the clinical trial.

In all of the presentations, it was made clear that bringing a gene therapy idea from the research stage to the clinic is an arduous undertaking and requires a tremendous number of skills and resources. One recurrent theme was the need for good communication. It usually requires a large number of people and organizations to successfully bring a product to the clinic, and good communication is essential. Also, while there may be a substantial cost to outsourcing for strategic planning, for regulatory and legal support, for outsourcing quality control, or for outsourcing the entire manufacturing process, the savings in time and mistakes may easily make up for the cost. Good communication is also important for interactions with the FDA. By frequent and open communication with the FDA, an investigator can avoid costly and time consuming delays and can get a lot of free advice. It is clearly important for each investigator to evaluate their own resources, expertise, facilities and goals so that they can determine the best course of action to bring their ideas to the clinic.

During this conference and during this session we have heard a sample of the support that can be utilized in the field of gene therapy, but it was not an exhaustive collection. We hope that through these presentations we have helped provide investigators with useful information that will bring their ideas from the bench to the bedside more effectively and that these therapies will eventually lead to improvements in the treatment of human diseases.

►AGENDA

The goals of the session center on enabling investigators to bring their ideas from the bench to the bedside. It is intended that this session will build on the lessons learned from he first day of the conference, where the FDA IND considerations and some NIH resources were presented. However, there are a considerable number of additional challenges that must be dealt with when planning to bring new therapies into human subjects. It is not unusual for an institution, organization, or corporation to lack the capabilities and expertise necessary to accomplish all the tasks involved in bringing a gene therapy product to the clinic. In such cases, the investigator leading the gene therapy project must turn to outside resources to provide the necessary expertise, support or services.

During this breakout session, there will be a series of presentations that will cover strategic planning of product and protocol development, important regulatory and legal considerations when preparing to submit an IND, resources to support IND submissions, and resources to support production of the gene therapy vectors and the quality control characterization of the gene therapy product. In addition to the presentations, there will be an opportunity for questions and discussion.

- Introduction.
  - Angus J. Grant, Ph.D., FDA/CBER
- Starting a Clinical Gene Therapy Program: Planning & Implementation.
  - Bruce Merchant, M.D., Ph.D., Merchant-Taylor International, Inc.
- Key Issues and Factors in Developing the Gene Therapy IND.
  - Gary E. Gamerman, M.S., J.D., Fenwick & West, LLP
- Contract Services Supporting Gene Therapy Development and Clinical Trials.
  - Jeffrey M. Ostrove, Ph.D.. Microbiological Associates, Inc.
- Implementation of a Phase I Gene Therapy Program in a GLP-GMP Continuum.
  - James A. Taylor, Ph.D., Merchant-Taylor International, Inc. -
- Monoclonal Antibody and Recombinant Protein Production Facility (soon to add viral vectors), National Cancer Institute, Frederick Cancer Research & Development Center.
  - Steven Creekmore, Ph.D., M.D., National Cancer Institute -
- Considerations for Process Development and Manufacturing.
  - Shawn L. Gallagher, MAGENTA Inc.

Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

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STARTING A CLINICAL GENE THERAPY PROGRAM: PLANNING AND IMPLEMENTATION

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ABSTRACT

Developing a new Clinical Gene Therapy program is a daunting project. In all likelihood, it will involve seeking objectives, marshaling resources, and confronting tasks that you have not confronted before. It will challenge your coalition-forming, team-building and communication skills in ways that they have not been previously challenged. It will set you face-to-face with new concepts, new frustrations, new opportunities, and new rewards.

Gene Therapy offers the potential to intervene in chronic diseases at a primary "first cause" level. Optimally, it will make available therapies for many diseases which currently lack effective treatments. By spearheading the development of a Clinical Gene Therapy program in your organization, you can take leadership in bringing "closure" between cutting-edge basic Molecular Biology and meaningful applications of this core science in the clinic. The whole program can be "tracked" by a special road map called a Master Project Schedule which can be used as an extremely effective tool both in planning and in implementation.

Making your Clinical Gene Therapy program a successful reality, will depend upon your being well informed, highly motivated, and adequately resourced. Included among essential resources are crucial funding, skilled and talented clinical and scientific colleagues, and a well motivated, team-oriented staff. You will need to develop a mind set that is open to new ideas, that values both afferent and efferent communication, and that espouses the principals of good project management. There will be much to learn as you proceed, including new scientific concepts, special clinical considerations, basic regulatory principles, good manufacturing practices, and patient-centered ethical sensitivities.

► STARTING A CLINICAL GENE THERAPY PROGRAM: PLANNING AND IMPLEMENTATION

- ◆ The Incentives are to:
  - ◇ Intervene in chronic diseases at a primary "first cause" level.
  - ◇ Provide important new "cutting edge" therapies to patients.
  - ◇ Make available therapies for many diseases lacking currently available treatment.
  - ◇ Effect "closure" between basic science and meaningful clinical applications.

► PLANNING: THE INITIAL STEPS—COMMIT TO WRITING

- ◆ Specific Objectives of the Program.
- ◆ Early - Intermediate - Long Term
- ◆ What constituencies will be served?
- ◆ What resources are available?
  - ◇ Financial—Institutional/Funding Sources
  - ◇ Space/Facilities
  - ◇ Human—Scientific/Clinical
  - ◇ Human—Managerial/Manufacturing/Quality Control

► PLANNING: THE INFORMATION GATHERING STEPS

- ◆ What internal resources are available and committed?
- ◆ What solid external resources can be counted on?
- ◆ What "mix" of these resources is right for your organization?
- ◆ How is that "mix" likely to evolve over the next several years?
- ◆ What contingencies are available in case the program evolves differently than expected?

► PLANNING: THE DECISION MAKING STEPS

- ◆ Who the Clinician/Scientists will be.

- What Departments/Divisions will be involved.
- What the Budget will be (& Funding Sources).
- Who the Project Leader will be (Accountability).
- What Space/Facilities will be available (When).
- What human talent will be provided.
  - Internal
  - External
- What contingencies will be provided for.

►IMPLEMENTATION

- Determine what the first clinical study will be.
- Determine if you will develop a Vector Production Facility.
- Develop a Master Project Schedule (Importance of cGMPs).
- Recruit Key Personnel.
  - Production Facility Manager
  - QA/QC Manager
- Design a Production Facility (Spirit of GMP).

►IMPLEMENTATION—FACILITY & DESIGN

- Decide What Equipment will be required.
- Get good advice on the basic Facility Design.
  - From colleagues "who've been there"
  - From qualified outside experts
  - From Workshops/Forums
  - From the FDA. (Pre-construction Package and Meeting)
- Engage an Architectural Firm.
- Settle on the final Plan.
- Conduct the bidding process.
- Obtain Permits.
- Do the Construction.
- Perform Validation on the Facility, the Equipment and on Key Production Processes.

►IMPLEMENTATION—PROCESS DEVELOPMENT

- Determine what vector(s) will be used.
- Determine which labs will be dedicated areas.
- Determine which labs will be "campaign" supporting.
- Develop Probable Production Scenarios and Flow Patterns.
- Determine a list of 25-50 Key Production Activities and develop SOPs for each.
- Understand that SOPs will evolve (both in content and in numbers).

►IMPLEMENTATION—CLINICAL/REGULATORY MANAGEMENT

- Move Clinical Plans from Outlines to Finished Protocols.
- Augment Dialogue with CBER (e.g. Pre-IND Meeting).
- Gain IRB and IBC approvals.
- Prepare and Submit an IND (Possibly also a separate DMF).
- Register Study with NIH ORDA.
- Initiate Gene Therapy Clinical Trials.

►PITFALLS TO BE AVOIDED

- Trying to do everything yourself. Teamwork is Essential.
- Assuming that Scientific Excellence assures Manufacturing Reliability (Two District Disciplines).
- Assuming that there will be no major glitches (Develop contingency Plans).
- Assuming that all your colleagues are marching to the same drummer.

- ◆ Failing to communicate thoroughly and frequently.
- ◆ Failing to recognize team accomplishments.
- ◆ Failing to develop follow and modify (as required) a Master Project Schedule.

► REWARDS

- ◆ Bringing cutting edge gene therapies to bear on problematic genetic & chronic diseases.
- ◆ Learning practical "nuts and bolts" realities about quality product Manufacture.
- ◆ Working as a team with skilled, and dedicated Scientists, Clinicians, Regulators and Outside Experts

Effecting "closure" between basic Molecular Biology and those Diseases which be favorably treated by gene

KEY ISSUES AND FACTORS IN DEVELOPING THE GENE THERAPY IND

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ABSTRACT

Developing a gene therapy IND requires consideration of a number of preclinical and clinical issues, some of which are common to all biotherapeutics, others are unique to this technology. This presentation will identify the most important issues and strategies for addressing them in the IND and product development plan. The discussion will be highlighted with examples of the types of problems that can sidetrack a gene therapy IND.

► DEVELOPMENT PROCESS MANAGEMENT AN INCREMENTAL DYNAMIC APPROACH

- ◆ Many Points for Binary/Multivariable Decisions During Development Cycle
  - ◇ Go/No Go
  - ◇ Process Selection
- ◆ Early Choices Channel Later Options and Selection
  - ◇ Development Candidate
  - ◇ Manufacturing Technology
  - ◇ Clinical Development Path
  - ◇ Cell Substrate
  - ◇ Formulation

► QUESTIONS TO GUIDE IND DEVELOPMENT & PLANNING

- ◆ Regulatory Science v. Bench Science
- ◆ Questions to Address Before Any Testing
  - ◇ What is the Product
  - ◇ How to use It
  - ◇ How to Test It
  - ◇ What will it Do
  - ◇ How to Make It
  - ◇ How to Sell It

► WHAT IS THE PRODUCT?

- ◆ Product Characteristics
  - ◇ Identity
  - ◇ Composition
  - ◇ Inherent Impurities
  - ◇ Activities

- ✣ Direct
- ✣ Collateral

◆ Process Characteristics
- ◇ Product Variability & Heterogeneity
- ◇ Process Limitations On Purity & Composition

► WHAT WILL IT DO?

◆ Intended Biological Effects
◆ Expected Collateral Effects
◆ Possible Risk Events
◆ Indicators of Activity
- ◇ Early Low Dose Treatment
- ◇ Higher Doses

◆ Indicators of Toxicity
◆ Traceability of Event Product

► HOW TO USE IT?

◆ Indication
◆ Population
◆ Dose
- ◇ Amount, Schedule, Route, Duration

◆ Formulation
◆ Setting

► HOW TO MAKE IT?

◆ Safe
◆ Consistent
◆ Validatable
◆ Scaleability
◆ Robust/Controllable
◆ Efficient/Cost-Effective
◆ Upstream/Downstream Considerations

► HOW TO TEST IT?

◆ Good Science Not Matrix Repetition
- ◇ Follow the Product's Nature
  - ✣ Preclinical: Laboratory Testing
    - Product Characterization
    - Process Characterization
  - ✣ Preclinical: Animal Testing
    - Model Human Biology
    - Model Human Protocols
  - ✣ Human Testing
    - Relevant, Goal-Oriented Design
    - Consider Practical Issues

► CONTRACTS IN GENE THERAPY DEVELOPMENT

◆ Types of Contracts
- ◇ Contract Manufacturing Organizations (CMO's)
- ◇ Contract Research Organizations (CRO's)
- ◇ Research Agreements:
  - ✣ Investigators, Institutions, Others, Patient Consent Forms
- ◇ Material Transfer Agreements
- ◇ Consultants/Employees

► **SELECTING CONTRACTORS**
- ◆ CRO's, Contract Manufacturers, Testing Labs:
  - ◇ Do They Have Relevant Expertise
  - ◇ Will They Service YOU
  - ◇ How Will Problems Be Resolved
  - ◇ Do They "Unsell" or Let You Fly Blind
  - ◇ What/How Have They Done Lately
- ◆ To Them It's a Contract, To You It's the Company:
  - ◇ You Cannot Surrender Control or Responsibility
  - ◇ You Need to Understand What Must Be & Will Be Done
  - ◇ Trust, But Audit!
- ◆ Contracts:
  - ◇ Everything is Negotiable
  - ◇ Regulatory Control is Essential

► **MAJOR CONTRACTING ISSUES**
- ◆ Respective Obligations
- ◆ Intellectual Property
- ◆ Regulatory Compliance
- ◆ Dispute Resolution & Enforcement
- ◆ Warranties, Indemnification's & Remedies

► **RESPECTIVE OBLIGATIONS**
- ◆ Need to Detail Mutual Responsibilities:
  - ◇ Who, What, Where, When & How
  - ◇ What Standards Will be Used for Performance
  - ◇ Task & Payment Schedule
- ◆ Procedures for Charges & Deviations
- ◆ Resource Allocation
- ◆ Procedures for Remedies If Faulty Performance:
  - ◇ Discussion, Arbitration, Litigation
  - ◇ Refund/Rework
  - ◇ Limitation of Liability

► **REGULATORY & PERFORMANCE COMPLIANCE**
- ◆ Qualify Contractors
- ◆ Performance Guaranty & Certification
- ◆ Contractor's QA Efforts
- ◆ Your Monitoring & Auditing
- ◆ Disclosure of FDA Inquiries & Inspections
- ◆ Non-FDA Obligations
  - ◇ EPA, OSHA, Employment, State, Local & Foreign Law

► **INTELLECTUAL PROPERTY**
- ◆ Primary Rights
  - ◇ Patents
  - ◇ Trade Secrets
  - ◇ Copyrights
- ◆ Derivative Rights
  - ◇ Product Related
  - ◇ Study Related
  - ◇ Other
- ◆ Use & Disclosure

- ◇ Technology & Data
- ◇ Publicity/Status

► DISPUTE RESOLUTION & ENFORCEMENT

- ◆ High Level Discussion
  - ◇ Most dispute can be resolved if addressed early
  - ◇ Evaluate the reason and significance for discrepancies
- ◆ Economic Leverage
  - ◇ Payment Schedules & Termination
- ◆ Arbitration
- ◆ Litigation
  - ◇ A very last resort
  - ◇ Keep good documentation
- ◆ Foreign Contractors?

► WARRANTIES, INDEMNIFICATIONS & REMEDIES

- ◆ Warranty = Promise on Current or Future Status
  - ◇ Patent
  - ◇ Regulatory Compliance
  - ◇ Performance
  - ◇ Capabilities
- ◆ Indemnifications = Coverage Against Third Party Actions
  - ◇ Standards, Causes
  - ◇ Respective Duties
- ◆ Remedies = Rights in Events of Breach
  - ◇ Injunctions
  - ◇ Money
  - ◇ Performance reprocess, rework)
  - ◇ Other (facility take over)
- ◆ Limitations of Rights
  - ◇ types & amounts

► CONTRACT MANUFACTURING ORGANIZATIONS

- ◆ Duties & Timing
- ◆ GMP & Process Definition Compliance
- ◆ Subcontracting
- ◆ Auditing
- ◆ Document
- ◆ Intellectual Property
- ◆ Warranties/Indemnification/Remedies

► CONTRACT RESEARCH ORGANIZATIONS

- ◆ Detailed Description of Tasks
  - ◇ What is Included
    - ✣ Monitoring & Audits
    - ✣ Data Handling & Timing
    - ✣ Report Generation
    - ✣ GDEA Certification
  - ◇ Who will be assigned
- ◆ Modifications
  - ◇ Extension & Alteration of Protocol
  - ◇ Ongoing, Interim & Final Reports
- ◆ Inspections & Documentation
- ◆ Payment Schedules

► **CLINICAL RESEARCH AGREEMENTS**
- ◆ Investigators
  - ◇ Compliance: Reference Investigators Brochure, Audits/Inspections
  - ◇ IP & Publications
  - ◇ Conflicts & Time
  - ◇ Payment Basis & Schedule
  - ◇ Patients & Study coordinators
- ◆ Institutions
  - ◇ IRB Compliance
  - ◇ Costs
  - ◇ Indemnifications
  - ◇ Accreditations
  - ◇ Employees

► **CONSULTANTS & EMPLOYEES**
- ◆ Intellectual Property
- ◆ Regulatory Compliance
  - ◇ Future Actions
  - ◇ Not debarred/disqualified
- ◆ Confidentiality
- ◆ Conflicts/Exclusivity
- ◆ Non-Compete Issues
- ◆ General Employment Concerns

► **MATERIAL TRANSFER AGREEMENT**
- ◆ Determine How Material Can Be Used
  - ◇ Will there be grant back of data rights?
  - ◇ Will use be consistent with regulatory objectives?
- ◆ State Who Owns Derivative IP
- ◆ Limit Who the Material Be Transferred To
- ◆ Include Warranty Disclaimers
- ◆ Ensure Proper FDA Labeling and/or End-User Certifications

► **PATIENT CONSENT FORMS**
- ◆ Only Protects Against Study Risks
- ◆ Does Not Protect Against
  - ◇ Unmentioned risks
    - ✣ associated with investigational product
    - ✣ associated with procedures
  - ◇ Negligence or wrongful act by company or investigator
  - ◇ Breach of regulatory of other obligations
- ◆ Must be Drafted and Executed In Compliance with FDA & Other Regulations
- ◆ Need to Update As New Risks are Discovered
- ◆ Do Not Minimize or Mischaracterize Risks or Benefits

► **KEY CONTRACTING PRINCIPLES**
- ◆ Avoid Mumbo-Jumbo
  - ◇ Clear, Concise, Complete, Correct
  - ◇ Check the Boilerplate
- ◆ Sign in Haste, Suffer in Leisure
  - ◇ Ask Questions Before, Not After
  - ◇ Verify Obligations and Remedies
- ◆ Communicate Regularly
  - ◇ Know What Your Contractor is Doing

◇ Let Them Know What You Expect
◇ Check Project Status

CONTRACT SERVICES SUPPORTING GENE THERAPY DEVELOPMENT AND CLINICAL TRIALS

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ABSTRACT

This presentation will focus on what is required to move a gene therapy project from the research laboratory into the clinic. The first step in this process is to ensure that the cell substrates that are being used to produce viral-based vectors for gene therapy are free of common, adventitious contaminants such as mycoplasma and replication-competent viruses. The next step is the establishment of a Master Cell Bank (MCB) and its characterization according to the FDA "Points to Consider" documents. The safety tests that are required to be performed on each lot of clinical supplies manufactured will also be described. Data on the incidence of common microbial and viral contaminants that have been detected in mammalian cell substrates used to produce gene therapy vectors and the incidence of replication-competent retroviruses (RCR) and adenoviruses (RAC) will be presented. Perhaps most important, the above process will be described with the context of the timing required for each step in the process. Planning and execution to a timetable are critical factors in reaching the clinic in a timely fashion.

Other services available for scientists developing gene therapies such as the biodistribution of gene therapy vectors administered to animal models and associated toxicology studies will be presented. Microbiological Associates has also developed assays for the monitoring of patients undergoing retroviral-mediated gene transfer. Western Blots have been qualified that can detect the presence of antibodies against amphotropic retrovirus-based vectors as well as PCR-based assays to detect the presence of retroviral sequences in clinical samples.

Microbiological Associates (MA) is a contract research and development organization which provides a full range of laboratory-based services to clients developing gene therapies. Our facilities and services are maintained according to the principles of Good Laboratory Practices (GLP) and current Good Manufacturing practices (cGMP). MA services span the spectrum of pre-clinical development of viral-based vectors through the manufacturing of clinical supplies and patient monitoring. MA laboratories are continually developing new assays to support the development of gene therapies.

► CONTRACT SERVICES SUPPORTING GENE THERAPY DEVELOPMENT AND CLINICAL TRIALS PROVIDED BY MICROBIOLOGICAL ASSOCIATES, INC.

- ◆ Provides GLP/GMP - compliant biological safety testing services to:
  - ◇ Pharmaceutical, biotechnology, chemical, cosmetics, medical device, and personal care products industries worldwide

► CONTRACT SERVICES

- ◆ PRECLINICAL DEVELOPMENT
- ◆ REGULATORY CONSULTANTS
- ◆ FACILITIES CONSULTANTS
- ◆ VALIDATION SERVICES
- ◆ PRECLINICAL TOXICOLOGY

► **CONTRACT SERVICES**
- ◆ SAFETY TESTING OF CELL SUBSTRATE
- ◆ CONTRACT MANUFACTURING
- ◆ LOT RELEASE TESTING
- ◆ CLINICAL TRIALS MANAGEMENT
- ◆ PATIENT MONITORING

► **REPLICATION COMPETENT RETROVIRUS TESTING BY MICROBIOLOGICAL ASSOCIATES (5/96)**

Amplification in Mus dunni	% Positive
mL (n=129)	0
> 100 mL (n=101)	11
Cocultivation (n=173)	4

► **PATIENT MONITORING**
- ◆ PCR FOR RCR
- ◆ Rapid DNA extract is prepared from PBMC
- ◆ PCR is performed using primers for amphotropic envelope
- ◆ Products are viewed on an agarose gel
- ◆ Positives confirmed by RE digest or hybridization
- ◆ Negatives confirmed by spiking with known positive DNA

► **PCR FOR RCR RESULTS**
- ◆ Dilutions of Positive control (plasmid) DNA show a 3-10 copy sensitivity in .5 μg cell DNA.
- ◆ Negative control DNA does not give a signal.
- ◆ DNA from amphotropic virus infected cells are positive.
- ◆ DNA from numerous cell lines are negative.
- ◆ The assay has been performed by 3 operators.

► **PATIENT MONITORING: WESTERN BLOT ASSAY**
- ◆ Murine amphotropic virus, 4070A provides antigen.
- ◆ Strips are incubated with positive control, negative control, or test serum.
- ◆ Secondary anti-Ig conjugated to peroxidase are incubated with the strips.
- ◆ Colored precipitate (band) of appropriate MW indicates positive.

► **PATIENT MONITORING: WESTERN BLOT RESULTS**
- ◆ Positive and Negative controls work
- ◆ Test serum spiked with positive control serum scores positive
- ◆ Nearly 100 patient samples tested

► **CONTRACT SERVICES: IN VIVO STUDIES**
- ◆ Preclinical Efficacy
- ◆ Toxicology
- ◆ Biodistribution

► **CONTRACT SERVICES: ADVANTAGES**
- ◆ Reduction in Time for Available Services.
- ◆ Utilization of Scientific Expertise Resident in Contract Testing Company.
- ◆ GLP, GMP and Regulatory Considerations.
- ◆ Better Utilization of Internal Resources.

► **CONTRACT SERVICES: DISADVANTAGES**
- ◆ Perceived Loss of Control
- ◆ Economic Implications

►HISTORICAL PERSPECTIVE ON QC TESTING ASSAY

- ◆Mycoplasma
  - ◇Research
    - ✣Cells (421/4412)
    - ✣Products (6/416)
  - ◇Regulatory
    - ✣Cells (53/1483)
    - ✣Products (42/5921)

►CGMPS - QUALITY

- ◆Quality, Safety, and Effectiveness Must be Designed and Built into Products.
- ◆Quality Cannot be Tested into the Finished Product.
- ◆Each Step of the Manufacturing Process needs to be Controlled.

►HUMAN CELL BANK CHARACTERIZATION

- ◆Sterility
- ◆Mycoplasma
- ◆TEM
- ◆In Vivo & In Vitro Adventitious viruses
- ◆Isoenzyme & Cytogenetic Analysis
- ◆Tumorigenicity (in vivo or in vitro)
- ◆Hep B, EBV, HIV, HTLV, AAV

►PRODUCT FLOW : PRELIMINARY TESTING

- ◆Research Lab Producer Cell Line
- ◆Preliminary Testing
  - ◇Mycoplasma
  - ◇Sterility
  - ◇Replication Competent
  - ◇Retrovirus

►MYCOPLASMA

- ◆Continuous Cell Cultures - Frequently Contaminated
- ◆(>3,000) Contaminated of >20,000 Cell Cultures Tested
- ◆) of 2.800 Isolated were Bovine Species
  - ◇Mycoplasma Arginini - 26%
  - ◇Acholeplasma Laidlawii - 8.5%
  - ◇Pirum - 7%
- ◆) of 2.800 Isolated Were Human Species
  - ◇Mycoplasma orale - 29.5%
- ◆of 2.800 Isolated Were of Porcine Species
  - ◇Mycoplasma Hyorhinis

►PRODUCT FLOW - MCB

- ◆Research Lab Master Cell Bank (MCB)
  - ◇(Produced Under GMPs)
- ◆Preliminary Testing
- ◆Master Cell Bank Cell Line Characterization (CLC)
  - ◇Mycoplasma
  - ◇Sterility
  - ◇In Vitro Adventitious Virus
    - ✣Co-Cultivation with Mus dunni
    - ✣Extended XC Plaque Assay
  - ◇Isoenzyme Analysis

- ◇ In Vivo Adventitious Virus
  - ✣ Mouse Antibody Production (MAP)

► **PRODUCTION RUN**
- ◆ Archive Samples
- ◆ Supernatant
- ◆ End of Production Cells (1% or 10e8, whichever is less)

► **LOT RELEASE TESTING (VECTOR-CONTAINING SUPERNATANTS)**
- ◆ Potency (titer)
- ◆ Identity
- ◆ Sterility
- ◆ General Safety
- ◆ Endotoxin
- ◆ Tests for Virus/Adventitious Agents
  - ◇ Includes Replication-Competent Viruses

► **FOOD AND DRUG ADMINISTRATION REQUIREMENTS: REPLICATION-COMPETENT RETROVIRUS TESTING**
- ◆ Current FDA Recommendations - 10/93
  - ◇ Master Cell Bank (MCB)
    - ✣ Cells - Co-Cult with Mus dunni/PG4 Endpoint
    - ✣ Volume tested - 1% or 108 cells - whichever is less
    - ✣ Supernatant - 5% Amplification on Mus dunni/PG4
    - ✣ endpoint
  - ◇ Manufacturer's Working Cell Bank (MWCB)
    - ✣ Either supernatant or cells (as above)
  - ◇ Qualification Run Including Transduction

► **EX VIVO TREATMENT**
- ◆ Transduced Cells (1%)
- ◆ Co-Cult Mus dunni/PG4 Endpoint
- ◆ General Consideration:
  - ◇ Tests should be complete prior to patient administration, if possible. If not, start testing

IMPLEMENTATION OF A PHASE I GENE THERAPY PROGRAM IN A GLP/GMP CONTINUUM

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ABSTRACT

Gene therapy product development, perhaps more than any area of therapeutic research, has arisen from academic institutions and newly-formed biotechnology companies. Implementation of early gene therapy programs, therefore, generally requires a large leap through the regulatory continuum from research to an environment of FDA-mandated Current Good Manufacturing Practice (CGMP) requirements established to produce investigational clinical supplies.

General concepts that impact the regulatory continuum from research to "Good Academic Research Practice" to "Good Laboratory Practice" to the "spirit" and "letter" of CGMPs will be discussed. Specific operational considerations that apply to gene therapy facilities will be addressed beginning with a facility design which can accommodate transduction and vector production laboratories. Emphasis will be placed on development of a Master Project Schedule and key Standard Operating Procedures as well as the importance of documentation and validation.

THE SCIENTIFIC-REGULATORY CONTINUUM

►ACADEMIC RESEARCH
- Scientific rigor
- Peer review
- Publication format

►GOOD ACADEMIC RESEARCH PRACTICES
- Reliance on protocols
- Enhanced documentation
- Facility impact
- Training
- Certification/calibration

►GOOD LABORATORY PRACTICES
- Standard operating procedures (SOPs)
- Quality assurance
- Qualification
- Release Criteria
- Product characterization

►GOOD MANUFACTURING PRACTICES
- Batch records
- Quality control
- Validation
- Specifications

►THE GLP-GMP CONTINUUM
- ◆ Spirit vs letter of GMP compliance
- ◆ Concepts differ by degree
  - ◇ Degree of SOP preparation
  - ◇ Degree of validation
  - ◇ Degree of product characterization

►MASTER PROJECT SCHEDULE
KEY FACILITY PROCEDURES
- ◆ Facility
  - ◇ Restricted access
  - ◇ Facility maintenance
- ◆ Systems
  - ◇ Environmental monitoring
  - ◇ Controller operations
  - ◇ Systems maintenance
- ◆ Equipment
  - ◇ Equipment calibration
  - ◇ Preventive maintenance
  - ◇ Use of dedicated equipment
  - ◇ Use of disposable labware
  - ◇ Use of preformulated media
- ◆ Manufacturing
  - ◇ Gowning
  - ◇ Product/waste transfer
  - ◇ Product changeover
  - ◇ Computerized records

- ◆ Personnel
  - ◇ GMP training
  - ◇ Employee training
  - ◇ Health & safety program
- ◆ Quality Control/Quality Assurance
  - ◇ Quarantine & release
  - ◇ Product specifications
  - ◇ Assay development
  - ◇ Document preparation
  - ◇ Audit function
- ◆ Validation
  - ◇ Cleaning validation
  - ◇ Systems validation
  - ◇ Assay validation
  - ◇ Equipment validation
  - ◇ Computer validation
  - ◇ Process validation

MONOCLONAL ANTIBODY AND RECOMBINANT PROTEIN PRODUCTION FACILITY NATIONAL CANCER INSTITUTE-FREDERICK CANCER RESEARCH & DEVELOPMENT CENTER

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► ABSTRACT

The Monoclonal Antibody and Recombinant Protein Production Facility (MARP) is an NCI-sponsored facility established at the NCI Frederick Cancer Research and Development Center to perform rapid and cost-effective production of clinical-grade biologicals for NIH-supported Phase I and Phase II clinical trials. Operating in temporary quarters for the past 18 months, MARP staff have manufactured several clinical-grade products: more than 500 gm of monoclonal antibodies, 5 recombinant vaccines, and 1 recombinant immunotoxin. The MARP has also vialed and released over 20 peptide vaccines. Recently, the MARP was relocated to a specially-designed and constructed building at NCI-FCRDC. When fully operational, the new facilities will triple the capacity to produce, purify, and vial clinical-grade biologics using mammalian, bacterial, or insect cell systems. Current projects include work on monoclonal antibodies, recombinant proteins, immunotoxins, peptides, and DNA and viral vectors. Working in close collaboration with the requesting scientific laboratories, MARP staff develop and validate production and purification procedures appropriate for scale-up from the laboratory models. MARP staff discuss the proposed approaches in pre-IND meetings with the FDA and the requesting scientific laboratory. Production, purification, formulation, and vialing are performed in dedicated suites according to current Good Manufacturing Practices (cGMPs). The MARP also provides cGMP documentation (Chemistry, Manufacturing, and Control) for incorporation in the IND. Cost accounting of MARP projects allows fair recovery of costs from users, and aids cost control measures. MARP projects have been supported by funds from several sources: NCI projects are authorized by the Decision Network Committee of the Division of Cancer Treatment, Diagnosis, and Centers (DCTDC). Projects from other governmental agencies or departments (e.g., NIAID) are funded through interagency agreements. Some projects are funded through budgeted costs in extramural grants. Other outside sources of funding may also be considered. Projects are prioritized by the Biological Response Modifiers Operating Committee (BOC) whose membership is drawn from NCI, NIAID, FDA, and other agencies.

For further information, contact Dr. Stephen Creekmore, Biological Resources Branch, DTP, DCTDC, NCI-FCRDC, Frederick, MD 21701-1201, (tel) 301-846-1098, (fax) 301-846-5429, (e-mail) Creekmor@ncifcrf.gov.

PRODUCTION OF CLINICAL AND LABORATORY-GRADE REAGENTS: SUMMARY OF CAPABILITIES OF BIOLOGICAL RESOURCES BRANCH, NCI

►NCI MONOCLONAL ANTIBODY/RECOMBINANT PROTEIN PRODUCTION FACILITY (MARP)

- ◆A contractor (SAIC)-operated and dedicated facility at NCI-FCRDC
- ◆Purpose: Small-to-Medium scale sterile/cGMP production facility
  - ◇Monoclonal antibodies
  - ◇Recombinant proteins
  - ◇Peptides
- ◆10-50 grams for NIH-sponsored Phase I and Phase II clinical trials and preclinical studies
- ◆A method resource in support of NIH-sponsored clinical research

►APPROACH TO CLINICAL DEVELOPMENT

- ◆Provide a range of services in support of investigator-initiated translational research
- ◆Keep costs down
- ◆Assist as many scientifically meritorious, approved projects as possible
  - ◇identified by peer review
  - ◇Decision Network Committee prioritizes projects requiring significant NCI resources
- ◆Identify promising candidates which may then be carried into full clinical development by CTEP and/or industry
  - ◇pilot clinical data help decide which projects to pursue

►ASSUMPTIONS

- ◆Success rate of agents entering clinical trials 5% or less
- ◆Lowering barriers to earlier clinical testing may:
  - ◇increase total number of successes
  - ◇stimulate interest in further development by industry and other investigators by "proof of concept"
  - ◇permit earlier refocusing of efforts by rapid "disproof of concept"
- ◆Trials by "inventor-clinicians" particularly need support, and represent an appropriate target of BRB efforts
- ◆Increasing demand for support investigator-initiated, peer-reviewed clinical research projects
- ◆Increased proportion from standard R01 pool
- ◆Continued but limited use of set-asides and other "targeted" approaches

Availability of low cost, cGMP production, IND filing, toxicology can determine whether a project is feasible (and fundable)

►PROJECT FLOW FROM THE INVESTIGATOR TO ITS INCORPORATION INTO THE MARP SCHEDULE

- Originator contacts the NCI-DCTDC-BRB
- The BRB chief submits proposal to MARP head
- Cost estimate of M&M, personnel, and opportunity costs is made
- Cost estimates are confirmed with originator—this takes about one week
- Project is reviewed by the Biological Operating Committee (BOC), and if necessary, the Decision Network (DN), prior to placement on the MARP calendar
- Once project is initiated, a particular technical services scientist is assigned and detailed discussions with originating laboratory begin

►WHO USES THE STERILE/CGMP BIOLOGICS MADE AT THE MARP/FCRDC?

- ◆Grantees
- ◆Intramural investigators
- ◆Contract clinical studies

Production priorities are set through the BOC

►EXAMPLES OF SERVICES AVAILABLE

- ◆Mammalian cell products
  - ◇Suspension culture
  - ◇Monoclonal antibodies
  - ◇Packaging cell lines
  - ◇DNA vaccines
  - ◇Viral production
- ◆Large Scale Production of monoclonal antibodies
  - ◇8 hollow fiber pathways
  - ◇hybridoma, CHO cells
  - ◇monoclonal antibodies 10-200 gm
- ◆Fermentation products
  - ◇Recombinant proteins
  - ◇immunotoxins
  - ◇recombinant vaccines
  - ◇cytokines
- ◆Protein purification/conjugation/vialing/release
  - ◇conjugation of antibodies with chelator
  - ◇peptide vaccines
- ◆Large-scale fermentation capacity
  - ◇300/3000 gallon fermentors (bulk pharmaceutical grade)
  - ◇natural products
- ◆Quality assurance and regulatory compliance
  - ◇pre-IND meetings with FDA
  - ◇process validation
  - ◇documentation
  - ◇preparation of CMC section of IND
  - ◇formulation and vialing
  - ◇testing and release
  - ◇toxicology

►BRB PRODUCTION PROJECTS

- ◆Finishing and vialing peptide vaccines (DCB, DCS)
- ◆Anti-lymphoma antibodies for immunotoxin production (extramural/DCS)
- ◆Anti-Hodgkin's (CD30) antibody (DCS)
- ◆k-ras proteins for vaccines (DCS)
- ◆Anti-ID vaccines for lymphoma and myeloma (DCS)
- ◆Anti-EGFR antibodies (extramural)
- ◆Chimeric anti-myeloma antibody (extramural)
- ◆Anti-colon antibody (DCB)
- ◆Anti-HIV antibody (NIAID)
- ◆Anti-Tac immunotoxin (DCB)
- ◆Anti-bombesin antibody (DCS)
- ◆IL-7 cytokines (DCS/extramural)
- ◆Retesting clinical grade cytokines: IL-2, IL-1, TNF (DCS, extramural)
- ◆Chelation of monoclonal antibodies

◆ Preclinical repository for cytokine standards, selected reagents (all)

For Further Information, Call:

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GETTING STARTED IN GENE THERAPY VECTOR DEVELOPMENT - CONSIDERATIONS FOR PROCESS DEVELOPMENT AND MANUFACTURING

Shawn L. Gallagher, MAGENTA Corp., Rockville, MD
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ABSTRACT

Once a transgene of interest is identified and the appropriate vector system for delivery is chosen, the process development phase of a gene therapy project must begin in earnest. During this exciting time period many decisions must be made without the benefit of complete information and missteps are inevitable. However, if consideration is given to clinical manufacturing issues early enough, major project delays can be avoided. Among the critical issues which must be addressed are the following:

◇ Clarification of regulatory environment
◇ Choice of host cell line (packaging, complementing, etc.)
◇ Selection of optimum virus strain
◇ Identification of appropriate facilities
◇ Optimization of production process
◇ Choice of in-house production or contract manufacturing option
◇ Development of appropriate formulation
◇ Selection of container/closure system
◇ Initiation of stability studies

CONSIDERATIONS FOR PROCESS DEVELOPMENT AND MANUFACTURING

► OUTLINE

◆ Process Considerations
◆ Production Options
◆ Key Factors for Evaluation of Alternatives
◆ Cost Analysis
◆ Some Thoughts Regarding Partnering for Production

- ◆ Recommendations

► KEY STEPS FOR PROCESS DEVELOPMENT EFFORTS
- ◆ Cell Line Development
- ◆ Virus Development
- ◆ Production System
- ◆ Purification Strategy
- ◆ Formulation
- ◆ Container/Closure

► OPTIONS FOR ACQUIRING CLINICAL MATERIAL
- ◆ In-House Manufacturing
- ◆ National Gene Vector Laboratories
- ◆ Corporate Partnering
- ◆ Academic Partnering
- ◆ Contract Manufacturing

► GLOBAL VARIABLES FOR A COMPARATIVE ANALYSIS
- ◆ Technology
- ◆ Time
- ◆ Regulatory Environment
- ◆ Product Development Strategy
- ◆ Money

► FIXED COSTS
- ◆ Depreciation
- ◆ Facility Operation
- ◆ Personnel

► FACILITY SCHEMATIC ADVANTAGES OF PARTNERING FOR MANUFACTURING
- ◆ Captive Technical and Regulatory "Consultants"
- ◆ Reduced Timeline to Product Availability
- ◆ Available Capacity / Optimized Technology
- ◆ Experienced Manufacturing Team
- ◆ No Need to Purchase Capital Equipment
- ◆ Full Quality Assurance Systems
- ◆ Validated Facilities

► BENEFITS OF IN-HOUSE PRODUCTION
- ◆ Flexibility to Select Technology
- ◆ Direct Control of Quality
- ◆ Reduced Cost
- ◆ Ability to Shift Resources to Accommodate Unforeseen Requirements
- ◆ Need for Control

► RECOMMENDATIONS FOR USE OF EXTERNAL RESOURCES
- ◆ Pilot Studies
- ◆ Special Projects
- ◆ Formulation and Filling
- ◆ Transient Needs
- ◆ Second Source

► IMPORTANT CHARACTERISTICS OF A MANUFACTURING PARTNER
- ◆ cGMP Experience

- ◆ Biologics Master File in Place
- ◆ Adequate Capacity
- ◆ Corporate Commitment
- ◆ Willingness to Transfer Technology

► THE COMMUNICATION BASICS

- ◆ Initial Information Exchange
- ◆ Establishing Confidentiality
- ◆ Project Definition
- ◆ Technology Transfer
- ◆ Use of Project Management Tools
- ◆ Production

► RELATED DOCUMENTS

- ◆ Points to Consider in:
  - ◇ Production and Testing of New Drugs and Biologics Produced by Recombinant DNA Technology, 1985 (Plus 1992 Supplement)
  - ◇ Human Somatic Cell Therapy and Gene Therapy, 1991 (Plus 1/96 Draft Addendum)
  - ◇ Characterization of Cell Lines Used to Produce Biologicals, 1993

► RELATED DOCUMENTS

- ◆ Cooperative Manufacturing Relationships for Biologicals (FDA Policy Statement - Federal Register 11/92)
- ◆ FDA Guidance Document Concerning Use of Pilot Manufacturing Facilities for the Development and Manufacture of Biological Products; Availability (Federal Register 6/95)

► SUMMARY RECOMMENDATIONS

- ◆ Spend the Time to Develop Robust Processes
- ◆ Define Release / Acceptance Specifications Early
- ◆ Develop a Change Control System
- ◆ Establish Project Review Checkpoints (Audits)
- ◆ Use Project Managers

These topics will be discussed with an emphasis placed on the resources available to assist in the successful completion of this challenging process.

BREAKOUT SESSION 5:
DEVELOPMENT OF NEW VECTOR SYSTEMS

SUMMARY

Neal DeLuca, University of Pittsburgh; Robert Anderson, FDA/CBER; Parris Burd, FDA/CBER

Part I. Herpes virus-based Systems

Four different HSV vector systems being developed for use in clinical trials were described. Neal DeLuca described his efforts towards constructing mutant Herpes viruses which do not express critical regulatory proteins (ICP4) and other IE proteins (ICP27 and ICP0) and described the biologic effects of the deletions. Elizabeth Rollinson described the construction, manufacture, and biologic properties of a 'non-infectious', disabled Herpes virus which does not express gH (DISC). This virus, when produced in a GH-complementing cell line, is able to introduce itself into a cell, but cannot complete a replication cycle. Alfred Geller described his studies of a virus encapsidated HSV-1 plasmid vector developed for the treatment of Parkinson's Disease. Robert Martuza described the development of replication-competent attenuated Herpes viruses for the purpose of tumor therapy. These viruses are able to grow in transformed cells, but do not replicate efficiently in normal cells.

Part II. AAV-based Vectors

The potential use of AAV vectors for gene transfer and stable expression in hematopoietic progenitor cells, neuronal and muscle cells was discussed. Jude Samulski gave a brief overview of AAV biology, described his efforts at producing AAV in the absence of Adenovirus and presented results of ongoing studies of long-term viral expression in brain and muscle. Saswati Chatterjee described efforts aimed at stable gene transfer into primitive hematopoietic progenitor/stem cells. Samuel Wadsworth described AAV development undertaken by Genzyme Corp and Gary Kurtzman described development of a helper-free AAV production system. Tom Reynolds discussed quantitation of infectious vector for titering and issues related to patient monitoring.

AGENDA

►PART I. HERPES SIMPLEX VECTORS

This session will discuss:
1.) the potential use of AAV vectors for gene transfer and stable expression in different tissues,
2.) production of AAV vectors in the absence of helper adenovirus, and
3.) quantitation of infectious vector for titering and patient monitoring.

- ◆Herpes Simplex Virus Mutants as Gene Therapy Vehicles
  - ◇Neal DeLuca
- ◆DISC-HSV a Novel Vector System
  - ◇Elizabeth Rollinson
- ◆Modifying Neuronal Physiology with a Helper Virus-free HSV-1
  - ◇Alfred Geller
- ◆Replication Competent Herpes Virus for Tumor Therapy
  - ◇Robert Martuza
- ◆Discussion

►PART II. ADENO-ASSOCIATED VIRAL VECTORS

This session will discuss and compare three different HSV vector systems:
1.) vectors with modified regulatory and immediate early genes,
2.) HSV-1 plasmid vectors (amplicons), and
3.) vectors with late gene deletions.

- AAV Vectors In Vivo
  - Jude Samulski
- Adeno-Associated Virus Vectors for Transduction of Hematopoietic Progenitor Cells
  - Saswati Chatterjee
- Development of AAV Vectors for Gene Therapy
  - Samuel Wadsworth
- AAV Vectors Can Be Efficiently Produced Without Helper Virus
  - Gary Kurtzman
- Development of Assays for the Detection and Quantitation of Recombinant AAV-CFTR Vector
  - Tom Reynolds

Discussion Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

GTINFO@A1.CBER.FDA.GOV

HERPES SIMPLEX VIRUS MUTANTS AS GENE THERAPY VEHICLES

Neal DeLuca
University of Pittsburgh School of Medicine, Pittsburgh, PA

Herpes simplex virus (HSV) is an extremely lytic virus that encodes approximately 75 gene products. However, given its capacity to establish latency in neurons, its broad host range, and the ability to obtain high infectious titers, HSV may be useful as a gene transfer vector. The cascade of viral gene expression and the infectious cycle of the virus can be blocked by deleting an essential regulatory protein of the virus, ICP4. Mutants in ICP4 only express five other viral genes (IE genes). The activities of the IE proteins all affect aspects of host cell metabolism, resulting in rapid cell death. The theme of our research in this area is to delete the genes that are expressed in the absence of ICP4 in order to help determine how the products of these genes function, and to eliminate the observed cytotoxicity, providing a more efficacious vector. Three of the five IE genes are essential for virus replication (ICP4, ICP27, and ICP0). Therefore, it was necessary to construct cell lines that would simultaneously complement all three. Using such cell lines it was possible to construct viruses deficient in different subsets of IE genes, efficiently propagate them, and determine their effects on host cell survival and gene transfer. Details on how these viruses were constructed, their phenotypes, and their usefulness as vectors will be discussed.

DISC-HSV A NOVEL VECTOR SYSTEM

Dr. Elizabeth A. Rollinson et al
Cantab Pharmaceuticals Research Ltd., Cambridge, England, CB4GN.

DISC-HSV is a Disabled Infectious Single Cycle Herpes Simplex virus from which the gene encoding gH, which is essential for virus entry, has been removed. A complementing producer cell line has been generated which allows for the production of phenotypically complete, infectious virions, but because there are no overlapping sequences between the cell line and virus, no replication competent virus is generated during manufacture. The virus is, therefore, capable of delivering its genome and heterologous genes into host cells but cannot spread or cause disease. The excellent safety profile of DISC-HSV has been demonstrated by extensive preclinical testing, including demonstration of non-neurovirulence/neuroinvasiveness, safety in immunocompromised animals, negligible probability of recombination, inability to reactivate from the latent state, antiviral drug sensitivity, absence of injection site reactivity and general safety in primates and laboratory animals. The DISC-HSV vector will enter Phase I/II trials shortly for genital herpes.

As well as this excellent safety profile, DISC-HSV offers additional advantages as a gene delivery vehicle, including: capacity to accommodate large amounts of foreign DNA (≥30 kb compared with 4-7 kb for AAV/AV/RV), broad host cell range (dividing and non-dividing, lymphocytic epithelial, neuronal, tumour cells), high transduction efficiency (for example, 60-100% transduction in primary leukaemia cells, compared with around 4% for an equivalent retroviral or adenoviral vector), and the added safety feature that incorporation of the heterologous gene into the disabling locus within the viral genome minimises the risk of gene transfer to wildtype HSV. In a murine tumor model, administration of a DISC-HSV expressing mGM-CSF resulted in inhibition of tumour growth.

In addition, Cantab is exploring means of expanding the range of possibilities for gene delivery with DISC-HSV, namely using HSV amplicons, which would allow delivery of DNA fragments up to 150 kb in the absence of HSV-encoded gene products, thereby minimising the potential problems associated with immunogenicity of the vector, and allowing long-term gene expression without the cytolytic effect of HSV replication. The DICS-HSV provides an excellent helper virus

system for this purpose. The amplicon gene delivery technique is potentially highly versatile; because the DISC-HSV vector and producer cell line have already been extensively characterized, a new gene therapy application would require only a new plasmid construct. The establishment of a manufacturing process and a regulatory framework for the clinical use of one amplicon-vectored gene would provide a very powerful basis for applications involving other genes.

MODIFYING NEURONAL PHYSIOLOGY WITH A HELPER VIRUS-FREE HSV-1 PLASMID VECTOR SYSTEM

Alfred I. Geller
Division of Endocrinology, Children's Hospital, Boston, MA 02115

We have developed a helper virus-free packaging system for HSV-1 plasmid vectors. After cotransfection into cultured fibroblast cells of a HSV-1 vector and a HSV-1 cosmid set lacking the DNA cleavage/packaging signals, the modified cosmid set can provide all the trans-acting functions required for the replication and packaging of the HSV-1 plasmid vector. The resulting vector stocks are free of detectable helper virus, and consequently, gene transfer into the rat brain is more efficient and causes reduced cytotoxic effects and immune responses compared to vector stocks prepared using a helper virus.

We are using this vector system to explore the potential for gene therapy of Parkinson's Disease (PD). Injection of a HSV-1 vector which expresses tyrosine hydroxylase into the partially denervated striatum in the 6-hydroxydopamine rat model of PD resulted in long-term (1 year) biochemical and behavioral recovery. Gene expression was maintained for this period and persistence of vector DNA was demonstrated.

HSV-1 vectors may also have potential for studying the molecular and cellular basis of specific behaviors. We have found that a HSV-1 vector that contains the tyrosine hydroxylase promoter can be used to target expression to substantia nigra pars compacta (SNc) neurons. A constitutively active protein kinase C (PKC) was delivered into Snc neurons, and resulting changes in dopaminergic neurotransmission caused changes in rat motor behavior. Thus, the PKC pathway in Snc neurons regulates motor behavior.

REPLICATION COMPETENT ATTENUATED HERPES VIRUS FOR TUMOR THERAPY

Martuza RL, Mineta T, Hunter W, Todo T, Todo M, Rabkin S.
From The Georgetown Brain Tumor Center, Molecular Neurosurgery Laboratory, and the Department of Neurosurgery, Georgetown University, 3800 Reservoir Rd. NW, Washington, DC 20007 USA (Fax: 202-687-3744)

In order to overcome some of the limitations of replication-incompetent retrovirus and adenovirus systems for tumor therapy, we have explored the progressive development, construction, and testing or replication-competent herpes virus for the purpose of tumor therapy. Our goals have been to develop a virus that is replication-competent in tumor cells and therefore can spread within the tumor, yet is replication-incompetent in the normal brain so that pathological effects will be minimized. We have progressively explored various single mutations in the herpes simplex virus including mutations or deletions of the thymidine kinase, DNA polymerase, ribonucleoside reductase, and gamma 34.5 genes. Using both in vitro and in vivo data from these studies, we have now constructed a viral vector with multiple mutations. This virus has been called G207 and contains deletions at both gamma 34.5 loci and a lac Z gene disruption of the ribonucleoside reductase locus. These deletions confer upon G207 a series of properties that are potentially useful for testing against human glioma and other tumors: 1, G207 can replicate in and kill human glioma (and other tumor) cells; 2, G207 is not able to replicate in rodent neurons or astrocytes in culture; 3, G207 is not toxic to mouse or primate

brain upon intracerebral inoculation; 4, G207 is hypersensitive to ganciclovir; 5, G207 is temperature sensitive. Toxicity studies have been done or are in progress in mice by the intracerebral, intraventricular, and intravenous routes. Sub-human primate toxicity has been done or is in progress utilizing high titer inoculation 107 and 109 pfu) intracerebrally in aoutus, a species known to be exquisitely sensitive to herpes virus.

AAV VECTORS IN VIVO

R. Jude Samulski, Ph.D.
Gene Therapy Center, University of North Carolina at Chapel Hill, CB#7352, Chapel Hill, NC 27599-7352 USA

We have utilized the parvovirus AAV as a vector in initial tests aimed at targeting neuronal and muscle cells for long term gene transduction and expression. Efforts aimed at characterizing gene transduction in non-dividing tissue has been achieved in both the rat and mouse model. Successful transductions with reporter gene and various therapeutic gene cassettes have been tested. Analysis from these studies suggest that AAV transduction in the brain demonstrated differential and persistent expression patterns for over a year. In no case were obvious signs of toxicity noted. Further studies suggest long term gene expression in muscle of immuno-competent animals. Molecular analysis of the AAV vector suggest integration as a mechanism for persistence. Continuing efforts in improving vector production has allowed rAAV production in the complete absence of Adenovirus infection. These studies and the important implications they have on AAV vectors in gene therapy will be discussed.

ADENO-ASSOCIATED VIRUS VECTORS FOR TRANSDUCTION OF HEMATOPOIETIC PROGENITOR CELLS

Saswati Chatterjee, Elizabeth Shaughnessy, Grace Fisher-Adams, Di Lu, Greg Podsakoff, and K. K. Wong
Divisions of Pediatrics, Hematology/BMT, and Surgery, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010

We are exploring the potentials of the use of adeno-associated virus (AAV) vectors for stable gene transfer into primitive hematopoietic progenitor/stem cells. AAV vectors encoding both RNA and protein based transgenes under the control of heterologous viral or cellular housekeeping gene promoters were constructed and encapsidated into transducing vectors. Transduction with AAV vectors was resistant to heat, DNase, and chloroform treatment but was abrogated by pretreatment with anti-AAV antibodies. Analysis of AAV transduction of human CD34+ cells revealed equivalent levels of transgene expression in both freshly isolated and cytokine pre-stimulated cells. In short term transduction assays, transgene expression was observed in 40-95% of cells from different donors, suggesting efficient entry, nuclear localization, and second strand synthesis of AAV vector sequences. Transduction of human bone marrow and cord blood-derived primitive myeloid progenitor cells tested in long term cultures (LTBMC) and long term culture-initiating cells (LTCIC) assays resulted in gene transfer at a frequency of 0.2-1 copy/cell at 6-8 weeks post-transduction in the absence of selective pressure. Fluorescent in situ hybridization (FISH) and Southern analyses indicated the presence of integrated AAV vector sequences in CD34 chromosomal DNA in transduced cells within one week after transduction and were detectable at comparable levels six weeks after transduction. These results indicated that AAV vectors are capable of stable integration into primitive hematopoietic progenitors. Transcription of the vector-encoded transgene was detectable in LTBMC at 6-8 weeks post-transduction, indicating the presence of transcriptionally active intracellular vector sequences. Cells from different donors transduced in parallel with the same batches of vector showed variability in transduction

frequencies, possibly indicating a genetic basis for AAV attachment/entry. Hematopoietic stem cell transduction was evaluated in a murine model of serial marrow transplantation. Lethally irradiated primary recipients of AAV vector-transduced marrow cells showed evidence of long term, multi-lineage reconstitution. Transgene-expressing cells were detected in the periphery at 6 months post transplantation. Serial transplants performed with marrow cells from primary recipients resulted in the reconstitution of secondary hosts with transduced cells. Transgene expression was detected in lympho-hematopoietic tissues at 6 months after secondary transplantation and 12.5 months after initial transduction. Interestingly, in a few mice, cells of both myeloid and lymphoid lineages revealed identical restriction fragment length polymorphisms, suggesting vector integration in a common progenitor cell, most likely a pluripotent hematopoietic stem cell. The ability to reconstitute animals long term with AAV-vector transduced cells and to detect vector-specific transcripts and integrated vector sequences in multiple lineages with similar RFLP patterns suggest the utility of this vector for stem cell gene therapy.

DEVELOPMENT OF AAV VECTORS FOR GENE THERAPY

Samuel C. Wadsworth
Genzyme Corporation

Gene therapy vectors based on adenoassociated virus (AAV) offer potential advantages over other viral and non-viral vectors. AAV vectors have a relatively simple protein coat and in general are deleted for all viral genes suggesting that they may be less immunogenic than other viral vectors. The parent virus can integrate into the human genome, apparently mediated by viral REP activity suggesting a mechanism for long-term vector gene expression. In addition, the parent virus is not associated with any known disease and there are reports that AAV vectors are less inflammatory. However, clear assessment of potential AAV advantages is clouded by the difficulty in producing sufficient quantities of vector for extensive preclinical studies at increasing dosage levels. Moreover, AAV vector preparations are frequently contaminated with adenovirus and wild-type AAV further complicating interpretation of gene transfer experiments. To address these limitations we have made modifications to the AAV helper plasmid used in vector production and in the production process itself. These changes allow increased vector production without contamination with wild-type AAV. More recently we have established a method for producing AAV vector in which Ad helper virus is replaced with isolated Ad early genes, eliminating adenovirus contamination. Progress in AAV vector production and characterization will be reviewed as well as progress in improving AAV vector gene transfer.

AAV VECTORS CAN BE EFFICIENTLY PRODUCED WITHOUT HELPER VIRUS

P. Colosi, S. Elliger, C. Elliger and G. Kurtzman
Avigen, Inc., Alameda, CA.

Vectors derived from adeno-associated virus (AAV) are being actively investigated for several gene therapy applications. Current methods for producing AAV vectors require that producer cells be infected with a helper virus, most commonly adenovirus. Adenovirus can be separated from AAV vectors by density gradient centrifugation and any residual infectious adenovirus inactivated with heat. However, the requirement for a helper virus in the AAV vector production process poses limitations for the production of vectors for clinical use. By identifying the adenoviral genes that are necessary for AAV vector replication, we developed an efficient method for producing AAV vectors that does not rely on helper virus infection. Human 293 cells, which stably express the adenovirus E1a and E1b genes, were systematically co-transfected with a plasmid containing the AAV vector and helper sequences and combinations of plasmids

containing the adenoviral early genes. This analysis revealed that the adenovirus E2a, E4orf6 and VA RNA genes were necessary and sufficient to achieve levels of AAV vector production equivalent to those obtained with adenovirus infection. No infectious adenovirus was generated. The AAV vectors produced by transfecting 293 cells with these adenoviral early genes ("Ad-Free" method) were as efficient at transducing cells in vitro as those produced with adenovirus infection. We are currently evaluating AAV vectors produced by the Ad-Free method in animals. Balb/c mice treated with a single intramuscular administration of an AAV vector containing the gene for human erythropoietin (hEpo) controlled by the CMV immediate early promoter show persistent elevation of serum levels of hEpo and concomitant increases in red blood cell production. The in vivo response to the administration of vector produced by either Ad-Free method or with adenovirus was identical. The Ad-Free method greatly simplifies AAV vector production and purification and may ultimately contribute to their acceptability for clinical use in gene therapy.

DEVELOPMENT OF ASSAYS FOR THE DETECTION AND QUANTITATION OF RECOMBINANT AAV-CFTR VECTOR

Daryn Debeiak, Molly Fee-Maki, Teresa Johnston, Tom Reynolds
Targeted Genetics Corporation, Seattle

AAV is a non-pathogenic human parvovirus that is being tested as a gene therapy vector in the setting of cystic fibrosis. AAV vectors are incapable of replication in the absence of either virally encoded rep genes or helper functions usually provided by adenovirus. We have developed an assay to quantitate infectious vector using a cell line inducible for AAV rep and cap functions. This assay has been further modified to rapidly detect the presence of infectious vector that could be shed by patients following vector administration.

BREAKOUT SESSION 6: RETROVIRAL VECTORS

SUMMARY

Estuardo Aguilar-Cordova, Ph.D., Baylor College of Medicine; Andra Miller, Ph.D., FDA/CBER; Carolyn A. Wilson, Ph.D., FDA/CBER

The goals of the session were to discuss the recommendations for detection of replication-competent retrovirus during retroviral vector production and patient follow-up, in order to provide a basis for revision of the current FDA recommendations for RCR resting.

Regarding recommendations on RCR detection during retroviral vector production, our goal is to move toward the development and definition of RCR detection systems which are scientifically based, can be standardized, and will allow a balance between sensitivity of RCR detection and risk of patient exposure. The FDA is working towards development of a standardized stock of RCR which would be titrated and distributed to all investigators performing RCR detection assays. This would allow investigators to "calibrate" their assay sensitivity. In particular, we had a discussion of our current recommendation of testing of 5% of the supernatant for RCR. Statistical predictions would suggest that RCR detection assays should be based on the sensitivity of the assay, and how many replicate samples need to be tested to achieve a limit concentration of RCR. The question of what the limit concentration or tolerated dose should be (i.e., 1 RCR/ml; 0.1 RCR/mL, etc.) was raised but not answered. It was suggested that the risk of RCR administration be compared to known risk factors for certain patient populations (i.e., cancer patients, relative risks from chemotherapy and radiation therapy). In a review of over three years of RCR testing by one contract laboratory, 3/384 samples were positive for RCR, and that each of these were due to contamination with the positive control. An additional issue which was raised was the current recommendation to perform both co-cultivation and supernatant testing at several stages during production—whether this dual testing adds value. Everyone agreed that this was an important question to address, but no one had data to support one test over the other. The presence of an endogenous virus in the Mus dunni cell line used for amplification of RCR was discussed. This virus is activated under circumstances where hydroxycortisone is present (≥1 μM). No instances of spontaneous activation have been reported, suggesting that this cell line, although not appropriate in indications where hydroxycortisone is present, is still a useful reagent in RCR detection assays.

Regarding data accumulated to date with patient monitoring for RCR, the available evidence suggests that no patients have received detectable levels of RCR. We had presentations which summarized the data from three different clinical trials, representing over 400 patients (with multiple time points tested for many patients). All patient samples examined by polymerase chain reaction for RCR sequences in their peripheral blood mononuclear cells were negative. Some of the patients who received either direct injection of vector or vector-producing cells had evidence of murine retrovirus-specific antibodies by Western blot or ELISA. Patients had received greater than one injection of material before developing antibody reactivity. The consensus expressed was that the antibody reactivity represented reactivity to vector, rather than authentic RCR, and that in cases where direct injection of vector and/or vector-producing cells was used, that serological assays could be misleading. Further, there was consensus that serological assays represented a convenient and simple alternative assay to PCR for sponsors administering retroviral vectors ex vivo. Data from an in vivo study of RCR infection of monkeys demonstrated that healthy, immuno-competent monkeys can be infected with RCR. The dynamics of infection were examined in the four monkeys on the study. In general infectious virus was isolated from the PBMCs only at early time points post-inoculation (2-6 weeks). Assays for RCR-specific antibodies and PCR assays detecting envelope sequences in the PBMCs were positive early (by 4 weeks) and remained positive in most animals for at least one year. Several

proposals for altering the frequency of patient monitoring were made, but no consensus was reached. Proposals ranged from having quarterly testing for the first year to having a one-time test 12 months after administration, and one proposal suggested linking patient monitoring to the type of vector production system used (i.e., rate of recombination and RCR generation from different packaging cells). Alternative proposals to our current recommendation of life-long patient monitoring were also made: patient follow-up for two years or as clinically indicated, and/or development of a patient tracking system which would report adverse events to the FDA and is linked to a patient identification card to allow for notification of the patient's participation in a gene therapy clinical trial.

The results presented in the workshop will provide the basis for a continuing dialog both within the FDA and with the gene therapy community to develop new recommendations for RCR testing. We encourage and welcome any additional data, comments, or ideas which might be pertinent to such discussions. Please submit all comments to the e-mail account GTINFO@A1.CBER.FDA.GOV by close of business September 30, 1996

►AGENDA

The goals of the session are to discuss the recommendations for RCR detection during retroviral vector production and patient follow-up in order to provide a basis for discussion of the current FDA recommendations for RCR testing.

- ◆ Specific questions for discussion include the following:

A. Regarding recommendations on RCR detection during retroviral vector production, our goal is to move toward the development and definition of RCR detection systems which are scientifically based, can be standardized, and will allow a balance between sensitivity of RCR detection and risk of patient exposure. In particular,

   1) What limit concentration of RCR should be achieved in RCR detection assays?

   2) Should volume tested be linked to lot size, and, if so, in what way?

   3) What virus(es) should be used as standards for determination of relative assay sensitivity?

B. Regarding our current recommendations for patient monitoring,

   1) What assays are most appropriate/sensitive? Should patient population and/or mode of vector administration be considered in deciding which assays should be applied?

   2) How often should patients be examined for RCR? Is our current recommendation for life-long monitoring reasonable and rational? RCR testing during retroviral vector production and in human clinical trials

- ◆ FDA/CBER Overview and goals of the session
  - ◇ Andra Miller, Ph.D.
- ◆ Statistical models for testing for replication-competent retrovirus
  - ◇ Tie Hua Ng, Ph.D., FDA/CBER
- ◆ Experimental issues in testing for replication-competent retrovirus
  - ◇ Carolyn Wilson, Ph.D., FDA/CBER
- ◆ Viral safety of biotechnology and gene therapy products-proposed conventions for sampling and testing for RCR
  - ◇ John Spaltro, Ph.D., Microbiological Associates, Inc.
- ◆ Sensitive assays for detection of replication competent retrovirus
  - ◇ Joe Hughes, Ph.D., Quality Biotech, Inc.
- ◆ Somatix Therapy Corporation, Replication competent retrovirus testing for manufacturing and gene monitoring of patients in clinical trials
  - ◇ Lawrence Cohen, Ph.D. and Joe Rokovich, Ph.D.
- ◆ Targeted Genetics, A novel murine retrovirus identified during testing for helper virus in human gene transfer trials

◇Tom Reynolds, M.D., Ph.D.

- ◆Infection of normal rhesus monkeys with a murine replication-competent retrovirus (RCR)
  ◇Arifa Khan, Ph.D., CBER
- ◆RCR testing in human clinical trials of a directly injected retroviral vector in patients with HIV: methods, results , and impact on frequency of patient sampling
  ◇Dale Ando, M.D., Chiron Corporation
- ◆Monitoring of cancer patients receiving retroviral vector gene therapy
  ◇Gerard J. McGarrity, Ph.D., Genetic Therapy, Inc.
- ◆Summary and Discussion
  ◇Estuardo Aguilar-Cordova, Ph.D., Baylor College of Medicine

Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

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TESTING FOR REPLICATION-COMPETENT RETROVIRUS

Tie-Hua Ng1, Carolyn Wilson2, Andra Miller3 and Peter Lachenbruch1
Divisions of 1Biostatistics and Epidemiology, 2 Cellular and Gene Therapies, and 3Application Review and Policy, CBER, FDA

Retroviral vectors are replication-defective viruses and are used in gene therapy protocols. In the process of producing these vectors, replication-competent retroviruses (RCR) sometimes arise, causing a public health concern. Therefore as part of the production process, the FDA requests all sponsors to perform a series of cell culture experiments to ensure that their material is RCR-free prior to administration in the clinic.

How much material needs to be tested in order to provide a reasonable confidence that the material administered will be RCR-free? Several years ago, this question was discussed with an advisory committee. It was decided that 5% of the cell supernatant and 1% (or 108) of the producer cells would be tested. We have chosen to examine our current recommendations in response to concerns from sponsors especially as they are producing larger lots of material. For example, for a 200 liter lot 5% of the supernatant is 10 liters of material for testing.

To determine the testing requirements, we begin with the following three characteristics.

(1) The concentration of RCR in the production lot; c (RCR/ml).

(2) The volume of the sample to be tested: vt (ml).

(3) The sensitivity of the assay: s (ffu/ml) (ffu: focus forming unit; represent detectable RCR).

The basic assumption regarding the sensitivity of the assay is as follows. The test on a sample volume vt will be positive if the number of RCR in the sample is greater than or equal to s vt. For example, if s = 3 ffu/ml and vt = 2 ml, then a single test of 2 ml will be positive if and only if the sample contains at least 6 RCR. The detection of RCR in the infectivity assay is therefore concentration dependent.

With this assumption, it is recommended that vt = 1/s be tested. The probability of detection (POD) with the recommended volume may be computed based on the Poisson distribution with parameter $\lambda = c\ vt = c/s$, and is given by $1- \exp(-c/s)$. This probability is very low when $c \ll s$. To increase the POD to a desired level, say $(1-\beta)$ for some small β (e.g., 0.05), it is necessary to perform k tests, where k is the smallest integer greater than or equal to $(-s/c)\ln(\beta)$ (i.e., rounding up) and ln denotes the natural log.

What value of c can we tolerate? For s = 1 and $\beta = 0.05$, if c = 0.1, then k = 30, which is manageable. However, if we cannot tolerate concentration higher than 0.001 (c = 0.001), then k = 2996, which becomes impractical. If we limit k to a reasonable number, say 50, and $\beta = 0.05$, then $s/c \leq -50 \ln(\beta) = 16.69$. Therefore, the sensitivity has to be no larger than 16.69 times the concentration or the concentration has to be no smaller 1/16.69 of the sensitivity.

The last question may be answered by specifying the patient risk of exposure to the RCR. For a patient dose of vd, if we specify that the risk of exposure to RCR is α, for some small α (e.g., 0.1), then $c = -\ln(1 - \alpha)/vd$. For example, if $\alpha = 0.1$ and vd = 100 ml, then c = 0.0010536. With the limitation of the assay sensitivity, there should be a balance between the desired limit concentration and the number of replicates which are reasonable to perform.

EXPERIMENTAL ISSUES IN TESTING FOR REPLICATION-COMPETENT RETROVIRUS

Carolyn A. Wilson*, Katie Whartenby°, and Andra Miller°
Divisions of Cellular and Gene Therapies* and Application Review and Policy°, CBER, FDA

Retroviral vectors can occasionally be contaminated with replication-competent retrovirus (RCR) due to rare recombinational events which occur in packaging cell lines used during production. Presence of RCR represents a safety concern for patients because of their ability to integrate into the host genome and potentially have a role in tumorigenesis, as well as the documented ability of murine retroviruses to pseudotype human retroviruses, such as human immunodeficiency virus. For these reasons, the FDA has made recommendations for RCR testing at several stages during retroviral vector production and in ex vivo transduced cells, where appropriate. We ask that 5% of the supernatant and 1% or 108 cells be examined in RCR detection assays.

We are currently evaluating our RCR testing recommendations. Data will be presented from experiments which are being done to test assumptions underlying our current recommendations, as well as to test predictions from a statistical model which has been developed (see abstract by Tie-Hua Ng). Using the PG-4 S+L- assay, we are examining several parameters affecting assay sensitivity, such as number or replicate samples, high titer retroviral vector-containing supernatant, and amplification on Mus dunni cells prior to detection on PG-4 cells.

VIRAL SAFETY OF BIOTECHNOLOGY AND GENE THERAPY PRODUCTS-PROPOSED CONVENTIONS FOR SAMPLING AND TESTING FOR RCR

John Spaltro, Ph.D.,
Microbiological Associates, Inc.

Viral safety and the risk of viral contamination are major issues in the manufacturing and control of biotechnology and gene therapy products. The potential for the occurrence of replication competent retrovirus (RCR) in retroviral gene therapy products of the possible introduction of viral contaminants from virus/cell banks, components of animal origin, or adventitious introduction during processing require that viral safety assurance testing be performed prior to product evaluation in human clinical trials.

Testing programs to detect the presence of RCR should require the use of sensitive and well-characterized methods for RCR detection, coupled with strategic sampling of production volumes in order to support viral safety assurance. Current requirements for RCR testing of gene therapy products requires the sampling of five percent of the total production volume. With increasing production volumes, test volume requirements are increasing and testing methodologies have been developed to allow for economical testing of larger test volumes. However, alternate methods have different sensitivities for the detection of RCR, and the extent of viral safety assurance for the product may be uncertain.

Results characterizing the sensitivities of a variety of amplification and end-point test procedures developed for RCR determination at Microbiological Associates will be presented. Test configurations including roller bottle, well, and dish techniques will be presented and statistical methodology used in the determination of the sensitivity or Probability of Detection (P.O.D.) for the tests will be discussed.

Using the assay P.O.D., a new strategy for the viral safety assessment of large volume production batches will be presented. Sampling issues will be addressed, as well as the impact of new methodologies with small test volume requirements and high throughput capability. Several examples of the proposed strategy implementation will be presented and discussed.

SENSITIVE ASSAYS FOR DETECTION OF REPLICATION COMPETENT RETROVIRUSES

Joseph Hughes, Lisa M. Rudderow, Teresa M. Byrne, Kim Bowers, Dilip Patel, Michael Burnham, Ingo Georgoff, Richard Metz, Fredrika McDevitt, and Michael D. Lubeck
Quality Biotech, Inc., Camden NJ 08104

Amplification of Mus dunni cells followed by a sensitive infectivity assay such as the PG4 S+L- focus forming assay has become one of the standard procedures for the detection of replication competent retroviruses (RCRs). This combined system is routinely used in many laboratories for testing gene therapy vectors and other biopharmaceuticals (i.e., murine hybridomas). In our laboratory this system has proven to be one of the most sensitive assays for the detection of infectious or recombinant murine leukemia viruses (MuLV). The 14 day test is used either as a co-cultivation for cells or an amplification for supernatants from cell and production lots; these assays have two subpassages supplying fresh cells at each passage. Additional passages (up to 5) have been performed on some Master and Working Cell Banks; validation studies have demonstrated that only a low number of passages is needed to achieve maximum sensitivity for many retroviruses.

We have demonstrated in validation experiments that the Mus dunni co-cultivation assay will enhance the detection of known amount of RCR by a least one log 10 unit. This enhanced detection of RCR has been demonstrated for small volumes as well as large volumes of sample. For these tests up to 20 ml or 100 to 125 ml of test material is plated on each T150 flask or roller bottle of Mus dunni cells, respectively. The PG4 S+L- assay has also been validated for a number of tests. The data from these studies show very good linearity for titrated virus (r2 of 0.95 to 0.99), reproducibility within or between tests of 0.35 and 0.45 log10 units respectively, and very good sensitivity of 2 to 3 focus forming units (FFU)/ml. Large volume sampling versus multiple small volume testing for the PG4 S+L- assay has also been examined. Introduction of a method to fix and stain the PG4 S+L- has significantly improved the recording of infectious retrovirus foci.

We have examined a variety of gene therapy products from a number of sources for the detection of RCRs. The tests have either included the amplification on Mus dunni cells followed by the PG4 S+L- assay or just the PG4 S+L- assay (on materials already amplified by the client). Current regulatory guidelines suggest that 1% of the producer cells (108 minimum) and 5% of a harvested supernatant are required for RCR testing. In our experience, the typical number for co-cultivations has ranged from 107 to 108 and vector lots have ranged from a few milliliters to 3 liters. Cell banks, production lots, and all supernatants have been routinely negative for the presence of RCR. The results from several hundred PG4 S+L- assays of client-amplified material demonstrated only three positive tests for RCR. These samples were found to be RCR positive due to cross-contamination with a retrovirus used by the sponsor company for co-cultivation controls.

In addition to evaluation of gene therapy vectors for RCR, we have examined a number of other products for evidence of infectious MuLV. Evaluations of other biopharmaceutical products, such as murine hybridomas, often demonstrate a small number of infectious units associated with high levels of retroviral particles. Initial sampling from the unamplified products may show undetectable to 10 FFU/ml. Upon amplification of these same lots, high levels of PG4 S+L- -positive retroviruses (typically 104 to 105 FFU/ml) are expressed. Thus the Mus dunni/PG4 S+L- system still remains a very sensitive assay for detection of infectious MuLV retroviruses in a variety of biopharmaceuticals. Highly sensitive PCR-based assays (QC-PCR, PERT) can also be used for the detection of specific RCR in recombinant viral preparations. The strategy for these tests would involve customizing the PCR protocol for the most likely RCR resulting from rare recombination events with the packaging cell line DNA. The development of PCR-based assays is

ongoing for a variety of gene therapy vectors. Such PCR-based tests should provide a rapid and highly sensitive RCR detection assay, which can be used alone or in combination with scaled-down co-cultivation studies.

REPLICATION COMPETENT RETROVIRUS TESTING FOR MANUFACTURING AND MONITORING OF PATIENTS IN CLINICAL TRIALS

L. Cohen and J. Rokovich
Somatix Therapy Corporation, Alameda, CA

Somatix Therapy Corporation has developed the MFG-S retroviral vector with the Ψ-CRIP packaging cell line as a delivery system for the huGM-CSF transgene. The MFG-S-huGM-CSF transducing particles have been employed to create genetically modified tumor cell vaccines for use in clinical protocols since late 1993. For each patient autologous tumor tissue is cultured ex vivo, transduced with MFG-S-GM-CSF, irradiated, and administered to the patient.

Replication competent retrovirus (RCR) testing is employed at two distinct stages of manufacture—at the end of transducing particle manufacture and at the end of the tumor tissue processing. The development of the RCR cocultivation and supernatant testing methods will be described, the controls identified, and the sensitivity of the assays discussed. Recommendations will be made as to the appropriate RCR testing samples and methods (cocultivation vs. supernatant) at various stages of manufacture, and the potential standardization of this testing.

On September 20,1993 the FDA requested that all IND sponsors using a retroviral vector monitor clinical trials subjects for evidence of replication competent retroviruses by three different methods. Somatix will relate its RCR monitoring experience with over 20 patient-years of experience. Additionally, recommendations for future monitoring will be presented including the type of testing and sampling frequency.

A NOVEL MURINE RETROVIRUS IDENTIFIED DURING TESTING FOR HELPER VIRUS IN HUMAN GENE TRANSFER TRIALS

A. Dusty Miller 1,2, Lynn Bonham1, John Alfano1, Hans-Peter Kiem1, Tom Reynolds3, and Greg Wolgamot1,2,4
Fred Hutchinson Cancer Research Center1, Department of Pathology2, and Medical Scientist Training Program4 and Targeted Genetics Corporation3, Seattle, Washington

Testing of retroviral vector supernatants and transduced cells used in human clinical trials for the presence of replication-competent retrovirus (RCR) is required. We have detected a novel inducible retrovirus produced under certain conditions during sensitive marker rescue assays using a Mus dunni cell line. The implications of this result and impact on RCR testing of retrovirus vectors and transduced cells will be discussed.

INFECTION OF NORMAL RHESUS MONKEYS WITH A MURINE REPLICATION-COMPETENT RETROVIRUS .

Arifa S. Khan1, Muhammad Shahabuddin1, Teresa A. Galvin1, Jeffrey Ostrove2, Exeen M. Morgan2, and Janet Hartley3
1Division of Viral Products, CBER, FDA, Bethesda, MD; 2Microbiological Associates, Inc., Rockville, MD; 3Laboratory of Immunopathology, NIAID, NIH, Bethesda, MD.

Replication-competent retroviruses (RCR) can arise in come cases unexpectedly and spontaneously from retrovirus-based packaging cell systems used in human gene therapy products. RCR containing an amphotrophic murine leukemia virus (MuLV) envelope possess a broad host range and can infect a wide variety of primate cells including human. To evaluate the potential of RCR infection in humans, four normal juvenile rhesus monkeys were inoculated

with a vector virus preparation (S3A) which contained RCR (provided by Genetic Therapy, INC., Gaithersburg, MD). The monkeys were monitored regularly for virus infections by 1.) isolation of infectious MuLV from the PBMC; 2.) Western blot analysis for antibody response; and 3.) DNA PCR for persistence of viral sequences in the host. Clinical changes were monitored by serum chemistry and hematology. The data indicate that all four animals were infected with RCR; although virus replications was early and transient, persistence of viral sequences was seen. Each animal responded uniquely with respect to the kinetics of infections and antibody response. The results of a three year study will be presented.

MONITORING HUMAN CLINICAL TRIAL SUBJECTS RECEIVING DIRECT ADMINISTRATION OF A RECOMBINANT RETROVIRAL VECTOR FOR THE PRESENCE OF REPLICATION COMPETENT RETROVIRUS (RCR) AND FOR THE INADVERTENT TRANSDUCTION OF GERMLINE CELLS: METHODS, RESULTS, AND IMPACT ON TESTING FREQUENCY

Dale G. Ando and Chiron HIV Immunotherapy Team

PCR assays capable of detecting a single copy of target sequence in the background of >11 x 105 cell equivalents have been developed for monitoring HIV-positive clinical trial subjects for the presence of RCR in PBMC and for the presence of vector in semen samples. These tests have been used to monitor the 297 patients enrolled in the Phase I and Phase II clinical trials for HIV-IT(V), an immunotherapeutic recombinant retroviral vector expressing the HIV-1 env/rep genes. Patients have been treated with cumulative doses of up to 180 million cfu/pt (Ph II). In Phase I studies, 79/81 patients have been tested for RCR at a minimum of three time points. In Phase II studies, all patients (216) have been tested for RCR. Tests were uniformly negative. Semen samples from 119 patients in the Phase II trial were analyzed for the presence of HIV-IT(V) vector sequences using PCR.

We have recently developed an ELISA that can detect antibodies to amphotrophic MLV. We have used this assay to screen serum samples from 46 Phase I clinical trial patients that had previously tested negative for RCR by PCR. Only one subject showed a positive change in anti-MLV titer after HIV-IT(V) with negative RCR by PCR. The ELISA test may be more appropriate for monitoring patients enrolled in ex vivo product trials, since ex vivo products should not express amphotrophic MLV proteins.

There has been no evidence of RCR infection in the HIV-infected Phase I and II clinical trial patients treated with HIV-IT(V). This is consistent with results from the Quality Control testing of the product; cocultivation of 108 post-production cells and direct testing of bulk HIV-IT(V) product with an MLV replication-permissive cell line, were negative. Semen analysis did not reveal any evidence of germline transduction. These data show that RCR infection and germline transmission have not been detected in patients treated with the HIV-IT(V). As such, a reduced testing frequency for RCR and germline transduction in patients seems reasonable.

MONITORING OF CANCER PATIENTS RECEIVING RETROVIRAL VECTOR GENE THERAPY

Gerard J. McGarrity, Ph.D.
Genetic Therapy, Inc., Gaitherburg, Maryland 20878

In 1993 CBER presented recommendations for monitoring of patients that have received retroviral vectors for gene therapy. These recommendations have included the type and frequency of assay for this patient population. Here we present the results of monitoring of 57 patients enrolled in brain cancer protocols involving the use of murine vector producer cells that deliver a retroviral vector encoding for Herpes simplex thymidine kinase. These 57 patients were

enrolled in six different protocols that involved the delivery of approximately 1 x 109 vector producer cells to patients that had recurrent glioblastoma multiforme and other neoplasms in the CNS. Patient assays have included the use of methods to detect antibodies to the mouse producer cells and to vector components as well as polymerase chain reaction (PCR) assays on peripheral blood lymphocytes to detect the presence of vector and replication competent retrovirus (RCR). RCR has not been detected in any of 263 specimens from 57 different patients by PCR. In addition, six different patient specimens were also negative in biological assays for PCR. Presence of vector was detected in certain patients. The majority of these, however, received two or more inoculations of producer cells. The methods and implications of these results will be discussed.

BREAKOUT SESSION 7: PHARMACOLOGY & TOXICOLOGY

SUMMARY

Anne M. Pilaro and Joy Cavagnaro
FDA/CBER

Speaker Presentations

Five speakers provided presentations, demonstrating how preclinical studies in animals have facilitated clinical development of adenoviral, retroviral, or adeno-associated viral vectors as therapeutic approaches in the treatment of cancer, cystic fibrosis, AIDS, or inborn errors of metabolism. Brendan Lee (Baylor College of Medicine) discussed how changes in the route of administration of adenovirus can affect the toxicity profile of the vector; it was concluded that the limiting toxicity of adenoviruses by any route was induction of a dose-related, inflammatory response. Additionally, a sharp threshold in the dose-related toxicity of adenovirus was noted after intravenous administration and was accompanied by a clinical picture resembling disseminated intravascular coagulation, which may limit the use of these vector by this route at high doses in the clinic. Using a murine model of oyster glycogen-induced peritonitis, Jeff Holt (Vanderbilt University) demonstrated that the presence of underlying disease could enhance the toxicity and dissemination of a retroviral vector used for treatment of peritoneal cancers. A similar, reversible toxicity (elevation of hepatic transaminases and infiltration of inflammatory cells) was noted in 1/6 patients treated with this vector, suggesting that the mouse model provided a relevant means to assess the potential clinical toxicities associated with this regimen. By contrast, Ron Crystal (Cornell Medical Center and GENVEC) discussed how the clinical toxicity noted in one cystic fibrosis (CF) patient treated with an adenoviral vector encoding the corrected CFTR gene was not predicted by studies conducted in Rhesus monkeys before the onset of the phase 1 trial. The toxicity observed in the patient resulted in a change in the strategy of dosing for future trials, such that the volume and the method of administration were vastly altered. These changes have resulted in successful administration of the vector to 12 CF patients without the clinical sequelae noted in the first trial, and effective re-treatment has been accomplished without toxicity. Studies in the Rhesus monkey with an adeno-associated viral vector (AAV) were presented by Tom Reynolds (Targeted Genetics), and focused on the mechanism of vector dissemination after intra-bronchial administration and the potential clinical and pathologic consequences of infection with AAV-based vectors. Data demonstrated that the low-level spread of the vector was most likely due to inadvertent inoculation of hematologic cells during the treatment procedure, was detected only transiently, and was without pathologic consequence. Assays to attempt to "rescue" and generate replication-competent AAV vector displayed only limited viral recovery in monkeys carrying latent infection with wild-type AAV and subsequent infection with very high levels of a host-range mutant, wild-type adenovirus. These parameters were identified from the preclinical studies as considerations to be followed during the clinical trial. Finally, Mike Blaese (NCHGR, NIH) presented clinical data from patients treated with retrovirally-modified, gene-transduced cells for several different indications, including adenosine deaminase deficiency and AIDS. Long-term follow-up (out to 6 years in one case) has demonstrated in some cases the persistence of the gene-modified cells, improvement in functions associated with the transduced genes, and no evidence of significant clinical toxicities or generation of replication-competent retrovirus recombinants, helper viruses, or pseudotypes. There was a wide degree of variability in response between patients, suggesting that as the animal models were not predictive of some clinical responses, so humans may not always predict other humans' responses either. In the absence of suitable animal models to study these issues, long-term safety of gene-modified cells will only be determined by clinical evidence, and will require careful clinical monitoring.

Discussion Session

Five questions were put to the audience for open discussion, and are presented individually below. A general conclusion was that for each of the issues discussed, the route, the dose, and the indication for which the vector was intended should be carefully considered and balanced with the risks to determine what would be acceptable.

1. How much RCA is "safe" to administer to patients? What preclinical data are useful to address the safety of RCA present in adenoviral vector preparations, especially after alternate routes of administration?

Conclusion: Regarding the administration of doses of adenoviral vector containing higher amounts of replication-competent virus (RCA) than the current standard allows, it was concluded that the risks may be more acceptable by some routes (e.g. subcutaneous) or in some indications (e.g. cancer) than in others (e.g. intra-bronchial in cystic fibrosis). However, increased amounts of RCA may be permitted in these situations if data are provided by the sponsor from experiments designed specifically to address the safety of RCA administration in the presence of the vector, and can provide an adequate, quantitative estimate of the risks. Preclinical data were cited by sponsors demonstrating that administration of up to 106 RCA in the lung in an excess of replication-defective particles was acceptable, while in the clinic, doses of vector which included RCA levels of 10 or 100 were administered without adverse effects. The group also concurred that RCA may no longer be as great a concern in the future, as further improvements in the way these vectors are produced and in the gene constructs themselves decrease the likelihood of recombination events.

2. What preclinical studies should we consider to demonstrate the delayed/long-term consequences of gene therapy? What animal models will be most useful for each vector class?

Conclusion: Discussion concurred that more preclinical data may be needed, depending on the duration and expected consequences of the proposed therapy. Animal models may be useful to study long-term effects, especially if predictive results of general organ toxicity are obtained in early studies. Current clinical monitoring practices in humans will also contribute to long-term safety assessment.

3. What safety studies should be required to demonstrate a lack of toxicity/developmental effects to germ line cells when positive gene transfer is seen.

after inadvertent distribution of vector to the gonads?

after intended targeting to germ cells?

Conclusion: Intended targeting of vectors to germ cells was not discussed at the present time. If a positive result of gene transfer to the gonads is obtained in an animal study, it was agreed that further preclinical studies need to be carefully defined to better assess the risks to the patient population. Study designs should employ the best available techniques, and explain or justify the limitations of detection for the method employed. These studies should initially focus on identification of the cell type in the gonadal tissue in which the signal is positive, with follow-up studies conducted if "positive" gene transfer is obtained, i.e. is there integration into the germ cell. Current practice is to include monitoring for potential targeting to the germ line (i.e. analysis of semen samples from male patients receiving gene vectors for the inserted construct) as part of the clinical protocol.

4. At what phase in development should full-scale reproductive toxicity studies of gene therapy constructs be considered?

Conclusion: Studies to define the effects of gene therapy on reproductive capacity and/or outcome will be based on the gene product, as well as the patient population intended for

treatment. It was concluded that traditional, reproductive toxicity study designs and timelines during drug development may not be appropriate for gene therapy vectors. An example was cited from the area of DNA vaccines, in which a problem-specific approach to the design of a reproductive study to support use of the vaccine in pregnant women was employed. By conducting a specific, targeted preclinical study designed to answer the safety of this product in pregnant animals, data should be generated that will facilitate its use in the intended population.

5. Is there a mechanism for "data-sharing" or conducting "investigator-neutral" studies to support various, recurrent questions about the safety of gene therapy, and if so, which areas would be most useful to focus on?

Conclusion: The group concluded that there was an overall willingness to share and/or publish information from preclinical studies, but acknowledged that there were differences between corporate and academic institutions' ability to make data publicly available. As products move into phase 2 and ultimately, phase 3, there was some concern that data would become less available to the field. The general consensus was that focus should be on the total "product", and not on individual vectors. Annual scientific conferences, such as are sponsored by the Cystic Fibrosis Foundation yearly in Williamsburg may be useful as a means to open communication in the field; alternatively, forums or conferences sponsored through ORDA/ODAC may achieve this end as well.

Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

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►AGENDA

►I. INTRODUCTION

◇Anne Pilaro

►II. SPEAKER PRESENTATIONS - 20 MIN EACH

- ◆How changes in route of administration of adenoviral vectors affect the toxicity in vivo
  - ◇Brendan Lee, Baylor College of Medicine
- ◆How a disease state may modify the distribution and safety of retroviral vectors in vivo, using a newly developed murine model system
  - ◇Jeff Holt, Vanderbilt University
- ◆Distribution studies in support of adeno-associated viral vector safety after administration to the lung
  - ◇Tom Reynolds, Targeted Genetics
- ◆Using in vitro data to support the safety or retrovirally-transduced cells in the absence of a suitable animal model for in vivo safety testing
  - ◇Eric Poeschla, UCSD
- ◆Preclinical studies with adenoviral vectors to define safety parameters for clinical monitoring after targeted administration
  - ◇Ron Crystal, GenVec/Cornell Medical Center
- ◆Long-term follow-up of patients receiving retrovirally-transduced cells: What have we learned?
  - ◇Mike Blaese, NCHGR

►III. PANEL DISCUSSION, JOY CAVAGNARO, CHAIR

- ◆Topics for panel discussion are as follows (approximately 5-10 min each):
  - ◇1. How much RCA is "safe" to administer to patients? What preclinical models are useful to address the safety of RCA present in adenoviral vector preparations, especially after alternate routes of administration?

- ◇ 2. What preclinical studies should we consider to demonstrate the delayed/long-term consequences of gene therapy? What animal models will be most useful for each vector class?
- ◇ 3. What safety studies should be required to demonstrate a lack of toxicity/developmental effects to germ line cells when positive gene transfer is seen—after inadvertent distribution of vector to gonads?--after intended targeting to germ cells?
- ◇ 4. At what phase in development should full-scale reproductive toxicity studies of gene therapy constructs be considered (if ever)?
- ◇ 5. Is there a mechanism for "data sharing" or conducting "investigator neutral" studies to support various, recurrent questions about the safety of gene therapy, and if so, which areas would be most useful to focus on?

► IV. SUMMARY AND CLOSING REMARKS—ANNE PILARO AND JOY CAVAGNARO

- ◆ The goal of this session is to understand how preclinical studies have helped to identify common areas of concern regarding the safety of gene therapy for each of the different vector types. Specific questions include:
- ◆ How changes in the route of administration of a vector may modify its toxicity.
- ◆ How the presence of a disease state may modify the safety of a given gene therapy and whether normal animals serve as good predictors of the clinical experience.
- ◆ When can pre-clinical studies be minimized?
- ◆ How preclinical data have identified parameters to follow in the clinic.
- ◆ What pharm/tox studies should be considered in support of future phase 2 and phase 3 gene therapy trials?

Questions, comments, and requests for further information about this session may be submitted after the conference by e-mail to:

GTINFO@A1.CBER.FDA.GOV

PHARMACOLOGY/TOXICOLOGY STUDIES FOLLOWING INTRAPERITONEAL INFUSION OF RETROVIRAL VECTORS.

J.T. Holt, D. Tait, P. Obermiller, S. Redlin-Frasier, R.A. Jensen, C.L. Arteaga, and M.C. King
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Interperitoneal infusion of retroviral vectors is a model system we have employed for initial preclinical and early clinical studies. Mouse studies demonstrate that spread of retroviral vector from the peritoneal cavity to blood and tissues (identified by PCR) is dependent on vector dose and enhanced by oyster glycogen-induced peritonitis. Although XM6:antifos and LXSN-BRCA1 retroviral vector infusion show similar spread to tissues, LXSN-BRCA1 produces a transient peritonitis and focal hepatocellular necrosis which is dose-dependent and enhanced by oyster-glycogen induced peritonitis. Clinical studies are just beginning, but 1 of 6 patients treated with intraperitoneal LXSN-BRCA1 for ovarian cancer developed a transient temperature (102.4) and focal peritoneal signs eight hours after infusion. Fluid cytology showed increased neutrophils consistent with a limited peritonitis. PCR/Southern studies show that vector can be detected in peritoneal fluid for 24 hours after infusion, and plasma studies show no development of antibody to retroviral envelope proteins in two patients (we have seen antibodies develop in one patient treated with intrapleural infusion of XM6: antifos vector). Dose escalation studies in patients are continuing along with concurrent animal studies aimed at further understanding of the mechanism of LXSN-BRCA1 peritonitis.

TOXICOLOGICAL STUDIES OF ADENOVIRUS MEDIATED GENE TRANSFER VIA A) INTRACEREBRAL AND INTRAPROSTATIC INJECTION IN NON HUMAN PRIMATES AND COTTON RATS, AND B) SYSTEMATIC DELIVERY INTO NONHUMAN PRIMATES, COTTON RATS, AND MICE

B. Lee, T.L. Timme, H.D. Shine, W. O'Neal, N. Carey, G.B. Hubbard, D.A. Carrier, C. Nyberg, R. Barrios, S. Rajagopaian, P.R. Wyde, A. Rojas-Martinez, C.A. Montgomery, S.L.C. Woo, A. Beaudet, T.C. Thompson, and E. Aguilar-Cordova
Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX 77030.

As part of preclinical studies for adenoviral mediated gene therapy, toxicological studies of E1 and E1/E2 deleted adenoviruses were carried out with different routes of delivery in several animal models. Up to 1.5 X 109 p.f.u. E1 deleted adenovirus expressing the Herpes virus thymidine kinase gene (Ad.RSV-tk) was delivered via intracerebral injection in nonhuman primates and cotton rats. Cotton rats were studied with and without preimmunization to wild-type adenovirus. Histologic inspection showed inflammation of the ependyma and choroid plexus and at the injection site. No differences were noted between the doses analyzed, gancyclovir (GVC) treatment, or status of preimmunization. Similarly, injection of higher doses up to 1.5 X 109 p.f.u. of Ad. RSV-tk with and without GVC treatment was carried out in baboons. Adverse clinical symptoms were only seen in the group injected with the highest dose and who were treated with GVC. However, MRI and necropsy revealed cystic changes at the higher dose and necrotic foci in all groups. These experiments showed toxicity associated with direct intracerebral injection of Ad.RSV-tk and GVC treatment.

Similar studies were carried out via injection into dorso-lateral prostates of C57BL/6 mice. No differences in local inflammation between the doses analyzed were noted in the groups without GVC. However, a greater degree of leukocytic infiltration was noted at higher doses treated with GVC. Vector sequences were localized primarily to the dorsolateral and ventral prostate and detected outside of local inflammatory response.

The effect of systemic delivery of up to 109 infectious units (i.u.) of Ad.RSV-tk via intracardiac injection with and without GVC treatment was studied in cotton rats. Primary distribution was in the heart, lung and thymus. Microscopic lesions were noted in the heart and spleen of

animals given the two higher doses with greater splenic hemosiderosis noted in the GVC treated group. Alternative systemic delivery via intravenous injection was studied in baboons and C3H/HeOuJ mice. Injection of 1.1 X 1013 particles/kg of an E1 deleted adenovirus expressing β-galactosidase (Ad.PGK-β-Gal) resulted in lethargy and petechial lesions which progressed to dyspnea by 48 hours. Elevated liver functions and abnormal hematological values were noted. Pathology analysis showed severe and diffuse vasculopathy.

Intravenous administration of E1 and E1/E2 deleted adenoviruses expressing human α-1-antitrypsin at up to 9 X 1013 and 1 X 1013 particles/kg, respectively, was analyzed in C3H/HeOuJ mice. Dose-related toxicity was noted primarily in the liver and spleen. A dose-independent transient thrombocytopenia was also detected. Potential safety advantage of E2 deleted adenovirus was evaluated by measurement of serum liver function markers and by histopathology.

An overview of these experiments will be presented and their cumulative significance to the safety of adenoviral vectors in gene therapy will be discussed.

DISTRIBUTION AND RESCUE STUDIES OF AN AAV-CFTR VECTOR IN RHESUS MACAQUES

S.A. Afionel, C.K. Conrad1, W.G. Kearns3, S. Chunduru1, R. Adams4, T.C. Reynolds5, W.B. Guggino2, G.R. Cutting3, B.J. Carter5, and T.R. Flotte1
Eudowood Division of Pediatric Respiratory Sciences1 and the Departments of Physiology2, Genetics3, and Comparative Medicine4, Johns Hopkins University School of Medicine, Baltimore, MD, and Targeted Genetics Corporation5, Seattle, WA.

AAV is a nonpathogenic human parvovirus that has potential application as a gene therapy vector. Preclinical studies were conducted to support human trials of an AAV-CFTR vector in the setting of cystic fibrosis. In the first study, vector was administered to the right lower lobe of rhesus macaques at several doses. No toxicity was observed at any dose level using a variety of clinical and laboratory measures. Vector DNA and RNA were detected in the lungs of treated animals for up to 6 months and no RNA expression was observed. Additional studies showed that low levels of vector DNA could be detected transiently in the peripheral blood lymphocytes of animals following vector administration. Vector shedding was not observed following bronchoscopic administration to the lung. The low level vector rescue was only observed following high dose anatomic co-administration of vector and wild-type AAV in the presence of adenovirus. These results indicate that in the rhesus model, AAV vectors are capable of mediating efficient and persistent gene transfer in the absence of toxicity, and that the risks of vector shedding and rescue appear to be low.

IN VIVO ADENOVIRUS VECTOR PREDICTABILITY OF SAFETY STUDIES IN EXPERIMENTAL ANIMALS

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The demonstration of the ability of replication-deficient adenovirus vectors to transfer genes in vivo led to the first human trials of adenovirus-mediated gene transfer in 1993. Since then, adenovirus vectors have been used to transfer genes to more than 120 humans, in trials directed toward the therapy of cystic fibrosis, colon cancer metastatic to liver, malignant mesothelioma and bronchogenic carcinoma. Safety studies have been carried out in experimental animals in order to evaluate the type and extent of possible adverse events that might be expected in humans. Now that there has been more than three years experience with adenovirus vectors in humans, it is useful to look back at the animal safety studies that have been carried out, and to

ask the question: are in vivo adenovirus vector safety studies in experimental animals predictable of adenovirus vector administration to humans?

The bulk of the experience with adenovirus vectors in humans is focused on cystic fibrosis (CF), a common recessive disorder caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, a 250 kb, 27 exon gene on chromosome 7. The major manifestations are on the airway epithelial surface, with purulent mucus, opportunistic infections, chronic inflammation and progressive loss of lung function. There is compelling logic underlying the concept that the respiratory manifestations of CF could be prevented by gene therapy. First, there is overwhelming evidence that the respiratory manifestations of CF result from insufficient CFTR function in the airway epithelium. Second, the deficiency in CFTR function in the airway epithelium can be corrected in vitro by transfer of the normal CFTR cDNA into the airway cells. Finally, a variety of strategies have been used to successfully transfer the normal human CFTR cDNA to the airway epithelium of experimental animals in vivo.

Collectively, the initial gene therapy studies using adenovirus vectors have had three goals: (1) to demonstrate the feasibility of gene transfer and expression in the respiratory epithelium; (2) to evaluate the ability of the transferred gene to function; and (3) to define the safety of the gene transfer process. Two respiratory epithelial targets have been evaluated: the airway epithelium (the site of the clinical abnormalities) and the nasal epithelium (a surrogate site used to evaluate function). Several important lessons have been learned. First, it is feasible to transfer the CFTR cDNA to the human respiratory epithelium in vivo, with expression of the CFTR cDNA at the mRNA and protein levels. Second, although not observed in all studies, trends toward functional correction of the electrophysiologic biologic abnormality have been observed following CFTR cDNA transfer to the nasal epithelium. Third, it is now possible to quantify vector-derived CFTR gene expression in bronchial epithelial samples at the mRNA level, and to determine the extent of vector-derived CFTR mRNA expression compared to endogenous CFTR mRNA expression, thus providing a quantitative goal to measure success of gene transfer to the airways. Finally, the "safety window" of doses and volumes has been approximated for administration of adenovirus vectors to the airways. Adverse events have been minimal, but they have been observed in both the nasal epithelium and the lung. In the nasal epithelium, mild, reversible inflammation has been observed at high doses. In the lung, parenchymal inflammation has been observed at high doses when the vector inadvertently reached the alveoli, rather than remaining in the bronchi (the target of the therapy).

Interestingly, although dose-dependent inflammation is observed in the lung parenchyma in animals receiving adenovirus vectors, studies with high doses in several species (mouse, cotton rat, and non-human primates) have failed to predict the adverse events observed in humans. From this experience, the following conclusions can be drawn. First, if toxicity is observed in experimental animals, human studies must be approached with caution. However, if no toxicity is observed in animals, the same caution must be applied to human studies, i.e., the animal studies may not predict the human responses. Second, no experimental animals are identical to humans in regard to adenovirus vectors, and there are no experimental animal models that exactly mimic human disorders, such as CF. Third, non-human primates do not seem to offer any advantages over rodents in regard to safety predictability for these vectors. Finally, despite the need to carry out studies in experimental animals before initiating human studies, it is only by studying humans that true safety profiles of biologic reagents, such as adenovirus gene transfer vectors, can be determined.

APPENDIX

Overview of Regulatory Documents Available by CBER FAX BACK

► FDA GUIDANCE DOCUMENTS

The Center for Biologics Evaluation and Review (CBER) of the Food and Drug Administration (FDA) provides specific guidance to sponsors for the purposes of aiding the development and evaluation of products. Specific guidance is based upon scientific principles and scientific experimentation derived from in-house as well as academic and industry sources. FDA does not intend that guidance documents be all-inclusive. Alternative approaches could be warranted in specific situations, and certain aspects might not be applicable in all situations. If an applicant believed the application procedure described in a guidance document was inapplicable to a specific situation for a particular product, the applicant could provide, for CBER's consideration, information supporting an alternative process. If an applicant chooses to use alternative processes, the applicant may wish to discuss the matter further with the agency to prevent expenditure of money and resources on activities that later might be determined to be inappropriate to FDA. Guidance documents do not create or confer any rights for or on any person, do not operate to bind FDA or the public, and represent the agency's current thinking.

- ◆ Points to Consider (PTC): Recommendations to follow or consider for areas where there is limited product experience or rapidly changing technology.
- ◆ Letter to Sponsor :A guidance document sent to sponsors, sometimes proprietary, describing the current regulatory approach to a specific issue.
- ◆ Other Guidance Documents Include guidelines and memoranda to manufacturers and sponsors.

► INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH)

- ◆ Guidelines developed by joint agreement of the US, Europe, Japan, and their industry representatives. Upon final acceptance, ICH documents supersede FDA guidelines and PTC where applicable.

► LAW/UNITED STATES CODE

- ◆ Code of Federal Regulations (CFR) (CFR Title 21- Food and Drugs) A compilation of all effective government regulations and interpretations of the law. A regulation is defined in 21 CFR 10.3 (a) as "an agency rule of general or particular applicability and future effect issued under a law administered by the Commissioner or relating to administrative practices and procedures". Regulations are usually subject to public comment before finalization. The CFR is binding upon both FDA and sponsors.
- ◆ Federal Register Notice A procedural announcement in the Federal Register of contemplated actions or policy decisions and which provides the opportunity for public comment.
- ◆ Food, Drug and Cosmetic Act and the Public Health Service Act Two of the major laws that mandate the responsibilities of the Food and Drug Administration. Specific interpretations of these laws are contained in the CFR. FDA and sponsors are bound by these laws.

DOCUMENTS AVAILABLE FROM THE CBER

NEW TELEPHONE NUMBER!
1-888-CBER-FAX
FAX INFORMATION SYSTEM - 1-301-827-3844
July 19, 1996

Document Number	Document Name
1000	Guideline for Quality Assurance in Blood Establishments, 7/11/95, 36 pages
1001	OELPS, Advertising and Promotional Labeling Staff Procedural Guidance Document (Draft), 8/94, 6 pages
1002	Points to Consider - Characterization of Cell Lines Used to Produce Biologicals, 7/12/93, 42 pages
1003	Points to Consider in Human Somatic Cell Therapy and Gene Therapy, 8/27/91, 21 pages
1004	Draft Guideline for the Validation of Blood Establishment Computer Systems, 9/28/93, 32 pages
1005	Points to Consider in the Production and Testing of New Drugs and Biologics Produced by Recombinant DNA Technology, 4/10/85, 14 pages
1006	Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability, 4/6/92, 9 pages
1007	Draft Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (8/94), 43 pages
1008	Points to Consider in the Production and Testing of Interferon Intended for Investigational Use in Humans, 7/28/83, 21 pages
1009	Guideline for Adverse Experience Reporting for Licensed Biological Products, 10/15/93, 56 pages
1010	ICH Guideline Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products, 2/23/96, 3 pages
1011	Draft ICH Document-Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products, 8/21/95, 19 pages
1012	ICH Guideline for Industry, Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, 3/1/95, 16 pages
1013	Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals, 8/22/95, 20 pages
1014	Draft Points to Consider for the Evaluation of Combination Vaccines: Production, Testing, and Clinical Study (1995), 10/3/95, 28 pages
1015	Guidance for Industry; Content and Format of Investigational New Drug Applications (INDS) for Phase 1 Studies of Drugs, including Well-Characterized, Therapeutic, Biotechnology-derived Products, 11/95, 17 pages
1016	Draft Guidance, Changes to an Approved Application, 1/96, 19 pages
1017	Draft Guidance, Changes to an Approved Application for a Well-Characterized Therapeutic Recombinant DNA-Derived and Monoclonal Antibody Biotechnology Product, 1/96, 14 pages
1018	Draft Addendum to The Points to Consider in Human Somatic Cell and Gene Therapy, 1/96, 18 pages
1019	ICH Final Guidance on the Need for Long-Term Rodent Carcinogenicity

	Studies of Pharmaceuticals, 3/1/96, 4 pages
1020	**ICH Guideline on Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility, 4/5/96, 3 pages**
1021	**FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-derived Products, 4/26/96, 10 pages**
1022	**Draft ICH Guideline on Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin, 5/10/96, 11 pages**
1023	**Guidance for Industry: The Content and Format for Pediatric Use Supplements, May 23 1996, 7 pages**
1024	**Guidance on Applications for Products Comprised of Living Autologous Cells Manipulated Ex Vivo and Intended for Structural Repair or Reconstruction, May 24, 1996, 10 pages**
2000	**Betaseron Approval Information, 7 pages**
2001	**1993 Biologic & Drug Approvals, 9 pages**
2002	**Advisory Committee Information Line Instructions, 1 page**
2003	**Baxter IGIV Talk Paper, 2/25/94, 2 pages**
2004	**How to Obtain a Certificate to Foreign Governments, 1 page**
2005	**How to Make a Freedom of Information Act Request, 1 page**
2006	**How to Use a Fax Modem with the CBER Fax Information System, 1 page**
2007	**How to Obtain CBER Documents by Internet Mail, 1 page**
2008	**How to Obtain CBER Documents by File Transfer Protocol (FTP), 1 page**
2010	**1994 CBER Approval Actions, 1/10/95, and FDA Talk Paper - 1994 Medication Approvals, 1/17/95, 7 pages**
2011	**Press Release and Approval Letter, Varicella Vaccine, 3/17/95, 5 pages**
2012	**Information about Export Applications and Requirements, 6/95, 21 pages**
2013	**1995 CBER Approval Actions and FDA Talk Paper - FDA 1995 Approvals, 1/16/96, 6 pages**
***2014**	**CBER User Fee Performance Goals, Actions as of 6/30/96, 15 pages**
2015	**Press Release and Approval Letter, Respiratory Syncytial Virus Immune Globulin Intravenous (Human), 1/19/96, 5 pages**
2016	**Press Release and Backgrounder, HIV Home Test Kit, 5/14/96, 6 pages**
2017	**Talk Paper, FDA Approves Second Interferon for Multiple Sclerosis, 5/17/96, 2 pages**
3000	**FEDERAL REGISTER notice - Alternatives to Lot Release, 7/20/93, 3 pages**
3001	**FEDERAL REGISTER notice - Application of Current Statutory Authorities to Human Somatic Cell Therapy, 10/14/93, 3 pages**
3002	**FEDERAL REGISTER notice - Interim Rule - Human Tissue Intended for Transplantation, placed on public display 12/10/93, 9 pages**
3003	**FEDERAL REGISTER notice - Final Rule - Adverse Experience Reporting Requirements for Licensed Biological Products, 10/27/94, 12 pages**
3004	**FEDERAL REGISTER notice - Proposed Rule - Adverse Experience Reporting Requirements for Human Drug and Licensed Biological Products, 10/27/94, 20 pages**
3005	**FEDERAL REGISTER notice - Biological Products; Allergenic Extracts Classified in Category IIIB; Final Order; Revocation of Licenses, 11/16/94, 10 pages**
3006	**FEDERAL REGISTER notice - FDA's Policy Statement Concerning Cooperative Manufacturing Arrangements for Licensed Biologics, 11/25/92, 3 pages**

3007	FEDERAL REGISTER notice - Home Specimen Collection Kit Systems Intended for Human Immunodeficiency Virus (HIV-1 and/or HIV-2) Antibody Testing; Revisions to Previous Guidance, 2/23/95, 2 pages
3008	FEDERAL REGISTER notice - Changes to be Reported for Product and Establishment License Applications; Guidance, 4/6/95, 4 pages
3009	FEDERAL REGISTER notice - FDA Guidance Document Concerning Use of Pilot Manufacturing Facilities for the Development and Manufacture of Biological Products; Availability, July 11, 1995, 4 pages
3010	FEDERAL REGISTER notice - July 27, 1995 - Subcommittee Meeting of the National Task Force on AIDS Drug Development on Drug Development Issues, September 13 & 14, 1995, 2 pages
3011	FEDERAL REGISTER notice - August 21, 1995 - International Conference on Harmonisation, Draft Guidelines, 11 pages
	1) Analysis of the Expression Construct in Cells Used for the Production of r-DNA Derived Protein Products
	2) Conditions Which Require Carcinogenicity Studies for Pharmaceuticals
	3) Detection of Toxicity to Reproduction: Addendum on Toxicity to Male Fertility
	4) Stability Testing of Biotechnological/Biological Products
3012	FEDERAL REGISTER notice - September 27, 1995 - Prominence of Name of Distributor of Biological Products, 6 pages
3013	FEDERAL REGISTER notice - October 25, 1995 - Characterization of Biological/Biotechnology Pharmaceutical Products; Notice of Public Workshop, 2 pages
3014	FEDERAL REGISTER notice - December 8, 1995 - Interim Definition and Elimination of Lot-by-Lot Release for Well-Characterized Therapeutic Recombinant DNA-Derived and Monoclonal Antibody Biotechnology Products, 6 pages
3015	FEDERAL REGISTER notice - December 8, 1995 - Advertising and Promotion; Draft Guidances, 2 pages.
3016	FEDERAL REGISTER notice - January 3, 1996 - Statement of Organization, Functions, and Delegations of Authority, 2 pages
3017	FEDERAL REGISTER notices - January 29, 1996 - pages 2733-2750, 1) Well-Characterized Biotechnology Products; Elimination of Establishment License Application; 2) Changes to an Approved Application; 3) Draft Guidance; Changes to an Approved Application for Well-Characterized Therapeutic Recombinant DNA-Derived and Monoclonal Antibody Biotechnology Products; Availability; 4) Changes to an Approved Application; Draft Guidance; Availability, 18 pages
3018	FEDERAL REGISTER notice - February 26,1996 - Draft Document Concerning the Regulation of Placental/Umbilical Cord Blood Stem Cell Products Intended for Transplantation or Further Manufacture into Injectable Products; Availability of draft document, 2 pages
3019	FEDERAL REGISTER notice - March 7, 1996 - Notice of Public Meeting March 15, 1996; Commissioner's Roundtable-Regulatory Approach to Products Comprised of Living Autologous Cells Manipulated Ex Vivo and Intended for Structural Repair or Reconstruction, 2 pages
3020	FEDERAL REGISTER notice - May 3, 1996 - Current Good Manufacturing Practice; Proposed Amendment of Certain Requirements for Finished Pharmaceuticals, 13 pages
3021	FEDERAL REGISTER notice - May 14, 1996 - Final Rule, Elimination of Establishment License Application for Specified Biotechnology and Specified

	Synthetic Biological Products, 7 pages
3022	**FEDERAL REGISTER notice - May 28, 1996 - Guidance on Application for Products Comprised of Living Autologous Cells Manipulated Ex Vivo and Intended for Structural Repair or Reconstruction; Availability, 2 pages**
***3023**	**FEDERAL REGISTER notice - July 10, 1996 - International Conference on Harmonisation; Final Guidelines on Stability Testing of Biotechnical/Biological Products; Availability; Notice, 5 pages**
***3024**	**FEDERAL REGISTER notice - July 17, 1996 - International Conference on Harmonisation; Guideline on Structure and Content of Clinical Study Reports; Availability; Notice, 25 pages**
4000	**Memorandum to Registered Blood Establishments, Deferral of Blood and Plasma Donors based on Medications, 7/28/93, 4 pages**
4001	**Memorandum to Registered Blood Establishments, Recommendations Regarding License Amendments and Procedures for Gamma Irradiation of Blood Products, 7/22/93, 20 pages**
4002	**Memoranda to Registered Blood Establishments, Revised Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leukocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV), 8/5/93 and 8/19/93, 9 pages**
4003	**Memorandum to Registered Blood Establishments, Guidance Regarding Post Donation Information Reports, 12/10/93, 3 pages**
4004	**Memorandum to Registered Blood Establishments, Donor Suitability Related to Laboratory Testing for Viral Hepatitis and a History of Viral Hepatitis, 12/22/93, 4 pages**
4005	**Memorandum to Registered Blood Establishments, Recommendations for the Invalidation of Test Results When Using Licensed Viral Marker Assays to Screen Donors, 1/3/94, 5 pages**
4006	**Memorandum to Registered Blood Establishments, Recommendations for Deferral of Donors for Malaria Risk, 7/26/94, 2 pages**
4007	**Memorandum to Registered Blood Establishments, Use of an FDA Cleared or Approved Sterile Connecting Device (STCD) in Blood Bank Practices, 8/5/94 with Transmittal Memo, 8/12/94 (This corrects Memorandum dated 7/29/94), 9 pages**
4008	**Draft Recommendations for the Quarantine and Disposition of Potentially HBV, HCV or HTLV-I Contaminated Units from Prior Collections from Repeat Donors with Repeatedly Reactive Screening Tests. (Intended for discussion at the meeting of the BPAC on 12/15/94.), 11/22/94, 5 pages**
4009	**Memorandum to Registered Blood Establishments, Recommendations to Users of Medical Devices That Test for Infectious Disease Markers by Enzyme Immunoassay (EIA) Test Systems, 12/20/94, 17 pages**
4010	**Memorandum to Registered Blood Establishments, Timeframe for Licensing Irradiated Blood Products, 2/3/95, 1 page**
4011	**Draft Discussion Points for Screening and Testing Donors of Human Tissue Intended for Transplantation and Human Reproductive Tissue, and for Establishment Registration, intended for discussion at the FDA workshop to be held 1:00 to 5:30PM on 3/24/95, 3/25/95, 8 pages**
4012	**Draft Background Information for Discussion at the Workshop on Leukoreduced Products on March 27, 1995, 3/9/95, 20 pages**
4013	**Memorandum to Registered Blood and Source Plasma Establishments, Revision of FDA Memorandum of August 27, 1982: Requirements for Infrequent Plasmapheresis Donors, 3/10/95, 3 pages**
4014	**Memorandum to Licensed Establishments Performing Red Blood Cell**

	Immunizations, Revised Recommendations for Red Blood Cell Immunization Programs for Source Plasma Donors, 3/14/95, 6 pages
4015	**Recommendations for the Deferral of Current and Recent Inmates of Correctional Institutions as Donors of Whole Blood, blood components, Source Leukocytes, and Source Plasma, 6/8/95, 5 pages**
4016	**Draft Document Concerning the Screening and Testing of Donors of Human Tissue Intended for Transplantation, 6/12/95, 9 pages**
4017	**Recommendations for Donor Screening with a Licensed Test for HIV-1 Antigen, 8/8/95, 14 pages**
4018	**Precautionary Measures to Further Reduce the Possible Risk of Transmission of Creutzfeldt-Jackob Disease by Blood and Blood Products, 8/8/95, 6 pages**
4019	**Disposition of Products Derived from Donors Diagnosed with, or at Known High Risk for, Creutzfeldt-Jakob Disease, 8/8/95, 3 pages**
4020	**Recommendations for Labeling and Use of Units of Whole Blood, Blood Components, Source Plasma, Recovered Plasma or Source Leukocytes Obtained from Donors with Elevated Levels of Alanine Aminotransferase (ALT), 8/8/95, 2 pages**
4021	**Guidance for Blood Establishments Concerning Conversions to FDA-Reviewed Software Products, November 13, 1995, 6 pages**
4022	**Memorandum to Registered Blood and Source Plasma Establishments, Donor Deferral Due to Red Blood Cell Loss During Collection of Source Plasma by Automated Plasmapheresis, 12/4/95, 2 pages**
4023	**Draft Document Concerning the Regulation of Placental/Umbilical Cord Blood Stem Cell Products Intended for Transplantation or Further Manufacturing Into Injectable Products, December, 1995, 18 pages**
4024	**Draft Document Concerning the Regulation of Peripheral Blood Hematopoietic Stem Cell Products Intended for Transplantation or Further Manufacture Into Injectable Products, February 1996, 15 pages**
4025	**Memorandum to Registered Blood Establishments, Additional Recommendations for Donor Screening With a Licensed Test for HIV-1 Antigen, 3/14/96, 6 pages**
4026	**Memorandum to Registered Blood and Plasma Establishments, Additional Recommendations for Testing Whole Blood, Blood Components, Source Plasma and Source Leucocytes for Antibody to Hepatitis C Virus Encoded Antigen (Anti-HCV), 5/16/96, 3 pages**
4027	**Memorandum to Registered Blood Establishments, Recommendations and Licensure Requirements for Leukocyte-Reduced Blood Products, 5/29/96, 12 pages**
***4028**	**Memorandum to Registered Blood and Plasma Establishments, Recommendations for the Quarantine and Disposition of Units from Prior Collections from Donors with Repeatedly Reactive Screening Tests for Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human T-Lymphotropic Virus Type I (HTLV-I)**
5000	**CBER Organizational Listing, January 1996, 13 pages**
5001	**CBER Organization Charts, January 1996, 28 pages**
5002	**CBER Mission and Vision Statements, 8/14/95, 2 pages**
5003	**CBER Telephone and Mail Routing Directory, May 21, 1996, 22 pages**
5004	**CBER Strategic Plan for 2004, 8/14/95, 23 pages**
5005	**CBER Annual Report FY94, 33 pages**
5006	**CBER Annual Report FY95, 52 pages**

6000	FDA December 17, 1993, Letter to Manufacturers: Bovine Derived Materials (BSE), 3 pages
6001	CBER December 28, 1993, letter describing preliminary position regarding Interim Rule - Human Tissue Intended for Transplantation, 3 pages
6002	Dr. Zoon's Slides - 1/27/95, The State of CBER 1994: The Year of Reinvention, 17 pages
6003	CBER Refusal to File (RTF) Guidance for Product and Establishment License
6004	CBER March 31, 1994, letter to Blood Establishment Computer Software
6005	Letter to Sponsors of INDs using Retroviral Vectors - 9/20/93, 2 pages
6006	Letter to Sponsors of INDs for Human Immunoglobulin Products - 5/23/94, 4 pages
6007	Review Performance Statistics, September, 1995, 27 pages
6008	Letter to Manufacturers of Licensed Anti-HIV Test Kits - 5/26/94, 4 pages
6009	Letter to IVIG Manufacturers, October 3, 1994, 3 pages
6010	Dear Colleague Letter, Update on the status of efforts to improve the efficiency and effectiveness of the biologic product review and approval program, 11/15/94, 17 pages
6011	Memorandum, OTRR, Alternatives to Lot Release Policy, October 27, 1994, 6 pages
6012	CBER Trends and the Managed Review Process, Dr. Zoon's Slides, 9/19/94, 16 pages
6013	CBER's International Activities, 9/23/94, 6 pages
6014	CBER Update, Dr. Zoon's Slides, 11/2/94, 3 pages
6015	The Role of Science in the Regulation of Biological Products, Dr. Zoon's Remarks at the Dedication of Building 29B, 11/22/94, 7 pages
6016	Statement on FDA Approval of AIDS Virus Test System Based on Oral Fluid Samples, 12/23/94, 2 pages
6017	Letters to Manufacturers of Immune Globulin (Human) - Testing for Hepatitis C Virus RNA, 12/27/94, 2 pages
6018	Letter to Blood Establishment Computer Software Manufacturers, 2/10/95, 5 pages
6019	Letter to Manufacturers of Immune Globulin (Human), 3/3/95, 2 pages
6020	Letter to Manufacturers of Immune Globulin (Human), 3/13/95, 3 pages
6021	Letter to Health Professionals - Immune Globulin (Human), 3/14/95, 7 pages
6022	Background Information - Poison Ivy/Poison Oak Extracts, 3/16/95, 14 pages
6023	Letter to Health Professionals - Market Withdrawal - Plasma Products Produced from Blood Derived from a Donor with Probable CJD, 3/29/95, 5 pages
6024	Addendum to March 29, 1995 Letter - Plasma Product Withdrawal Associated with Probable CJD Donor, 3/31/95, 4 pages
6025	Memorandum to Licensed Manufacturers - Extended Expiration Dating of U.S. Standard Pertussis Vaccine, Lot No. 11, 4/6/94, 1 page
6026	Error and Accident Reports: Summary for First Quarter FY 95 3/22/95, 5 pages, Summary for FY 94, 10/26/94, 22 pages (Total : 27 pages)
6027	Dr. Zoon/Mr. Beatrice Slides, FDA Workshops on Regulatory Policy Issues in the Development & Manufacture of Biopharmaceuticals & Other Biotechnology-Derived Products, 5/95, 6 pages
6028	Dr. Zoon Slides, Drug Information Association, 6/26/95, 6 pages

6029	Error and Accident Reports: Summary for Second Quarter FY-95, 9/22/95, 17 pages
6030	Error and Accident Reports: Summary for Third Quarter FY-95, 9/22/95, 17 pages
6031	Letter to Specific Sponsors, Changes in Lot Release Requirements for Well-Characterized Therapeutic Recombinant DNA-derived and Monoclonal Antibody Products, 11/9/95, 2 pages
6032	Reinventing the Regulation of Drugs Made from Biotechnology; President Bill Clinton, Vice President Al Gore, 11/95, 12 pages
6033	Dr. Zoon Slides, Well-Characterized Biotechnology Products: Evolving to Meet the Needs of the 21st Century, 12/11/95, 6 pages
6034	Dear Colleague Letter, Regarding Reverse Transcriptase Activity in Viral Vaccines Produced in Chicken Cells, 1/4/96, 2 pages
6035	Dr. Zoon Slides, BioEast, January 16, 1996, 6 pages
6036	Dr. Zoon Slides, Northwest Biotech, January 18, 1996, 12 pages
6037	Error and Accident Reports: Summary for Fourth Quarter FY-95, 1/17/96, 18 pages
6038	Meeting Notice; Gene Therapy Conference: Development and Evaluation of Phase I Products; July 11, 1996 and Workshop on Vector Development; July 12, 1996, 4 pages
6039	Reinventing the Regulation of Cancer Drugs - Accelerating Approval and Expanding Access; President Bill Clinton and Vice President Al Gore, March 1996, 9 pages
6040	Error and Accident Reports - Annual Summary for FY95, April 17, 1996, 42 pages
6041	Letter to Manufacturers of FDA-Regulated Drug/Biological/Device Products, Bovine Spongiform Encephalopathy (BSE), May 9, 1996, 2 pages
7000	Individual Sponsor/Investigator IND Packet, 38 pages
7001	Commercial IND Packet, 73 pages
7002	Application for Establishment License for Manufacture of Biological Products, Form FDA 3210 (5/94), 18 pages
7003	Review Checklists from FDA/CBER Workshop for Licensing Blood Establishments, January 30 & 31, 1995, 27 pages
7004	Establishment & Product License Applications for Manufacturers of Blood and Blood Components [Forms 2599, 2599(a), 2600, 3098, 3098(a), 3098(b), 3098©, 3098(d), 3098(e), and General Instructions], 28 pages
7005	FDA/CBER Workshop for Licensing Blood Establishments, January 30 & 31, 1995, Slides describing request to use computer crossmatch, 9 pages
7006	Reviewer Guidance, Computer Software, 4/26/95, 4 pages
7007	Draft Reviewer Guidance, Changes in Personnel, 10/95, 1 page
7008	Draft Reviewer Guidance, Informed Consent for Plasmapheresis/Immunization, 10/95, 4 pages
7009	Draft Reviewer Guidance, Disease Associated Antibody Collection Program, 10/95, 2 pages
7010	Blood Registration Information and Form FD 2830 (08/95), 4 pages
7011	Application to Market a New Drug, Biologic or an Antibiotic Drug for Human Use, FDA3439, 5/14/96, 2 pages
7012	Draft Reviewer Guidance for a Premarket Notification Submission for Blood

	Establishment Computer Software, 4/12/96, 7 pages
8000	**Guide to Inspections of Blood Banks, Division of Field Investigations, Office of Regional Operations, Office of Regulatory Affairs, September, 1994, 18 pages**
8001	**Methods of the Allergenic Products Testing Laboratory, October, 1993, 120 pages**
8002	**Guide to Inspections of Source Plasma Establishments, Division of Field Investigations, Office of Regional Operations, Office of Regulatory Affairs, December, 1994, 17 pages**
8003	**Computer Assisted Product License Application (CAPLA) Guidance Manual, March 1996, 88 pages**
***8004**	**Guide to Inspections of Infectious Disease Marker Testing Facilities, 6/1/96, 11 pages**
9001	**Guidelines, Points-to-Consider, and other guidance documents for Human Biological Products available from the Division of Congressional and Public Affairs, 6/96, 16 pages**
9002	**Memorandum and Related Documents Pertaining to Human Blood & Blood Products, 7/5/96, 7 pages**
9003	**List of Guidelines available from the Center for Drug Evaluation and Research, 20 pages**
9999	**Documents Available from the CBER FAX Information System, 10 pages**

*Items added since 7/10/96

Who Do You Call to...

►REQUEST AN IND PACKET?

- Office of Communication, Training, and Manufacturers Assistance
  301-827-1800

►SET UP A PRE-IND MEETING?

- Joyce Frey Vasconcells,
  acting Chief, Cytokine and Gene Therapy Branch, CBER
  301-594-0830

►DISCUSS PRODUCT-SPECIFIC QUESTIONS?

- Cell and Gene Therapy Branch, CBER
  301-594-0830

►DISCUSS PRE-CLINICAL PHARMACOLOGY/TOXICOLOGY ISSUES?

- Pharm/Tox Branch
  Division of Clinical Trial Design and Analysis, CBER
  301-594-5599 or 301-594-5600
  fax. 301-594-0513

►DISCUSS CLINICAL TRIAL DESIGN ISSUES?

- Division of Clinical Trial Design and Analysis
  301-594-5600

►INQUIRE ABOUT CENTER JURISDICTION ISSUES?

- Joy Cavagnaro, acting Director, Quality Assurance, CBER
  301-827-0379 (tel. and fax.)
  e-mail: CAVAGNARO@A1.cber.fda.gov
- Eugene Berk, CDRH
  301-594-1190
  301-594-0379 (fax.)
- James C. Morrison, CDER
  301-594-5443
  301-594-5298 (fax.)

►DISCUSS PROPOSED CHANGES TO AN EXISTING IND?

- Specific IND reviewers, contact CSO assigned to the IND (see IND acknowledgment letter)

►ASK REALLY WEIRD QUESTIONS?

- John Bishop, Assistant to the Director, Division of Cellular and Gene Therapies, 301-827-0687