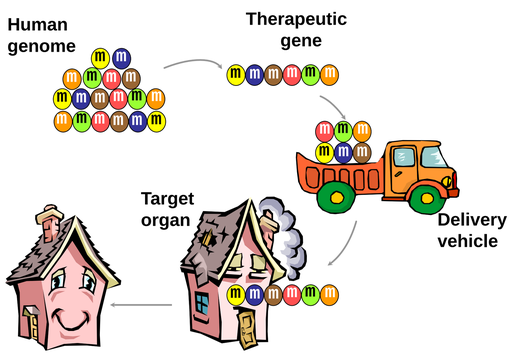
Weill Medical College of Cornell University
Belfer Gene Therapy Core Facility

Core Director: Ronald G. Crystal, MD; Chairman, Department of Genetic Medicine
Belfer Gene Therapy Core Facility, 515 East 71st Street, S-1000, Weill Medical College of Cornell University, NY, NY 10023

Mission

- To provide to the Weill Cornell, Cornell Ithaca and associated centers the infrastructure to carry out basic, translational and clinical research utilizing gene transfer for therapeutics and vaccines

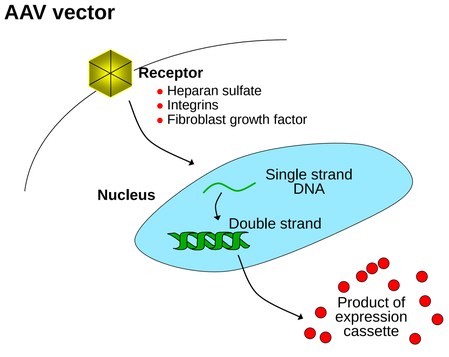
Gene Therapy



Vectors as Drug Delivery Vehicles

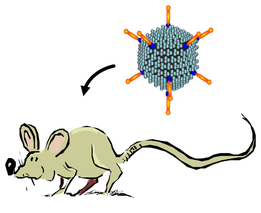
Vector	Gene expression	
	Level	Time
Plasmid ± liposome	Low	Transient
Retrovirus	Moderate	Persistent
Lentivirus	Moderate	Persistent
Adeno-associated virus	Moderate	Persistent
Adenovirus	High	Transient

Example of Gene Transfer Adeno-associated Virus Vectors

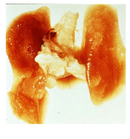


Gene Transfer with a Marker Gene

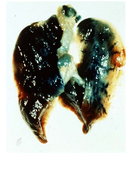
Adenovirus coding for "blue" gene (β-galactosidase)



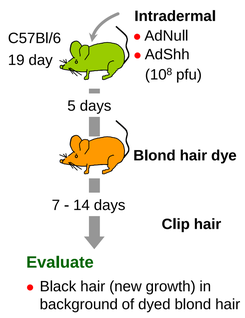
Uninfected



Ad.RSVβgal



Effect of Intradermal AdShh Administration on Hair Growth



- Black hair (new growth) in background of dyed blond hair

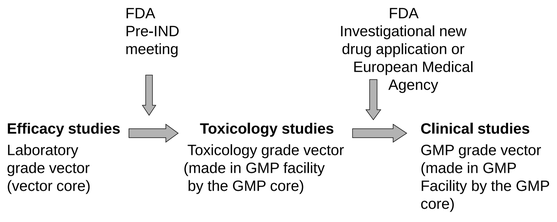
Description of the Facility

- **12,000 ft², 9th and 10th floors, S building**
- **Main components**
  - Vector Core –laboratory vectors
  - GMP Core – clinical vectors
- **Focus**
  - Production of GMP vectors
  - Enabling faculty, fellows, students, technicians in gene therapy technology
  - Providing common gene transfer biologic reagents
  - Molecular/cell analysis

Vector Core

- The vector core aims to provide the Cornell community with access to gene transfer vectors primarily for *in vivo* gene therapy experiments.
- It also serves as an educational facility and will train personnel in the relevant technology
- The core is committed to adopting innovative technology developments in the field and introducing new applications
- The vector core has a fully equipped and supported laboratory and cell culture spaces, a training program, protocols and the biological materials required for making adenoviral, adeno-associated virus, retrovirus, lentivirus, plasmid gene transfer and stable cell banks for therapeutics and vaccines

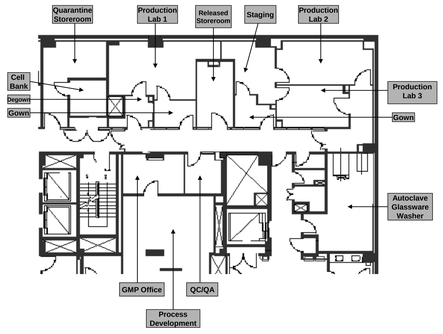
Gene Therapy Clinical Development



GMP Core

- Produces clinical grade adenovirus, adeno-associated virus and plasmids
- ~2,400 square foot GMP facility, plus associated office space and non-GMP laboratory space

Map of the GMP Suite



Environmental Monitoring is Performed to Ensure Product Quality

- Real-time 24/7 monitoring of critical facility parameters by wireless R&D Scientific Datatron system
- Data accessible via the internet and in QA/QC office in the facility
- System contacts GMP personnel by phone to notify of parameters out of specification
- All data is stored permanently, locally, and at R&D Scientific
- In addition to Datatron there is weekly assessment of viable and non viable particles

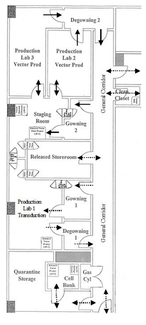
GMP Technicians in the Facility Being Observed by Supervisor

Production Requirements Under Good Manufacturing Practice

- Document Control (SOPs, Batch Records, etc)
- Tracking of Materials
- Flow of Materials/Personnel
- Stability Testing
- Test Parameters and Specifications
- QC Testing
- Environmental Monitoring
- Equipment Maintenance
- Personnel Training

An Example of Process Control Facility Flow of Personnel

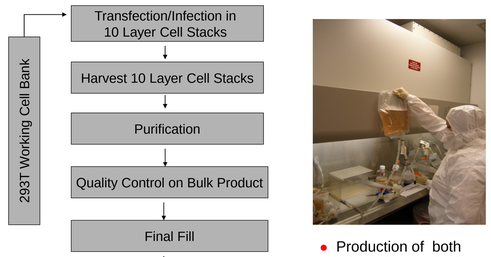
- **Personnel flow within the production facility**. The arrows on the map of the facility indicate the allowed direction of personnel flow. These rules are enforced by SOP and hardware. Doors within the facility have no hardware on the side of forbidden entry to prevent SOP violation.



Mandatory Record Keeping Production Data Records

- Released upon QA/QC Approval
  - Assures the use of the most current revision
- Records are modular
  - Can be easily varied based on project needs
- Quality Systems Documents include:
  - **Nonconformance Report:** Documents non-compliance with standard operating procedures and captures a risk analysis to product safety and quality
  - **Change Request Approval:** Revision of an existing or implementation of a new process is controlled by a change control mechanism. If the change is major, validation may be required.
  - **Corrective and Preventative Action (CAPA):** Serves to identify the root cause of systemic problems and implement a corrective or preventative action

Typical Vector Production Method



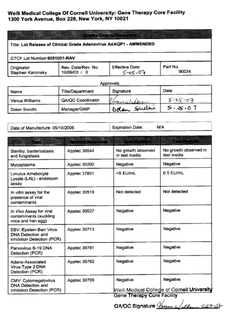
- Production of both adenovirus and adeno associated virus follows the same basic pattern

Vector Quality Control

Safety testing for gene therapy products are no different from other biopharmaceuticals. However, there are unique challenges and certain tests are necessary

- **Characterization of the Cell Lines Used**
  Usually a two-tier system of master and working cell banks which must be validated before use.
- **In-process Testing**
  Intermediate products (i.e. cell harvests) are tested for mycoplasma, bioburden, and adventitious viruses
- **Lot Release**
  Before release the final product is assessed for safety and characterized. Safety testing includes sterility, endotoxin, and residuals testing. Final product characterization includes tests for identity, purity, potency, titer, and infectivity

Example of Lot Release/Certificate of Analysis



Weill Medical College Of Cornell University: Gene Therapy Core Facility
1300 York Avenue, Box 228, New York, NY 10021

Title: Lot Release of Clinical Grade Adenovirus AAAGPI - AMENDED

QTCF Lot Number:MB50001-RAV

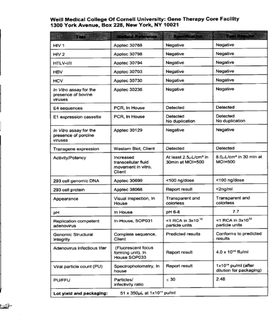
Originator	Rev. Date/Rev. No.	Effective Date	Part No
Stephen Kaminsky	10/06/03 / 0	5-10-03	90024

Approvals

Name	Title/Department	Signature	Date
Venus Williams	QA/QC Coordinator		5-12-03
Dolan Sondhi	Manager/GMP		5-10-03

Date of Manufacture: 05/10/2006 — Expiration Date: N/A

Test	Method, Reference	Specification	Test Results
Sterility, bacteriostasis and fungistasis	Applied: 30044	No growth observed in two media	No growth observed in two media
Mycoplasma	Applied: 30300	Negative	Negative
Limulus Amebocyte Lysate (LAL) - endotoxin assay	Applied: 37851	<5 EU/mL	0.5 EU/mL
In vitro assay for the presence of viral contaminants	Applied: 30519	Not detected	Not detected
In Vivo Assay for viral contaminants (using mice and hen eggs)	Applied: 30027	Negative	Negative
EBV: Epstein-Barr Virus DNA Detection and Inhibition Detection (PCR)	Applied: 30713	Negative	Negative
Parvovirus B-19 DNA Detection (PCR)	Applied: 30791	Negative	Negative
Adeno Associated Virus (AAV) DNA Detection (PCR)	Applied: 30792	Negative	Negative
CMV: Cytomegalovirus DNA Detection and Inhibition Detection (PCR)	Applied: 30705	Negative	Negative
HIV 1	Applied: 30768	Negative	Negative
HIV 2	Applied: 30768	Negative	Negative
HTLV-I/II	Applied: 30794	Negative	Negative
HBV	Applied: 30703	Negative	Negative
HCV	Applied: 30730	Negative	Negative
In Vitro assay for the presence of bovine viruses	Applied: 30236	Negative	Negative
E4 sequences	PCR, In House	Detected	Detected
E1 expression cassette	PCR, In House	Detected No duplication	Detected No duplication
In Vitro assay for the presence of porcine viruses	Applied: 30129	Negative	Negative
Transgene expression	Western Blot, Client	Detected	Detected
Activity/Potency	Increase in extracellular fluid impedance in vitro, Client	At least 2 ΔCL/cm² in 30min at MOI=500	8 ΔCL/cm² in 30 min at MOI=500
293 cell genomic DNA	Applied: 30699	<100 ng/dose	<100 ng/dose
293 cell protein	Applied: 38566	Report result	<2ng/ml
Appearance	Visual inspection, In House	Transparent and colorless	Transparent and colorless
pH	In House	pH 6-8	7.7
Replication competent adenovirus	In House, SOP#201	>1 RCA in $3x10^{10}$ particle units	>1 RCA in $3x10^{10}$ particle units
Genomic Structural Integrity	Complete sequence, Genome	Confirms to predicted sequence	Confirms to predicted sequence
Adenovirus infectious titer	CPE(transient focus assay), In House SOP#203	Report result	4.0×10^{10} ifu/ml
Viral particle count (PU)	Spectrophotometry, In house	Report result	1×10^{12} pu/ml (after dilution for packaging)
PU/IFU	Particle/infectivity ratio	≤ 30	2.48
Lot yield and packaging:	51 x 350µL at 1×10^{12} pu/ml		



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Weill Medical College Of Cornell University
Gene Therapy Core Facility
QA/QC Signature

Summary of Ongoing and Complete GMP Manufacturing

User/Affiliation	Product/Transgene	Purpose	Total Amount -Status
Dolan Sondhi & Ronald Crystal- Weill Cornell	AAV2$_{CU}$hCLN2	Clinical	4.3×10^{13} pu -complete
Ronald Crystal- Weill Cornell	AAV5α1-antitrypsin	Toxicology	5.5×10^{13} gc -complete
Stefan Worgall- Weill Cornell	AdOprF.RGD.Epi8	Toxicology Clinical	1.0×10^{13} pu -complete 1.0×10^{13} pu- ongoing
Todd Rosengart, Evanston NorthWestern	AdCUVEGF.1	Clinical	1.0×10^{13} pu -complete
Robert Korst- Weill Cornell/ Valley Health	AdCD40L	Toxicology Clinical	1.8×10^{13} pu -complete 2.0×10^{13} pu -ongoing
GeneVec	(TNFerade) AdTNFα	Toxicology	4.7×10^{13} pu- complete
Bruce Baum NIDCR	AdGH (growth hormone)	Clinical	4.0×10^{13} pu- complete
Bruce Baum NIDCR	AdAQP1	Toxicology Clinical	9.3×10^{12} pu- complete 3.0×10^{12} pu- complete
Dolan Sondhi & Ronald Crystal- Weill Cornell	AAVh.10$_{hu}$hCLN2	Toxicology	1.0×10^{13} gc -complete
Kirk Hammond	AdAC6	Clinical	1.0×10^{13} pu -complete
Confidential - France	AAVh.10hARSA	Toxicology Clinical	4.0×10^{13} gc –ongoing 2.0×10^{13} gc –ongoing
Confidential-France	AAV1-MPS	Toxicology Clinical	4.0×10^{13} gc –ongoing 2.5×10^{13} gc –ongoing

Contact Information

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