Ottawa Health Research Institute
OHRI IRSO
Institut de recherche en santé d'Ottawa

Gene Therapy

New Approaches to the Treatment of Genetic Disease

Robin J. Parks

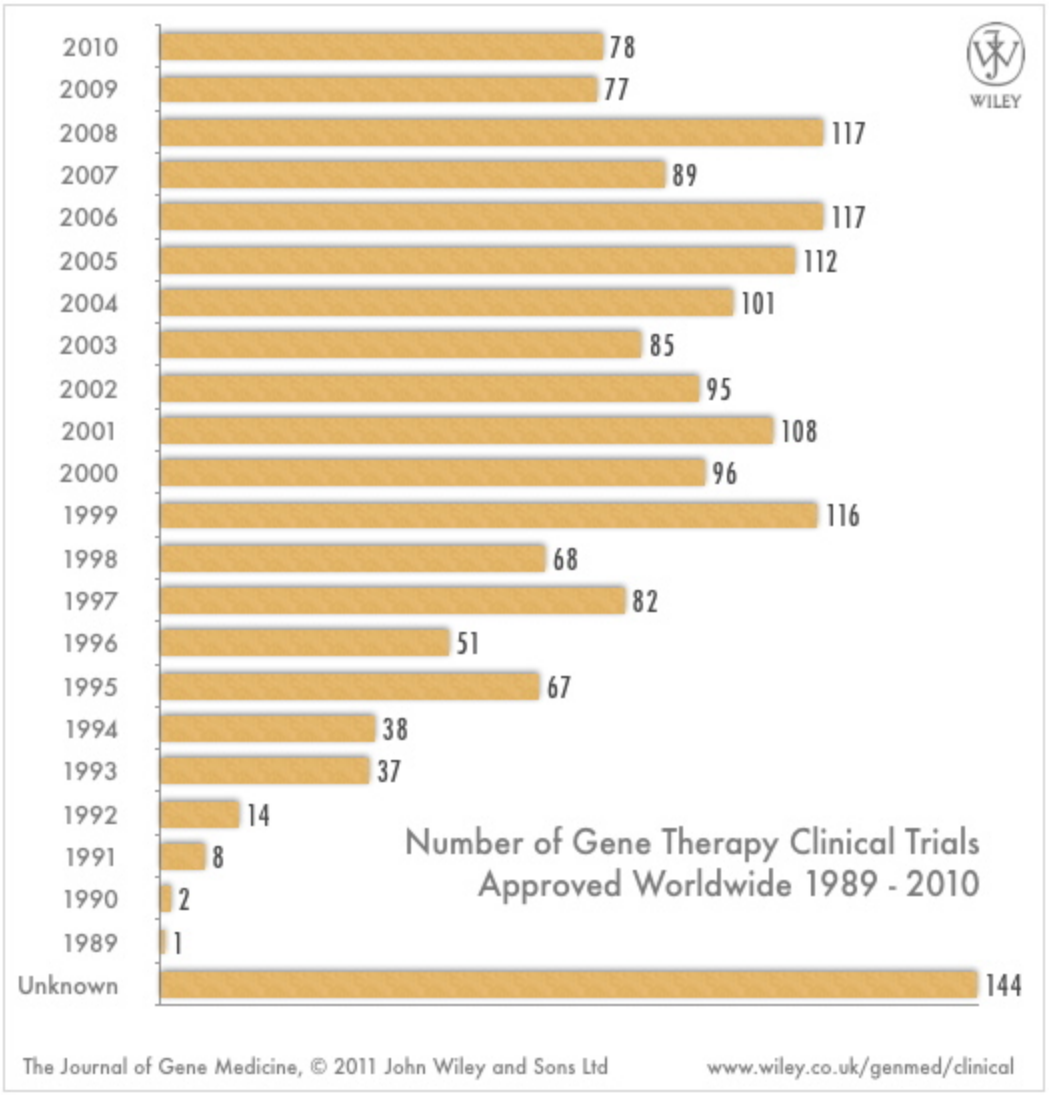
Points we will address

- Lecture 1:
  - What is gene therapy?
  - How do you deliver DNA to cells?
  - Discuss some of the “land-mark” gene therapy studies.
    - This will include gene therapy successes and failures.
  - “Evolution” of a gene therapy vector.
- Lecture 2: How can gene therapy be used to treat muscle disease?
  - before this lecture, please read “The ethics of human gene transfer” by Jonathan Kimmelman in Nature Reviews Genetics Volume 9 pages 239-244.

Gene Therapy

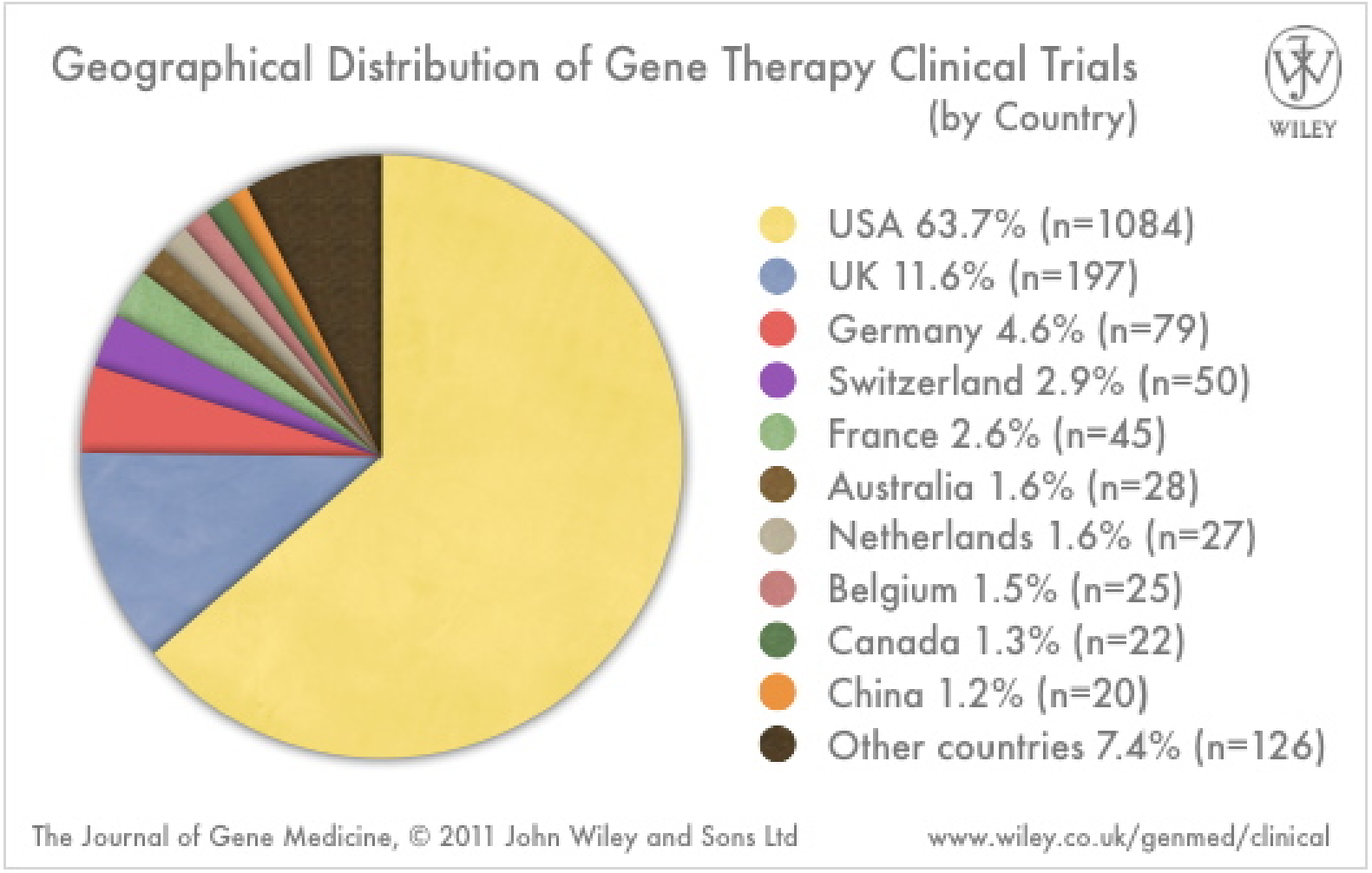
- Gene therapy involves delivering therapeutic DNA to a patient.
- Gene therapy may be used to:
  - alter or supplement the function of a mutated gene by providing a copy of the normal gene
  - directly alter and/or repair the mutated gene
  - provide a gene that adds missing functions or regulates the expression of another gene
- Success is dependent on two factors:
  - ability to get the gene into an appropriate cell
  - ability to express the transgene

Number of Gene Therapy Clinical Trials Approved – by Year



Gene Therapy By Country

(March 2011)



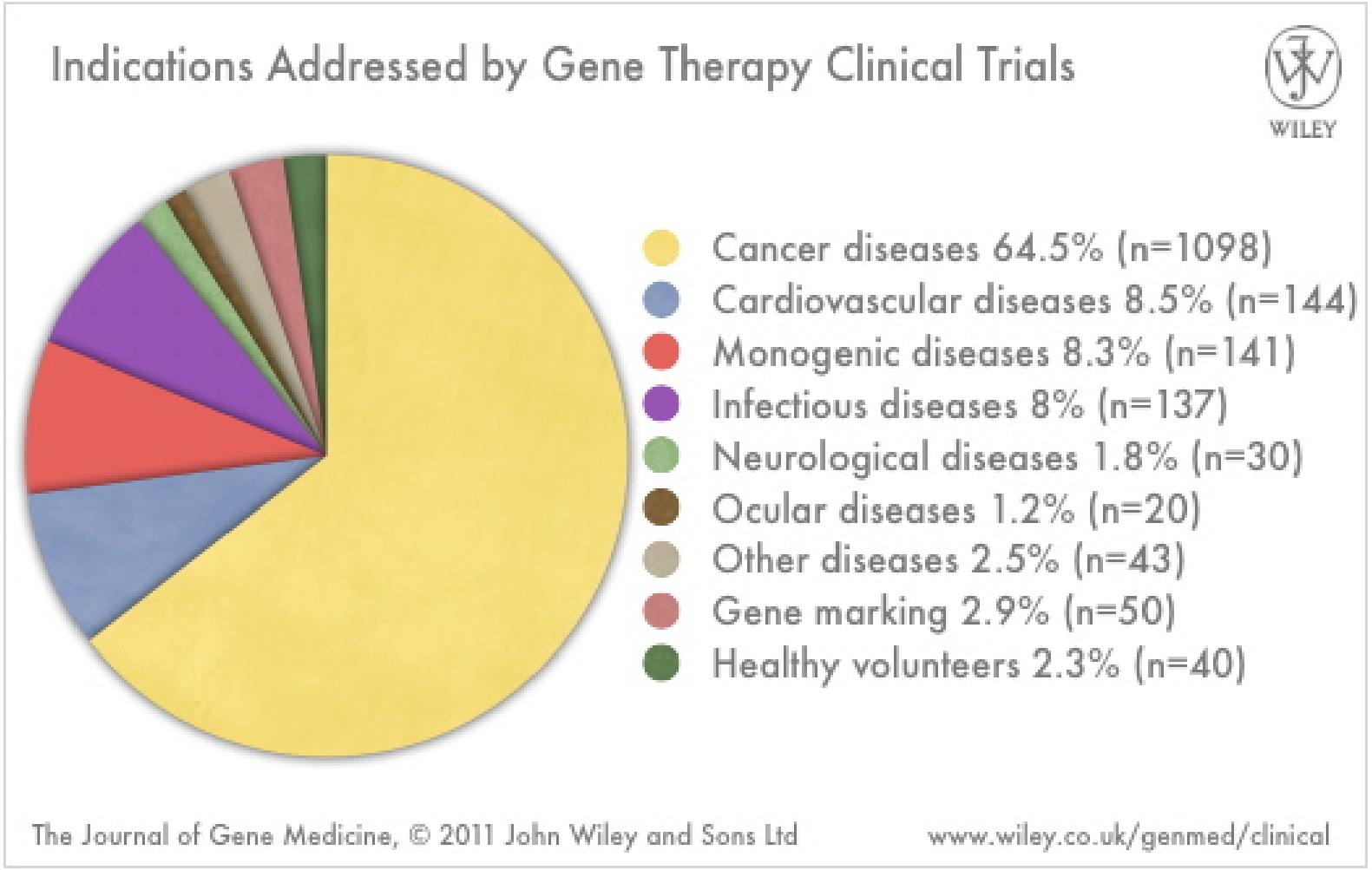
Total number of trials: 1703

Classes of Gene Therapy Target Diseases

- Genetic disease
  - e.g. Cystic fibrosis, Duchenne muscular dystrophy
  - require life-long expression of corrective gene
- Acquired disease
  - e.g. cancer, HIV
  - may only require expression of corrective gene for as long as the disease persists

Gene Therapy Clinical Trials

(March 2011)



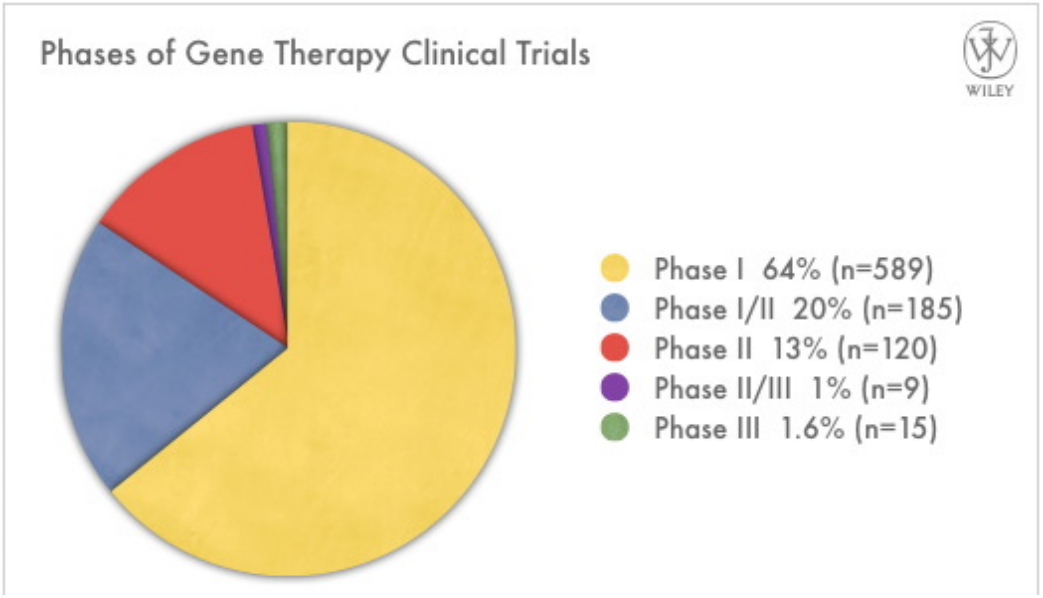
Total number of trials: 1703

Stages of Gene Therapy Studies

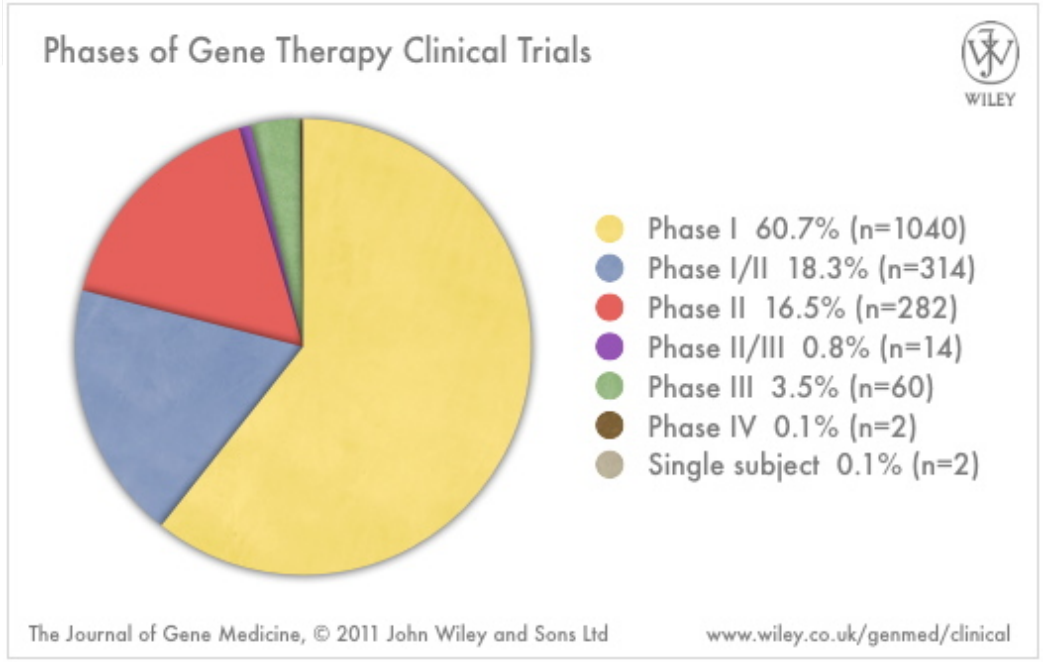
- Preclinical
  - demonstrate efficacy and safety in at least two animal models
  - refine experimental protocol (e.g. vector choice, single or multiple doses, route of administration, etc)
- Clinical
  - Phase I - assess safety and toxicity (few patients)
  - Phase II - assess safety and efficacy (larger number of patients)
  - Phase III - assess efficacy
  - Phase IV - "postmarketing surveillance"

  - At all stages, serious adverse events must be reported immediately

Phases of Clinical Trials



2004 Data



2011 Data

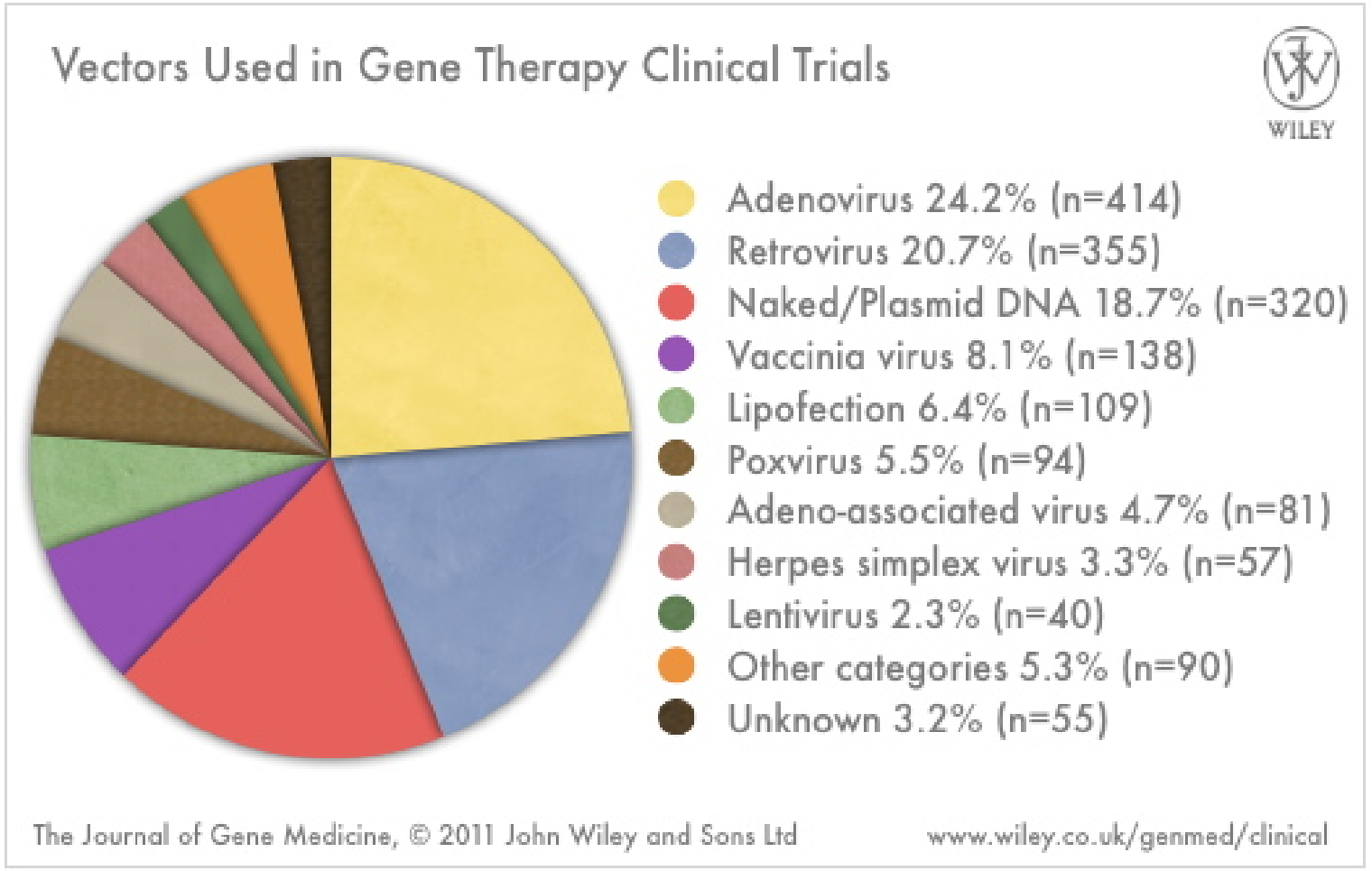
Factors to consider when designing a gene therapy strategy

- Therapeutic transgene expression?
  - Short- versus long-term
  - organ/tissue-specific or ubiquitous
  - regulated expression (how much is expressed or when during development)
- Many cells or only a few?
- *Ex vivo* versus *in vivo*?

These factors will affect the choice of gene delivery system (often referred to as a “vector”)

Gene Delivery Vectors used in Human Clinical Trials

(March 2011)



Choice of vector

- Non-integrating
  - e.g. plasmid DNA, adenovirus, poxvirus, adeno-associated virus (AAV)
- Integrating
  - e.g. retrovirus, lentivirus

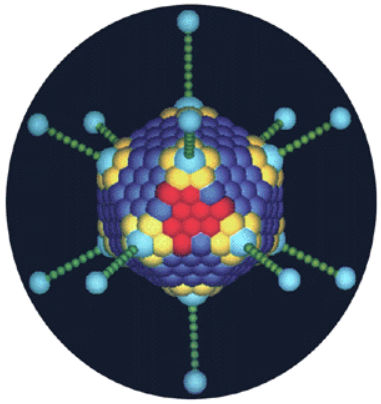
DNA

- Direct injection of plasmid DNA can lead to expression of transgene
- advantages: simple, relatively safe, ease of large-scale production, lack of specific immune responses
- disadvantages: relatively inefficient, typically transient expression, “patchy”
- good as DNA vaccines

Improvement to DNA delivery

- Physical
  - gene gun
  - electroporation
  - hydrodynamic injection
- Chemical
  - cationic lipids
  - polymer-based systems

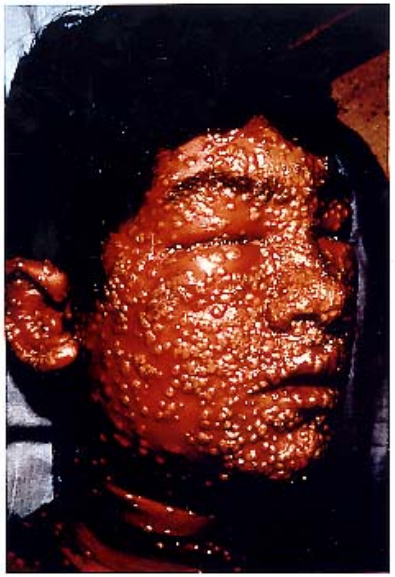
Adenovirus Vector



- Advantages
  - can be made replication defective
  - high transduction efficiency
    - both dividing and non-dividing cells
    - many different cell types and tissues
  - relatively large cloning capacity – up to 36 kb
  - can be grown to high titer
- Disadvantages
  - transient transgene expression (few days to weeks)
  - frequently leads to inflammatory and immune responses

- Recent advances in vector design
  - “fully-deleted” Ad vectors can provide very long-term therapeutic gene expression

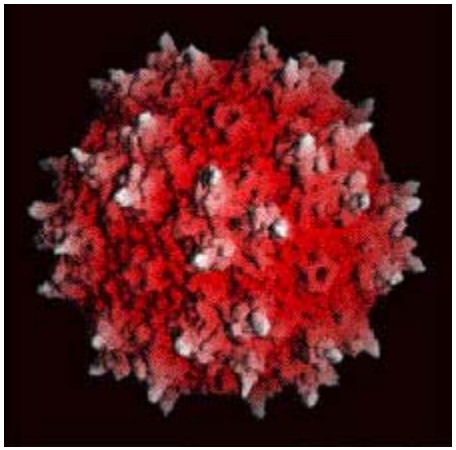
Poxvirus



- Large linear, dsDNA genome (~175 to 300 kb)
- Human poxvirus – Smallpox
  - 20 and 60% mortality rate, over 80% in children
  - During the 20th century, smallpox caused 300–500 million deaths
  - Eradicated in 1977
- The vaccine for smallpox is “vaccinia virus”
  - derived from a cowpox virus
- Current gene therapy vectors are based on vaccinia or other species (fowlpox or canarypox) and are used mostly as vaccines (anti-HIV or anti-cancer)
- More recently, vaccinia has been developed as an oncolytic virus
  - engineered to specifically replicate in and kill cancer cells but not normal cells.

Adeno-associated virus

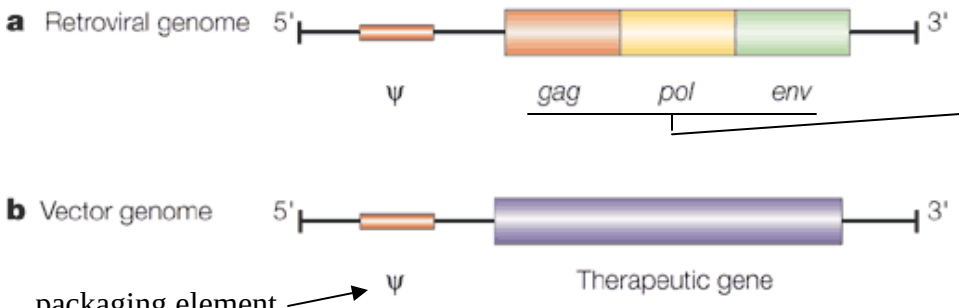
- Non-enveloped, 4.7 kb ssDNA genome
- Advantages
  - Does not elicit strong inflammatory or immune responses
- Disadvantages
  - Small cloning capacity (only ~5.0 kb)
  - Somewhat difficult to grow up large quantities of vector



Retrovirus

- e.g. Moloney murine leukemia virus
- produced in packaging cell lines
  - these cell lines produce all the viral proteins – you just need to introduce your therapeutic gene-of-interest in an appropriate plasmid
- can be “pseudo-typed” to alter cell tropism
  - change the virus attachment protein so that the virus can bind more efficiently to a variety of cells (e.g. VSV G-protein)

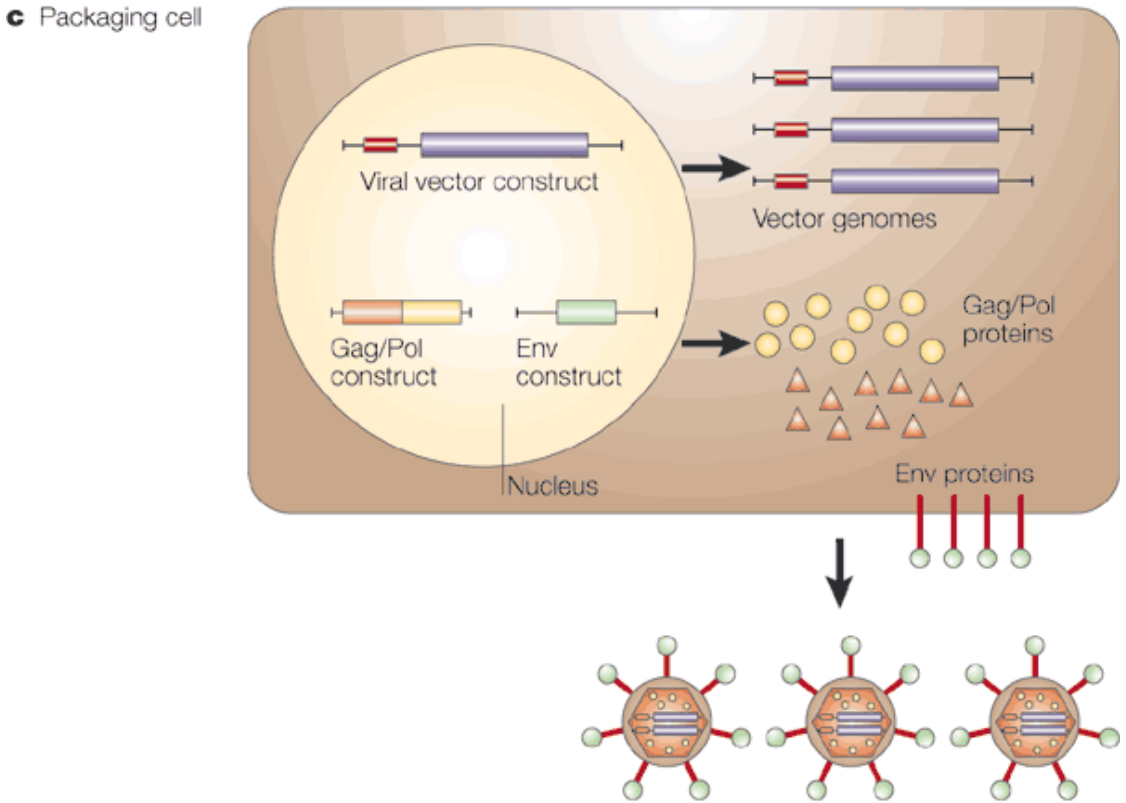
Retrovirus



proteins required for replication and packaging of the viral genome

5' and 3' inverted terminal repeats – involved in genome replication

packaging element
- gets the DNA into the virion



Retrovirus

- Advantages
  - 10 kb cloning capacity
  - integrate into genome of target cell
  - do not transfer virus protein coding sequences

- Disadvantages
  - only integrate in actively dividing cells
  - integration could lead to gene activation or inactivation
  - low titer
  - sometimes leads to production of replication competent retrovirus (RCR)

Lentivirus

- e.g. human immunodeficiency virus type I
- similar in many respects to MoMLV
- main advantage: can transduce non-dividing cells
- main disadvantage: it's HIV
- alternatives: SIV, FIV

Very promising preclinical results have been obtained using HIV-based vectors

Gene Therapy – The first real success?

The New England
Journal of Medicine

VOLUME 346 APRIL 18, 2002 NUMBER 16

SUSTAINED CORRECTION OF X-LINKED SEVERE COMBINED IMMUNODEFICIENCY BY EX VIVO GENE THERAPY

SALIMA HACEIN-BEY-ABINA, PH.D., FRANÇOISE LE DEIST, M.D., PH.D., FRÉDÉRIQUE CARLIER, B.S., CÉCILE BOUNEAUD, PH.D., CHRISTOPHE HUE, B.S., JEAN-PIERRE DE VILLARTAY, PH.D., ADRIAN J. THRASHER, M.D., PH.D., NICOLAS WULFFRAAT, M.D., RICARDO SORENSEN, M.D., SOPHIE DUPUIS-GIROD, M.D., ALAIN FISCHER, M.D., PH.D., AND MARINA CAVAZZANA-CALVO, M.D., PH.D.

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ABSTRACT

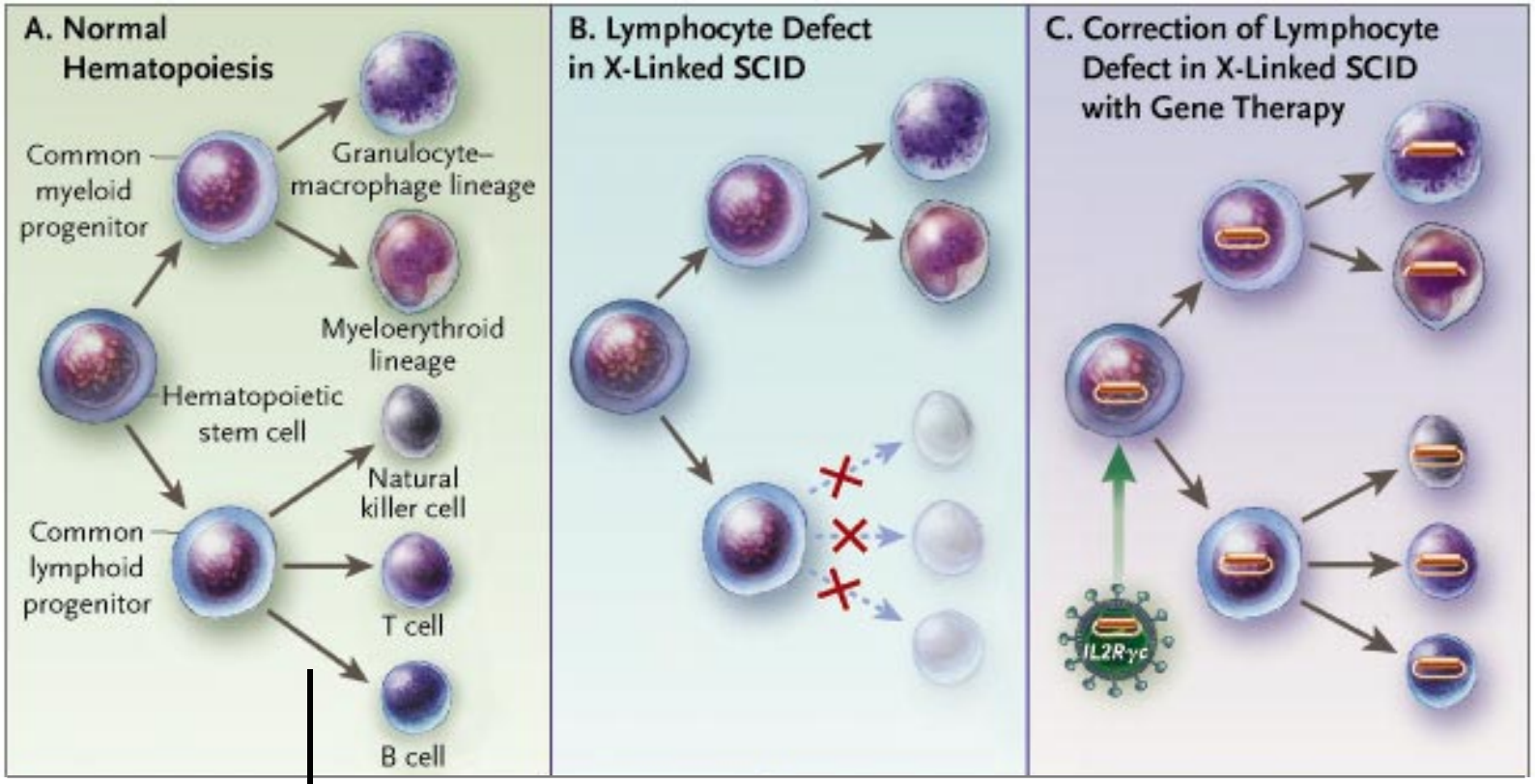
Background X-linked severe combined immunodeficiency due to a mutation in the gene encoding the common γ (γc) chain is a lethal condition that can be cured by allogeneic stem-cell transplantation. We investigated whether infusion of autologous hematopoietic stem cells that had been transduced in vitro with the γc gene can restore the immune system in patients with severe combined immunodeficiency.

Methods CD34+ bone marrow cells from five boys with X-linked severe combined immunodeficiency were transduced ex vivo with the use of a defective retroviral vector. Integration and expression of the γc transgene and development of lymphocyte subgroups and their functions were sequentially analyzed over a period of up to 2.5 years after gene transfer.

Results No adverse effects resulted from the procedure. Transduced T cells and natural killer cells appeared in the blood of four of the five patients within four months. The numbers and phenotypes of T cells, the repertoire of T-cell receptors, and the in vitro proliferative responses of T cells to several antigens after immunization were nearly normal up to two years after treatment. Thymopoiesis was documented by the presence of naive T cells and T-cell antigen-receptor episomes and the development of a normal-sized thymus gland. The frequency of transduced B cells was low, but serum immunoglobulin levels and antibody production after immunization were sufficient to avoid the need for intravenous immunoglobulin. Correction of the immunodeficiency eradicated established infections and allowed patients to have a normal life.

Conclusions Ex vivo gene therapy with γc can safely correct the immune deficiency of patients with X-linked severe combined immunodeficiency. (N Engl J Med 2002;346:1185-93.)

SCID Patients Lack Key Immune Cells



These cells are essential for a healthy immune system

The New England
Journal of Medicine

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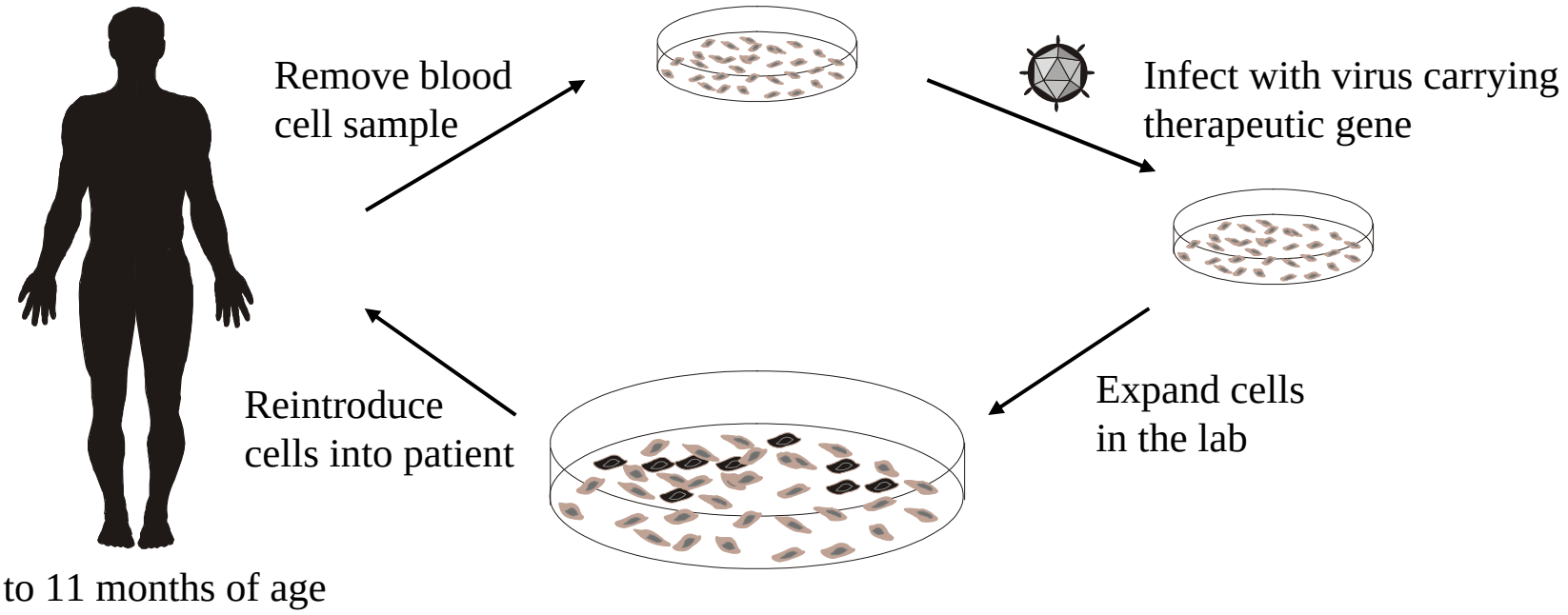

TABLE 1. CHARACTERISTICS OF THE PATIENTS.

PATIENT NO.	AGE AT TREATMENT	CLINICAL STATUS BEFORE TREATMENT	ENGRAFTMENT OF MATERNAL T CELLS	MUTATION	γc EXPRESSION BEFORE TREATMENT	INFUSED CELLS		CLINICAL STATUS AFTER TREATMENT	FOLLOW-UP
						CD34+	CD34γc+		
	mo		cells/mm³			cells/kg			yr
1	11	*Pneumocystis carinii* pneumonitis Protracted diarrhea Failure to thrive	0	Arg 289→stop	Yes	15 million	7 million–14 million	Well Normal growth	2.5
2	8	*Pneumocystis carinii* pneumonitis Protracted diarrhea Graft-versus-host disease–like lesions Failure to thrive	<10	Deletion of exon 6	No	16 million	5 million	Well Normal growth	2.3
3	10	Disseminated bacille Calmette–Guérin infection Adenovirus and respiratory syncytial virus infections in the lungs Protracted diarrhea Failure to thrive	0	Deletion of exon 4	No	14 million	5 million	Improving*	0.7
4	1	Well Free of infection	0	Tyr 219→stop	No	27 million	14 million	Well Normal growth	1.8
5	3	Graft-versus-host disease–like lesions	2000	Gln 285→Ala	No	38 million	20 million	Well Normal growth	1.6

T-lymphocyte counts after treatment

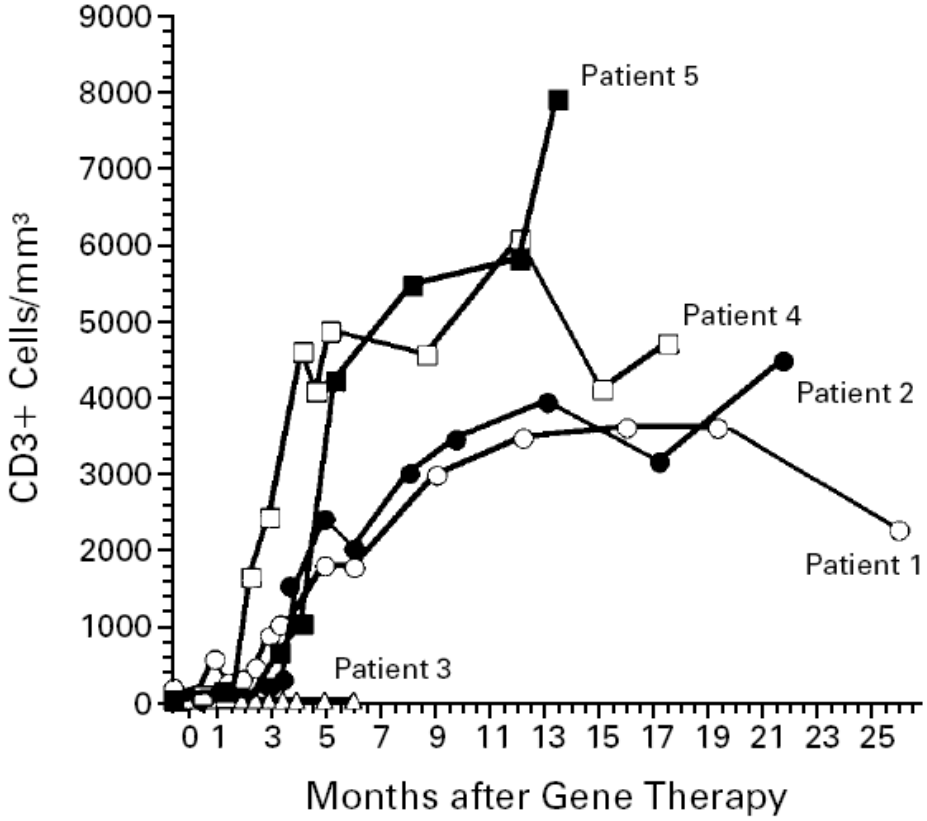

Figure 1. Absolute Numbers of CD3+ Cells after Gene Transfer in Patients 1 through 5.

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SUSTAINED CORRECTION OF X-LINKED SEVERE COMBINED IMMUNODEFICIENCY BY EX VIVO GENE THERAPY

RESULT – 2.5 years after treatment

In 9 treated patients, correction of the immunodeficiency eradicated established infections and allowed patients to have a normal life

CONCLUSION

Gene therapy can safely correct the immune deficiency of patients with SCID

CLINICAL RESEARCH

Gene Therapy a Suspect In Leukemia-like Disease

A French gene-therapy team that was hailed in 2000 for its breakthrough in curing children of a lethal immune deficiency reported a serious adverse event this week. One of 10 children they treated has developed a blood disorder resembling leukemia. Concerned

Science - October 4, 2002

NEWS

GENE THERAPY

Second Child in French Trial Is Found to Have Leukemia

Science - January 17, 2003

Gene Therapy Experiments Put on Hold

Federal authorities have temporarily suspended three gene therapy experiments — two of them in Los Angeles — following news that a third child in a similar French study has developed leukemia and that one of the three has died.

Los Angeles - Times March 4, 2005

GENE THERAPY

Seeking the Cause of Induced Leukemias in X-SCID Trial

Details of a second case of cancer in a gene-therapy trial in France, revealed last week, raise the odds that both were therapy induced. In both cases, a retrovirus engineered to shuttle corrective genes into cells inserted itself in or near a cancer-causing gene, apparently triggering uncontrolled cell growth. The risks seem "surprisingly high," says pediatrician Alain Fischer, who with Marina Cavazzana-Calvo led the trial at the Necker Hospital for Sick Children in Paris.

The French team has restored the immune systems of nine of 11 boys with X-linked severe combined immunodeficiency disease (X-SCID), making this the first clear success in gene therapy. But the appearance of two cancers, one in September and a second in December, is a major setback for the field (*Science*, 17 January, p. 320).

Last week, the U.S. Food and Drug Ad-

which in turn boosts the production of T cells. In a cell in which the vector has landed near and also activated the *LMO2* gene, *LMO2* may be cooperating with γc, giving cell growth an extra kick. If so, even if just one cell in 100,000 carried the insertion in or near the *LMO2* gene, it could multiply quickly enough to dominate the T cell population, notes von Kalle.

This gene insertion may seem "frightening" because it can lead to cancer, says Theodore Friedmann, a gene-therapy researcher at the University of California, San Diego, and chair of the RAC group. But there is also reason for optimism: The technique is producing therapeutic results, and the adverse effects might be "specific to the X-SCID trials," von Kalle says. Other SCID trials that don't involve the same vector sequences might be safe, he and others suggest. Moreover, it may be

Science - January 24, 2003

A fourth child in this trial also developed leukemia.

March 2007

HUMAN GENE THERAPY 19:3–4 (January 2008)

DOI: 10.1089/hum.2007.1221

Commentary

Case of Leukaemia Associated with X-Linked Severe Combined Immunodeficiency Gene Therapy Trial in London*

Board of the European Society of Gene and Cell Therapy,[1]
Executive Committee of the Clinigene Network of Excellence,[2]
and Executive of the Consert Integrated Project[3]

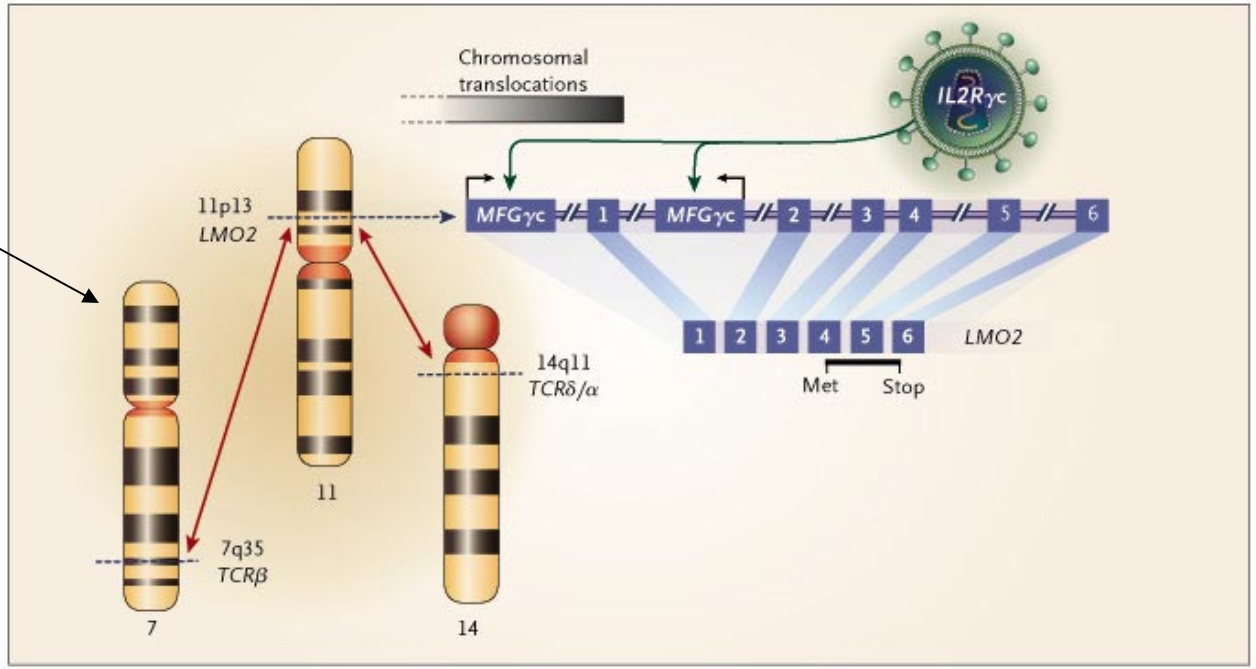
- "There was speculation that protocol differences may have distinguished it from the French trial in terms of leukaemia risk. The current leukemia case proves this hypothesis to be qualitatively wrong, and indicates that SCID-X1gene therapy in its current form…carries a high risk of leukaemia induction."

LMO2 and Leukemia

Four patients with leukemia in the SCID trial had retroviral insertion near the LMO2 gene

- LMO2 is a bridging molecule in some transcription factor complexes
- it is not clear why LMO2 causes T-cell leukemia

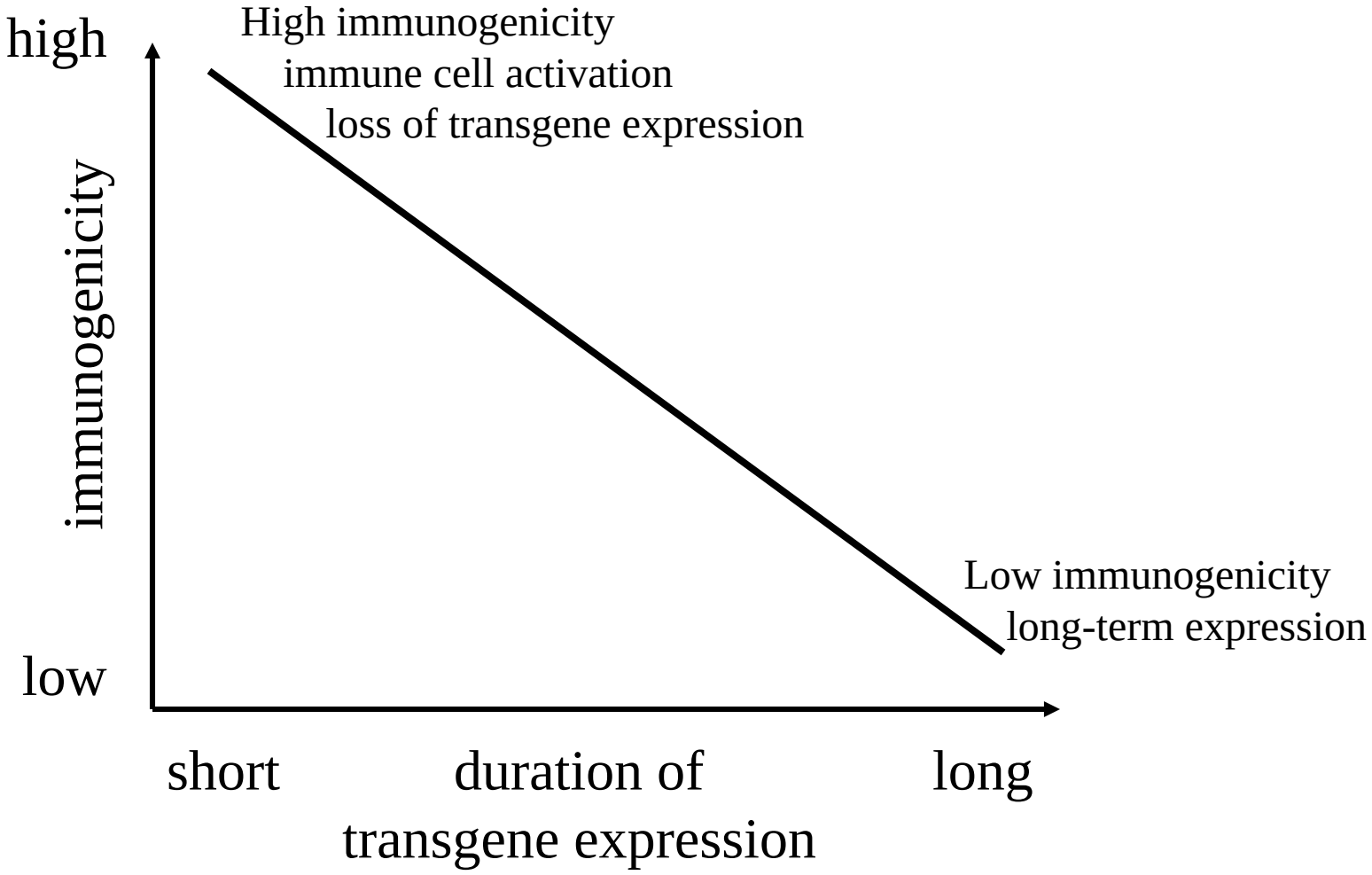
LMO2-induced leukemia can also occur “naturally” through chromosomal translocation.



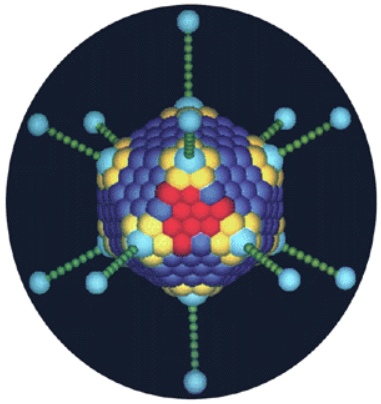
The risk of oncogenic insertional mutagenesis in humans as a consequence of gene therapy with the use of a retrovirus vector was deemed to be low.

-Subsequent studies have shown that retrovirus vectors preferentially integrate into promoter regions of genes

Vector Immunogenicity vs. Duration of Transgene Expression



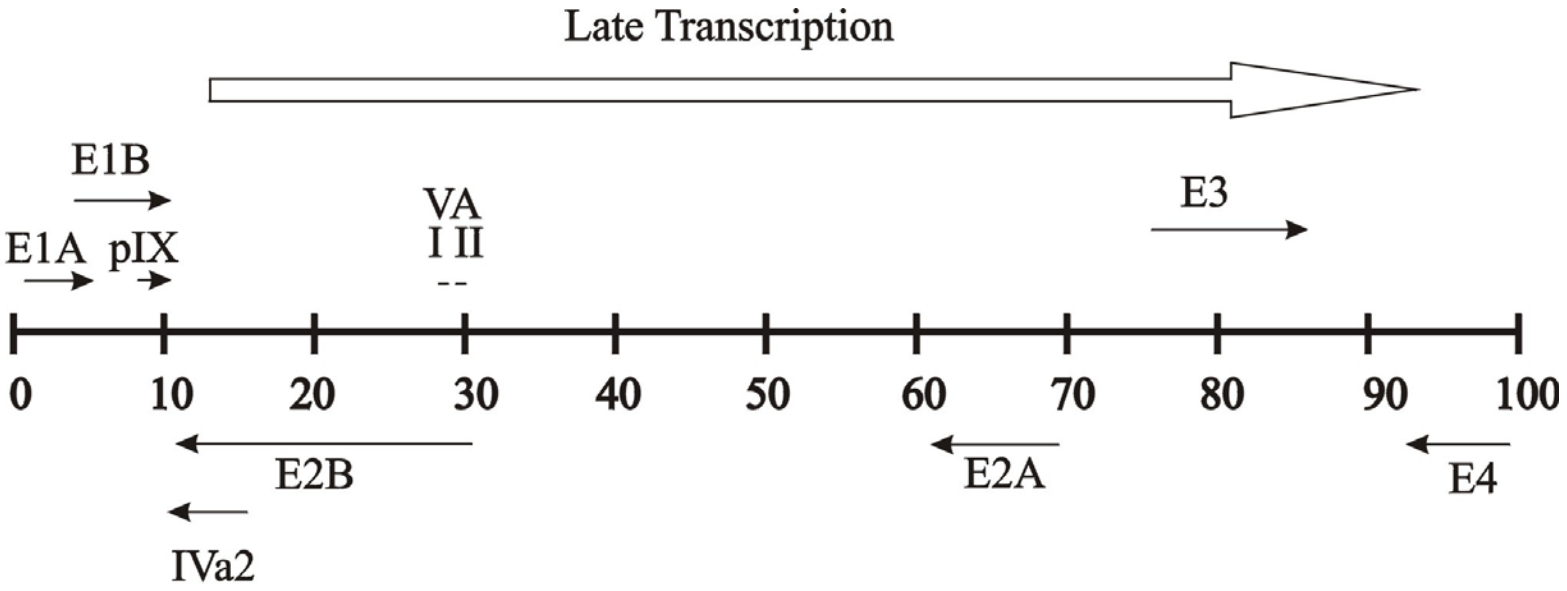
Adenovirus



- Icosohedral, non-enveloped virion of ~70 to 100 nm
- linear dsDNA of ~36 kb
- viral genes divided into early (E1-E4) and late (L1-L5) transcription regions, depending on whether they are expressed before or after DNA replication
  - early proteins are involved in turning-on other regions of the viral and cellular genome (E1 and E4), modulating the host immune response (E3), or DNA replication (E2).
  - late proteins are structural proteins for virion formation
- cause only mild, self-limiting illness in infected individuals
  - respiratory illness, keratoconjunctivitis, or gastroenteritis
  - not associated with neoplasmic disease

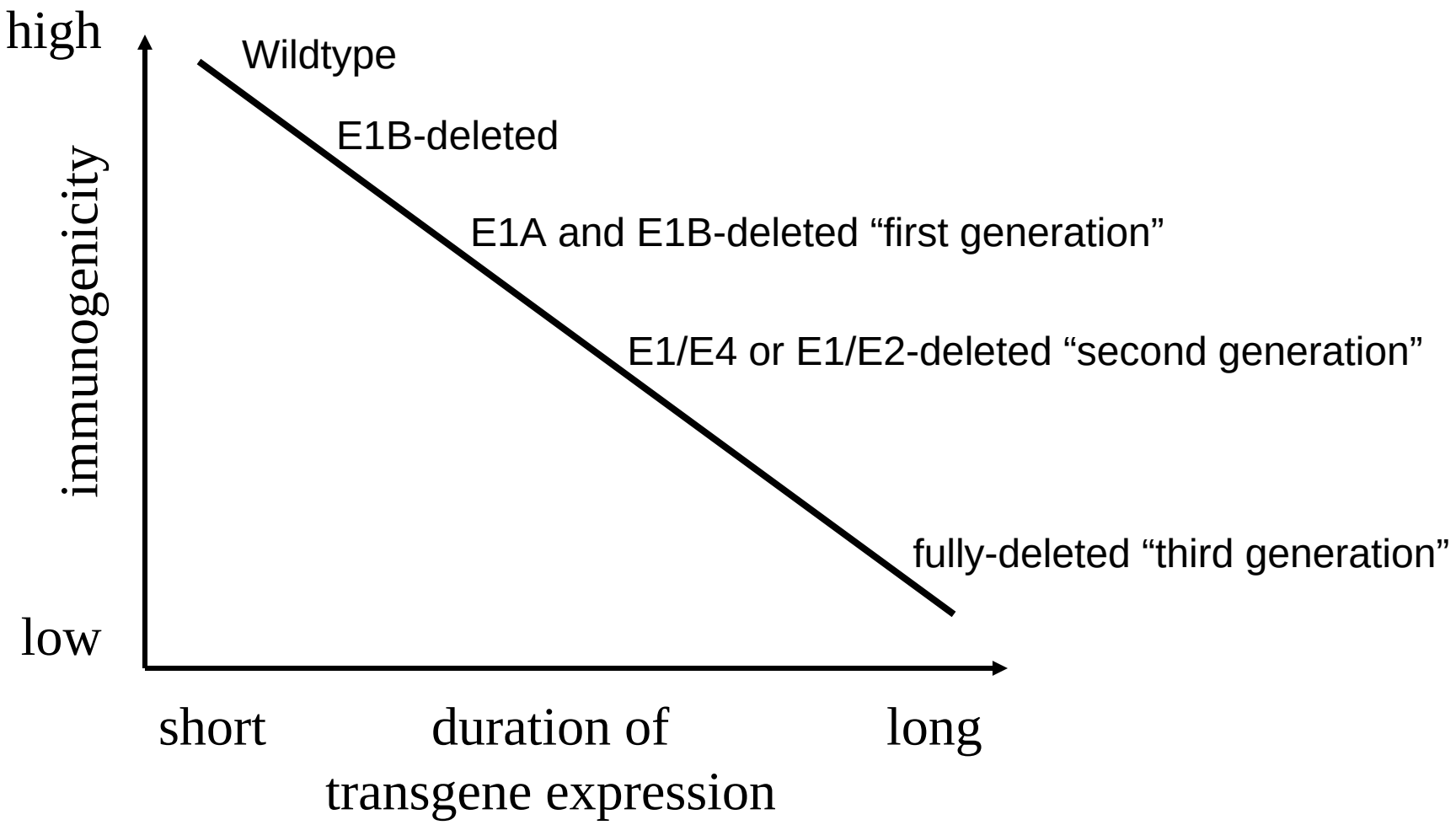
Human Adenovirus Type 5

Linear double-stranded genome of ~36 kb



E1, E2, E3 and E4 are “early” genes which are expressed before viral DNA replication during wildtype virus infection.

Vector Immunogenicity vs. Duration of Transgene Expression

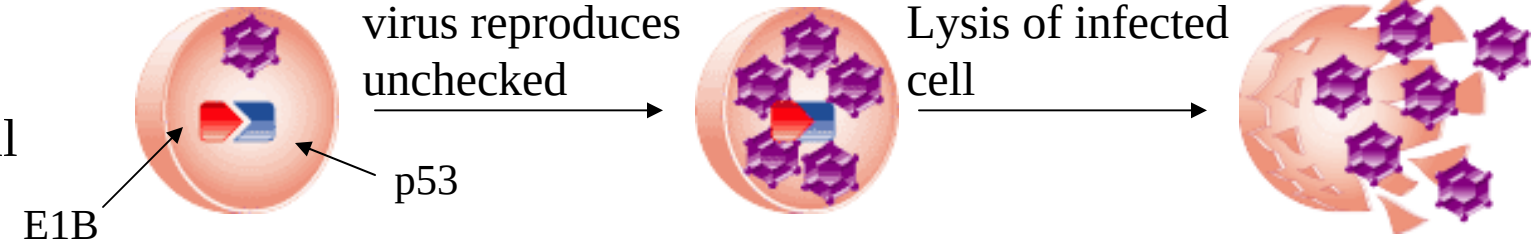


E1B-deleted Ad

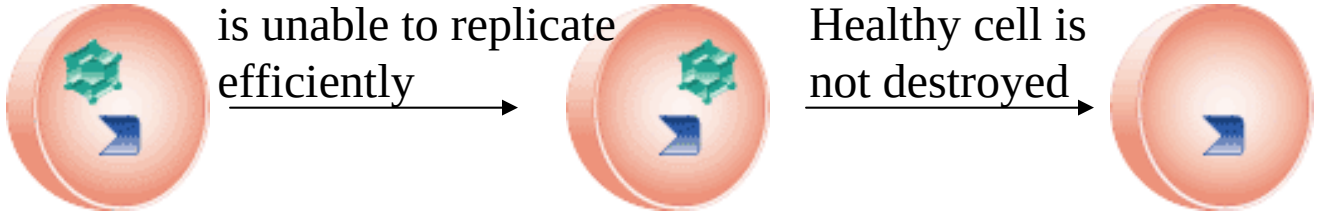
- Conditional replication
- E1B-deleted Ad will not replicate in p53+ cells
  - will replicate in, and kill, p53- cells
- p53 is mutated in about half of all cancers
  - these tumors are refractory to chemotherapy or radiation
- provides a mechanism to selectively kill tumor cells

Replication of E1B-deleted Ad

Wildtype Ad in normal cell



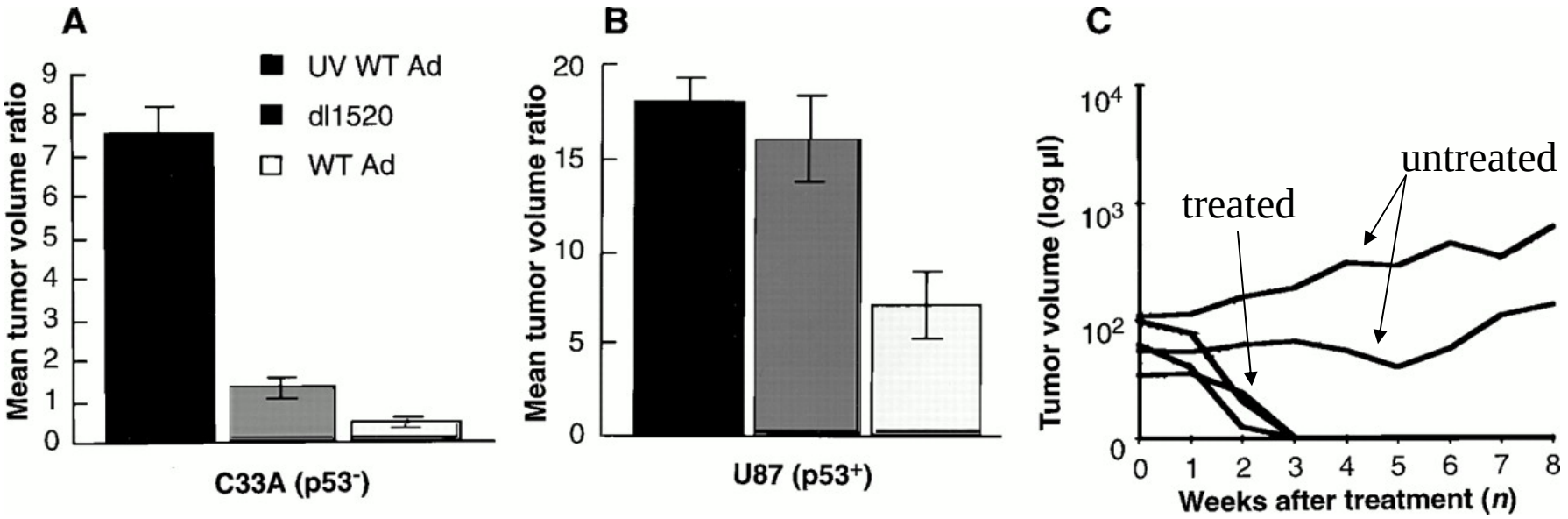
E1B-deleted Ad in normal cell (p53+)



E1B-deleted Ad in tumor cell (p53-)

Note: the actual mechanism of action for this virus is more complex

ONYX-O15 (dl1520) and Tumor Growth in Mice



Tumor cells (C33Ap53- or U87p53+) were injected subcutaneously into immunodeficient mice. After the tumors had reached ~150 microlitre volume, the tumors were injected with ultraviolet-inactivated wildtype Ad, wildtype Ad or E1B-deleted Ad (dl1520) - three doses of 10^8 pfu administered every other day. Tumor volume was monitored 5 weeks after injection (Panel A and B) or weekly (Panel C) after injection.

A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer

- 22 patients treated with E1B-deleted vector
  - 10^7-10^{11} plaque forming units (pfu) per patient
- evidence of viral replication in 4 of 22 patients, all 4 were mutant for p53
- using “conventional” response criteria
  - no response was observed
- using “non-conventional” response criteria
  - 3 patients showed partial response (necrosis within injected tumor)
  - 2 showed minor response
  - 8 patients had stable disease for 8 weeks
- no adverse events (minor flu-like symptoms in some patients)

Ganly et al. Clinical Cancer Research 6:798, 2000

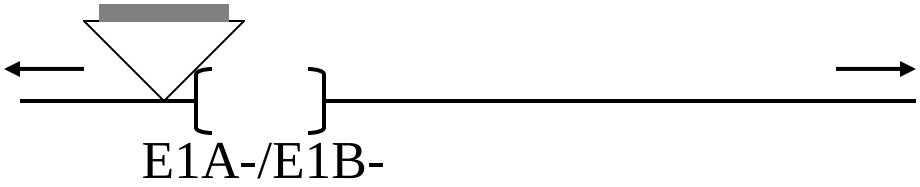
MOLECULAR THERAPY
The Journal of the American Society of Gene Therapy

The End of the Beginning: Oncolytic Virotherapy Achieves Clinical Proof-of-Concept

MOLECULAR THERAPY Vol. 13, No. 2, February 2006

In November 2005, Shanghai Sunway Biotech (Shanghai, China) announced the approval of H101 by Chinese government regulators, specifically for the treatment of nasopharyngeal carcinoma in combination with cisplatin-based chemotherapy. H101 is an oncolytic adenovirus with an E1B-55kD gene deletion, similar to that present in the Onyx-015 (dl1520) oncolytic adenovirus. The E1B-55kD deletion may result in tumor-selectivity by varied mechanisms, although results clearly vary depending on experimental methods.

E1-deleted Ad



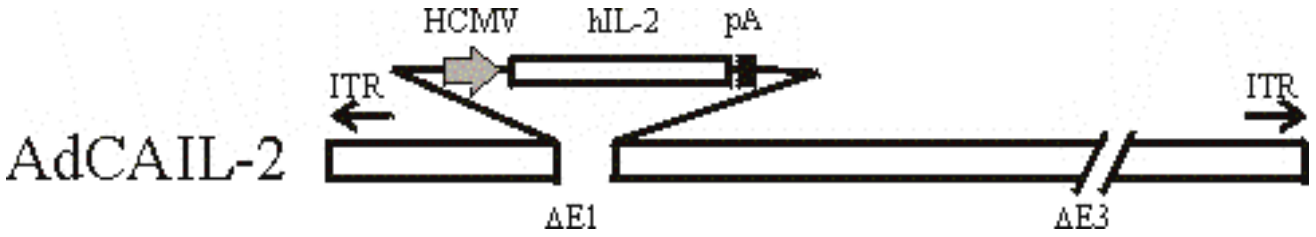
- Replication defective
- a.k.a. first-generation Ad vectors
- must be propagated in an E1-complementing cell line (e.g. 293 cells)
- cloning capacity of ~8kb
- even though the vectors are replication defective in normal cells, this does not affect their ability to get the viral DNA to the nucleus and express some viral (and therapeutic) proteins

E1-deleted Ad Vector Characteristics

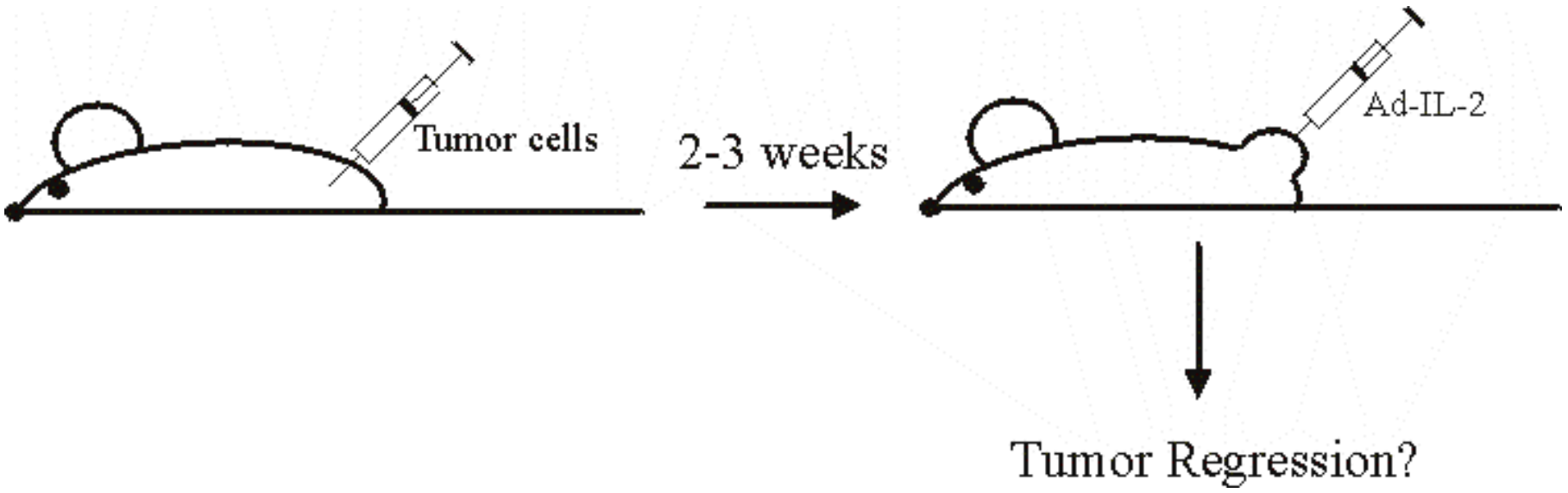
- Advantages
  - replication defective (E1 deleted)
  - high transduction efficiency
    - both dividing and non-dividing cells
    - many different cell types and tissues
  - relatively large cloning capacity (8 kb)
  - can be grown to high titer

Immunotherapy for cancer (Ad-IL-2)

- Interleukin-2 (IL-2)
  - cytokines
    - soluble, hormone-like proteins that act as a messenger between cells
    - stimulate or inhibit the growth and activity of various immune cells
    - essential for a coordinated immune response and can also be used as immunologic adjuvants
  - IL-2 stimulates activity of a variety of immune cells, including cytotoxic T-lymphocytes (T-killer cells)

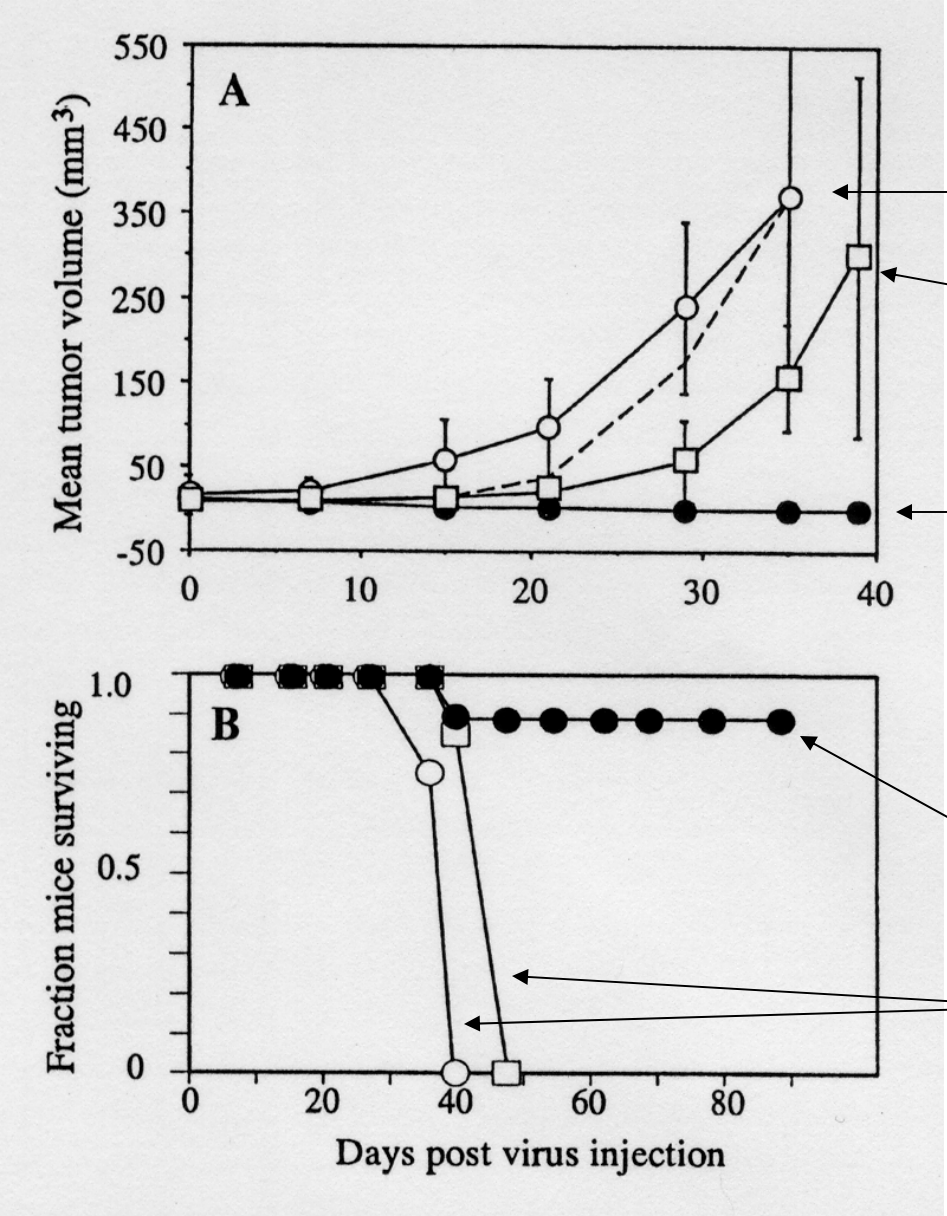


Tumor Model



Tumor model: tumor cells were injected into the hind flank of animals, 3 weeks later the tumor was injected with AdIL-2, control Ad or no vector.

Tumor Regression mediated by Ad-IL-2



Tumor Size

No virus

Control Ad

Ad-IL-2 treated

Mouse Survival

Ad-IL-2 treated

Controls

Addison et al. PNAS 92:8522, 1995

Gene Therapy (1999) 6, 350–363

http://www.stockton-press.co.uk/gt

Adenovector-mediated gene delivery of interleukin-2 in metastatic breast cancer and melanoma: results of a phase 1 clinical trial

AK Stewart[1], NJ Lassam[2], IC Quirt[1], DJ Bailey[1], LE Rotstein[1], M Krajden[1], S Dessureault[3], S Gallinger[3], D Cappe[1], Y Wan[4], CL Addison[4], RC Moen[5], J Gauldie[4] and FL Graham[4]

[1]The Toronto Hospital/Princess Margaret Hospital; [2]Sunnybrook Regional Cancer Center; [3]Mount Sinai Hospital, University of Toronto, Ontario; [4]McMaster University, Hamilton, Ontario, Canada; and [5]Baxter Health Care Gene Therapy Division, Round Lake, IL, USA

- 23 patients, 10^7-10^{10} virus particles
- 60% of patients showed local inflammation
- 24% incomplete tumour regression at site of injection
- biopsies
  - tumor necrosis
  - lymphocytic infiltrates (CD3 and CD8)
  - hIL-2 mRNA detected in 80% of samples at 7 days
  - no hIL-2 protein detected in tumours after 7 days
- elevated Ad5 antibodies in all patients

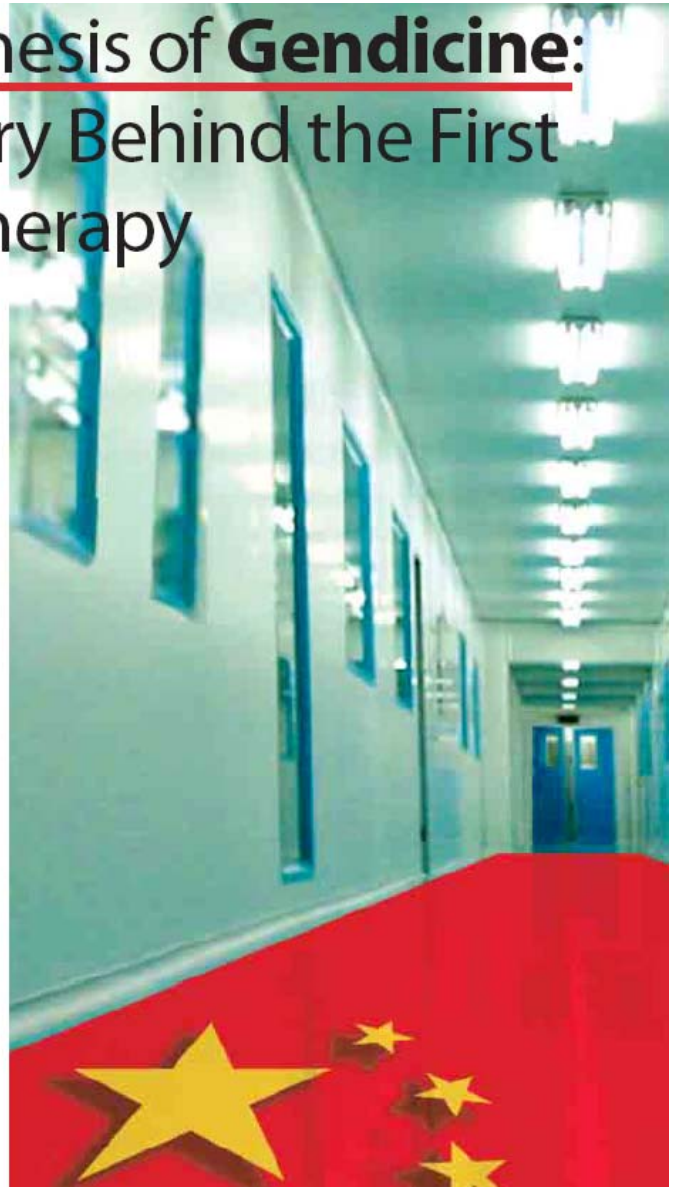
Table 1 Characteristics of patients

Study No.	*Diagnosis*	*Dose*	*No. inject*	*Day of biopsy*	*Toxicity*	*Lymphocyte infiltration*		*Response*
						Before	*After*	
1	Melanoma	10^7	1	7	Local inflammation	0	1	LR
2	Melanoma	10^7	1	7	Local inflammation	1	2	NR
3	Melanoma	10^7	1	7	Local inflammation swelling at injection site	0	0	NR
4	Breast	$10^{7.5}$	1	7	None	0	0	NR
5	Breast	$10^{7.5}$	1	7	Local inflammation	0	2	NR
6	Breast	$10^{7.5}$	2	7	None	0	2	NR
		10^{10}		7	None			NR
7	Melanoma	10^8	1	7	Pain (grade 3) at injection site cellulitis	0	1	NR
8	Melanoma	10^8	1	7	Local inflammation	0	3	LR
9	Breast	10^8	1	7	Local inflammation	0	2	NR
10	Melanoma	$10^{8.5}$	2	7	Local inflammation, cellulitis,	0	2	LR
		10^9		ND	fever (grade 2)			NR
11	Breast	$10^{8.5}$	1	7	Local inflammation, erythema tissue necrosis	1	3	NR
12	Melanoma	$10^{8.5}$	2	7	Local inflammation	0	2	NR
		10^9		ND				
13	Breast	10^9	1	7	Local inflammation, necrosis	2	4	LR
14	Melanoma	10^9	1	7	Injection site pain (grade 3) bone/joint pain, headache, fever	0	3	LR
15	Melanoma	10^9	1	7	None	0	1–2	NR
16	Melanoma	$10^{9.5}$	1	7	Fever (grade 2)	0	2	NR
17	Melanoma	$10^{9.5}$	1	7	Nausea (grade 1) pain (grade 1)	1	2	NR
18	Breast	$10^{9.5}$	1	7	Local inflammation pain grade 2, fever grade 2	0	0	NR
19	Melanoma	10^{10}	1	14	Local inflammation myalgia, lethargy, diaphoresis, fever, hiccups	0	1	NR
20	Melanoma	10^{10}	1	7	none	0	ND	NR
21	Breast	10^{10}	2	7	none	ND	3	LR
		10^{10}		7	none	ND	ND	NR
22	Melanoma	10^{10}	1	7	none	1	3	LR
23	Melanoma	10^{10}	5	2	Local inflammation	1	1	NR
		2×10^8						
		2×10^8						
		2×10^8						
		2×10^8						

The Genesis of **Gendicine**: The Story Behind the First Gene Therapy

In this *BioPharm International* exclusive interview, SiBiono's founder relates the science and manufacture of his company's innovative cancer therapy.

Dr. Zhaohui Peng is the chairman, chief executive officer, and founder of Shenzhen SiBiono GeneTech Co., Ltd., Langshen Road, Shenzhen Hi-Tech Industrial Park, Shenzhen, China, 0755.2696.8818, fax: 0755.2696.8808, sbn@sibiono.com; www.sibiono.com.



In October 2003, Shenzhen SiBono GenTech (Shenzhen, China) obtained a drug license in China for its Ad-p53 gene therapy vector for treatment of head and neck squamous cell carcinoma

Total patients treated to date: (2003-mid 2007): 8,700
Foreign patients treated to date: (2003-mid 2007): 1,800

“Record $3.8 M in Gendicine(R) Orders in One Day Sells Out All Available Product; Promising Reported Results Drive Unprecedented Demand”
(September 2007)

Gendicine

the most prominent observed side effect is self-limited fever; in nearly 80% of the cases, temperatures ranged from 37.5C to 39.5C occurring usually 2 to 4 hrs after administration and lasting for approximately 2 to 6 hrs. Other rare side effects include chills, pain at the injection site, discomfort, fatigue, nausea, and diarrhea.

How long do the treatments last?

Treatments may last from 1 to 3 months, depending on the patient’s condition and his/her specific response to treatment.

How much will treatment cost?

The amount of treatment costs will be based on diagnosis and treatments undergoing. Generally speaking, it will be around USD 25,000-35,000 / month.

Beijing GreatWall International Cancer Center
http://www.cancertherapychina.com

First generation Ad vectors

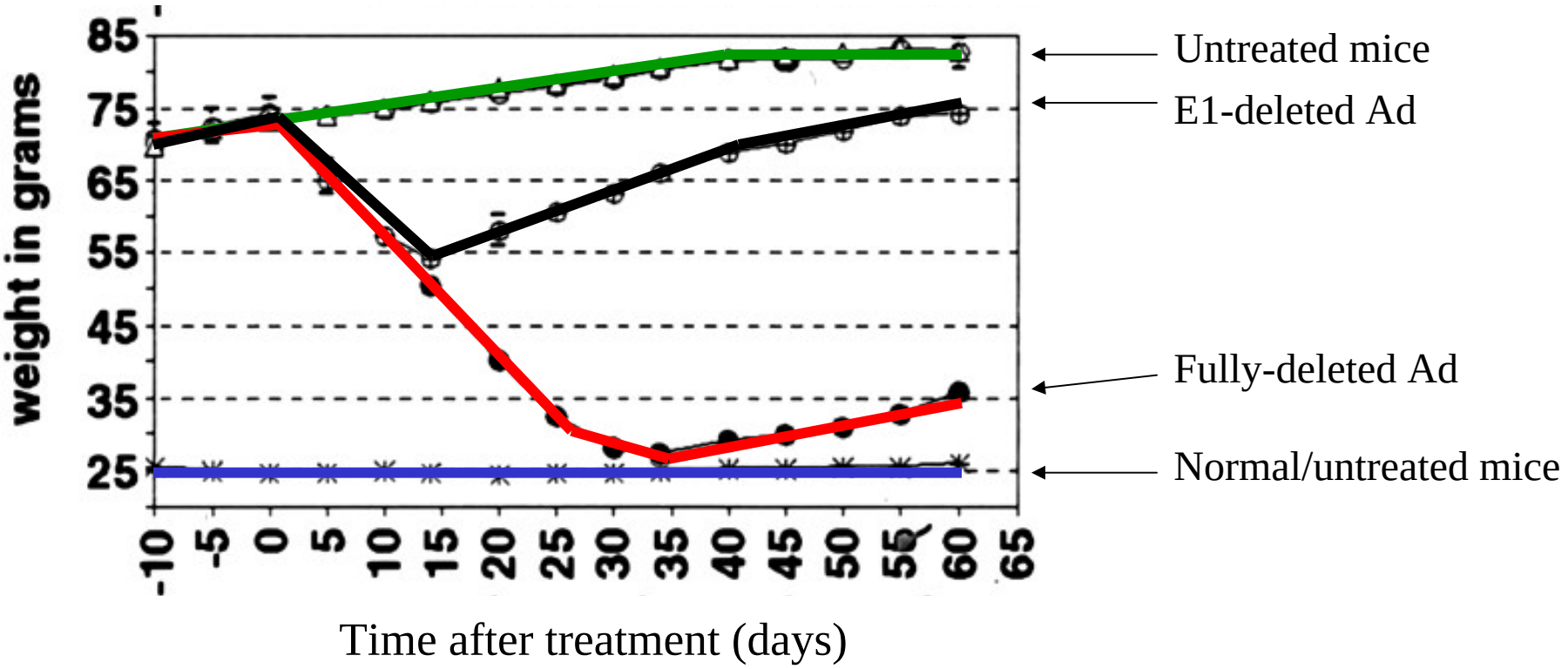
- Even though E1-deleted vectors cannot replicate, they still stimulate strong immune responses which result in elimination of the “infected cell”
  - good for cancer therapy
  - bad for treatment of genetic diseases (e.g. cystic fibrosis)
- Immune responses include
  - Humoral (antibody)
    - Prevents readministration of the vector
  - Cellular
    - Formation of cytotoxic T-lymphocytes which kill infected cells
    - Caused, at least in part, by expression of viral protein off the vector backbone
- Solution? Attenuate the virus more extensively

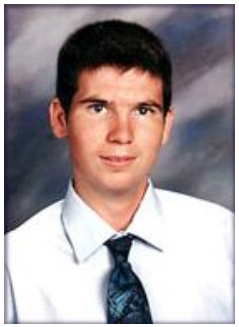
Ad vectors deleted of all protein coding sequences

- Advantages:
  - very large capacity for foreign DNA (~36 kb)
  - safe
  - no chance of viral gene expression
- Problem:
  - How do you grow a virus that lacks all viral genes?
    - Must co-replicate the fully-deleted Ad in the presence of a “helper” virus

Gene Therapy for Obesity

- Leptin – potent modulator of weight and food intake
- Mutation of the leptin gene results in morbid obesity
- Can gene therapy be used to correct leptin deficiency in mice?



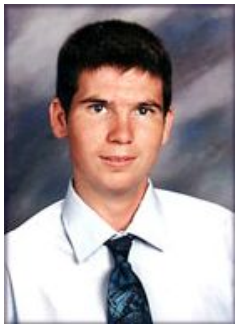


Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer

Recombinant Adenovirus Gene Transfer in Adults with Partial Ornithine Transcarbamylase Deficiency (OTCD)

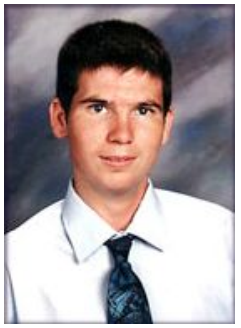
- OTC involved in urea metabolism
  - OTCD results in build up of ammonia in blood and brain
  - Severe cases are lethal (usually die within 2 weeks of birth)
  - Mild disease can be somewhat managed by drugs and protein-restricted diet
    - patients are at a continual risk of life-threatening encephalopathy, coma and brain damage
- Phase I trial
- “second-generation” Ad vector
- dose escalation study starting at $2x10^9$ particles/kg
- direct injection into portal vein

Jesse Gelsinger



- Received highest dose - 3.8 x 10^{13} particles of Ad-OTC ($6x10^{11}$ particles/kg)
- September 17, 1999
  - Within 12 hours of receiving the adenoviral vector, patient experienced fever, nausea, and back pain.
  - The following morning, patient experienced elevated ammonia levels and jaundice.
  - During days 2-4, patient experienced disseminated intravascular coagulation, adult respiratory distress syndrome, and kidney and liver failure.
  - Patient died four days after vector administration.

Jesse Gelsinger



- What went wrong?
  - The researchers and institute were cited for several procedural problems
    - Jesse should not have been treated
      - As a symptomatic male, he should have been the third patient treated in his cohort – he was treated second
      - His blood ammonia levels were above permitted levels
    - The researchers did not report two previous severe adverse events
    - The consent form did not properly outline the risks
      - Two non-human primates treated with a related vector died
- Other issues
  - Could Jesse provide informed consent?
  - Did researchers have a conflict of interest?

GENE THERAPY

As Gelsinger Case Ends, Gene Therapy Suffers Another Blow

Five years after 18-year-old Jesse Gelsinger died in a gene therapy experiment, the U.S. Department of Justice has reached a settlement with the researchers and with their institutions. The department announced last week that the University of Pennsylvania (U. Penn) will pay fines of $517,496, and Children's National Medical Center in Washington, D.C., will pay $514,622. The settlement also restricts the clinical research of the three investigators.

The Department of Justice alleged that toxic reactions in humans should have halted the trial earlier and that the lead investigators misrepresented clinical findings to the study's overseers, such as the National Institutes of Health (NIH) and the Food and Drug Administration (FDA). James Wilson of U. Penn, who had a financial interest in a company that stood to profit if the trial was successful, has agreed not to lead any FDA-regulated clinical trials for 5 years and be monitored for 3 years. Steven Raper of U. Penn and Mark Batshaw of Children's face less severe restrictions. Under the agreement, the scientists do not admit responsibility for Gelsinger's death. "Outrageous," responds Gelsinger family attorney Alan Milstein, who said the family had hoped for a formal apology and the release of the clinical trial documents.

While the Gelsinger case drew to a close, the field of gene therapy suffered another setback last month: A third child in a French trial for X-linked severe combined immunodeficiency (X-SCID) developed leukemia, French authorities reported on 24 January. Seventeen children have been successfully treated for SCID using gene therapy, making it the field's bright spot. But two patients in the French trial developed leukemia in late 2002 after a vector inserted near an oncogene; one child died last October. In response to the third leukemia case, the French trial has been halted again and FDA has suspended three U.S. SCID trials, but a trial in Britain continues.

The two previous leukemia cases in France occurred in infants treated at 3 months of age or less, which led to speculation that cells with the oncogene insertion proliferate more readily in very young children. But the third child was treated at 9 months, suggesting that older children may also be at risk, says Harry Malech of NIH, who heads one of the U.S. trials. Experts expect to discuss the case when FDA's gene therapy advisory committee and NIH's Recombinant DNA Advisory Committee meet in March.

–JENNIFER COUZIN AND JOCELYN KAISER

CREDIT: UNIVERSITY COLLEGE LONDON

Adverse Results Do Not Invalidate the Rationale of Gene Therapy

- “Apparent "failures" in early phaseI/II or even phase III studies do not necessarily indicate a therapeutic wild-goose chase. Because gene therapy is highly experimental and many patients are desperately ill, serious adverse events and even deaths will occur.

  It is vital to understand the reasons for unexpected results or clinical failures to allow the development of corrected procedures and improved experimental methods.

  For example, problems with polio vaccines due to persistence of live disease-causing poliovirus in incompletely inactivated preparations and the presence of SV40 in the vaccine were identified early, corrected, and used to develop improved programs.”

- Theodore Freidmann, Science March 24, 2000