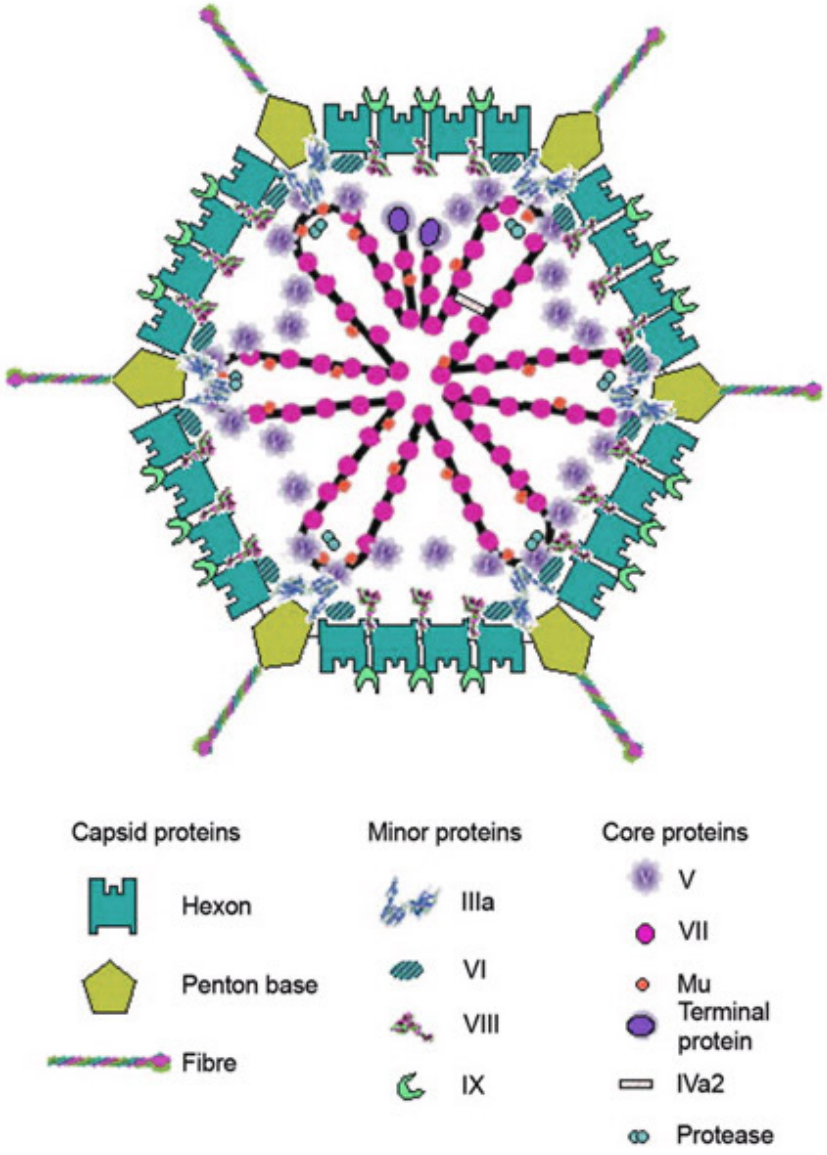
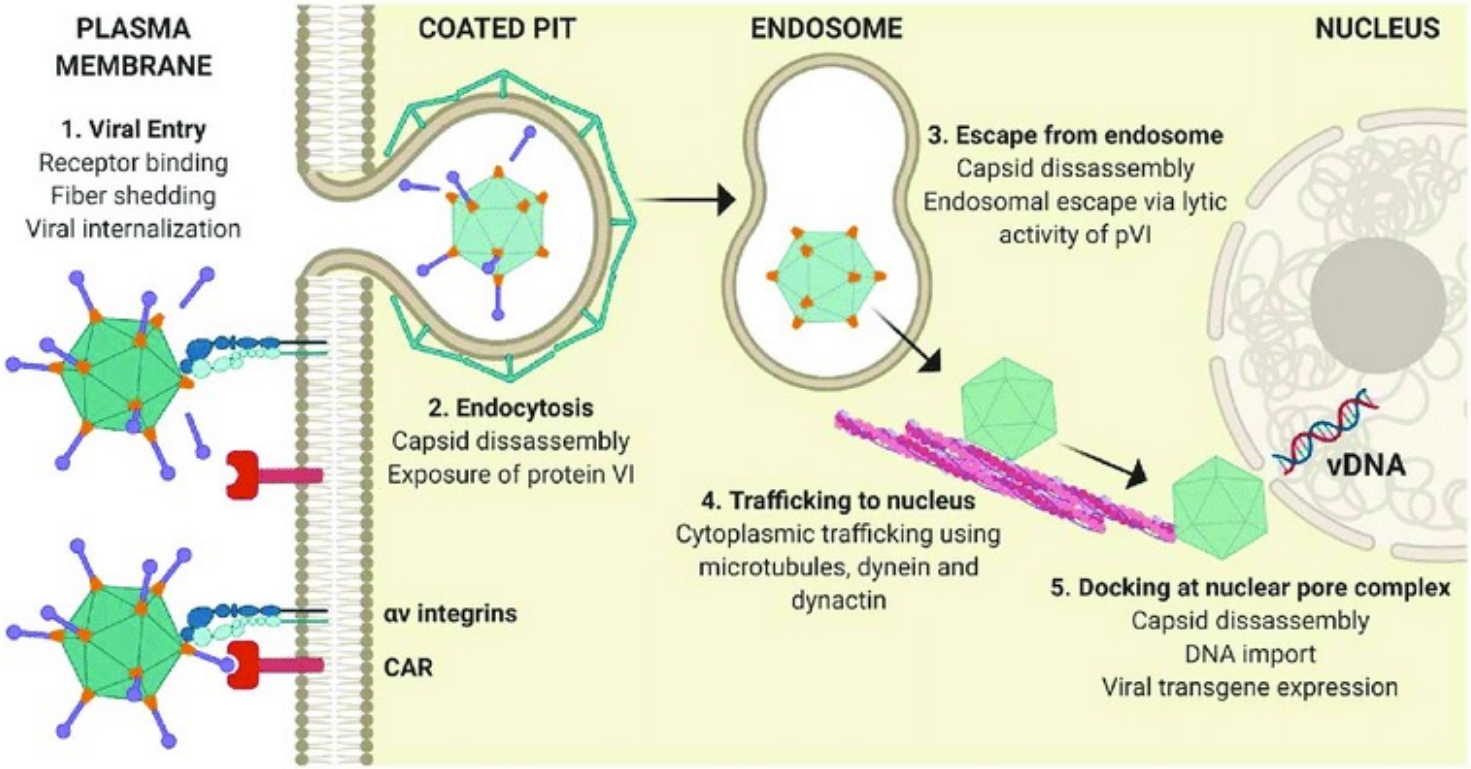
Cherish your doubts, for doubt
is the attendant of the truth

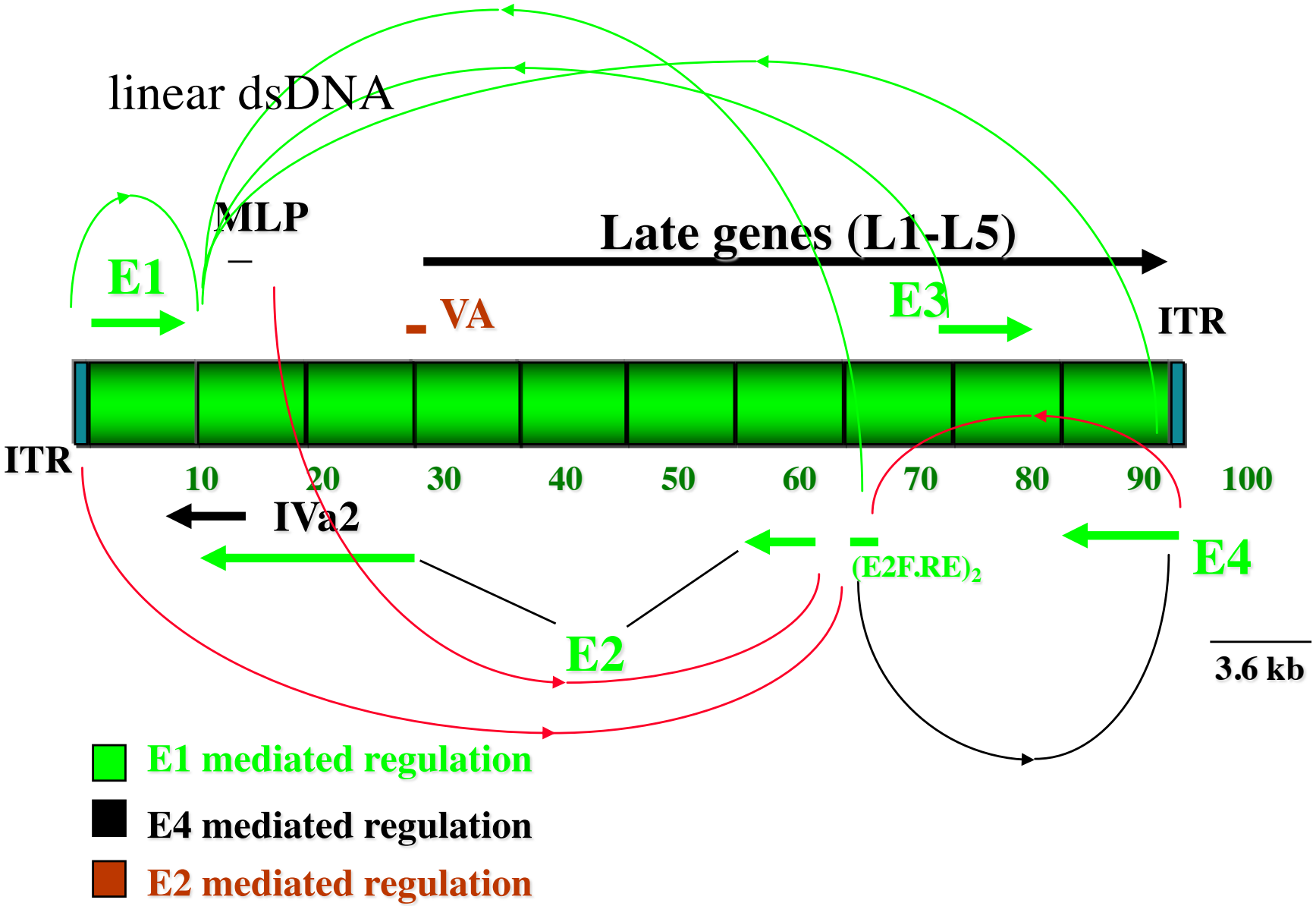
Adenovirus


Capsid proteins
Hexon
Penton base
Fibre
Minor proteins
IIIa
VI
VIII
IX
Core proteins
V
VII
Mu
Terminal protein
IVa2
Protease

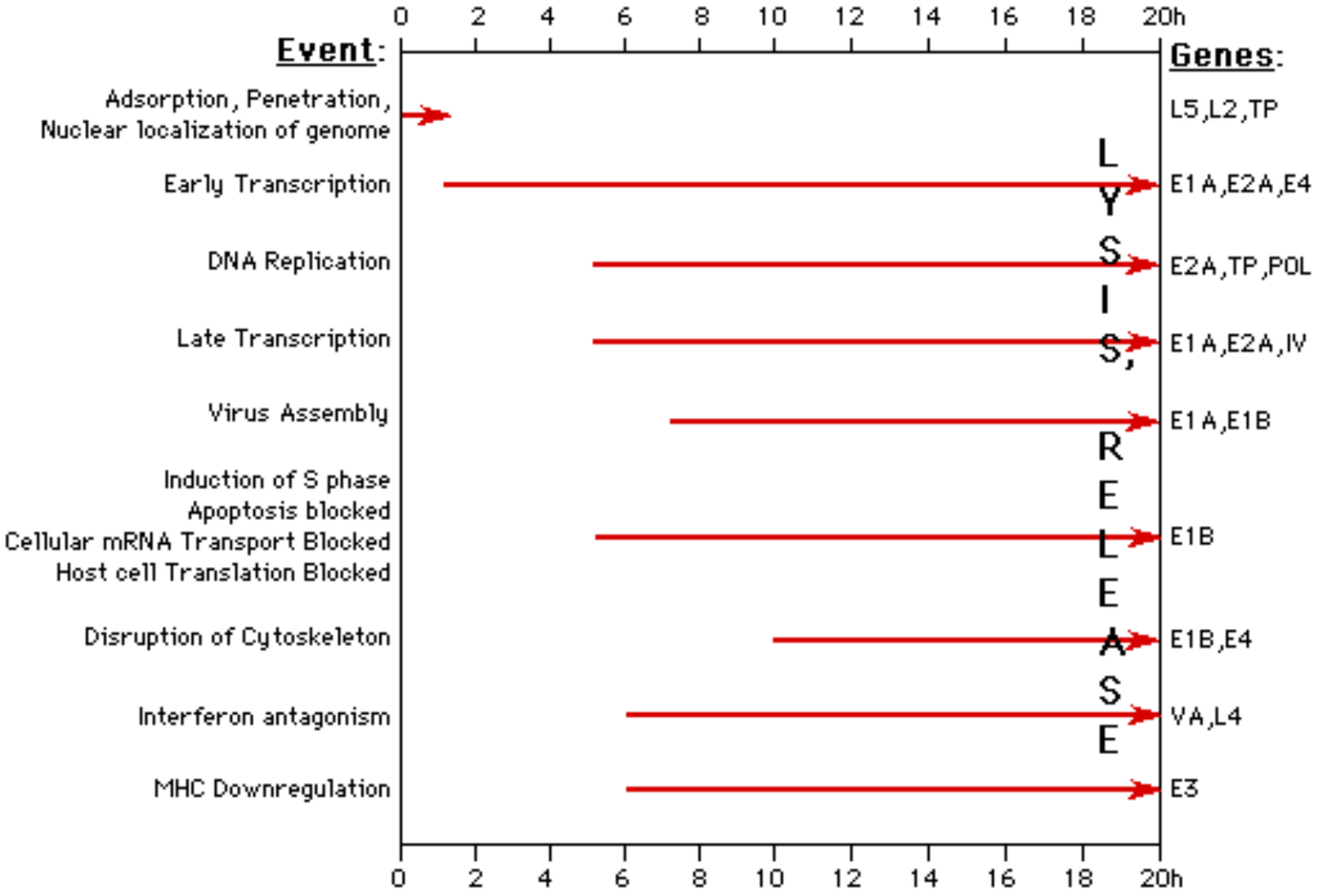
Adenovirus


PLASMA MEMBRANE
COATED PIT
ENDOSOME
NUCLEUS
1. Viral Entry
Receptor binding
Fiber shedding
Viral internalization
2. Endocytosis
Capsid dissassembly
Exposure of protein VI
3. Escape from endosome
Capsid dissassembly
Endosomal escape via lytic activity of pVI
4. Trafficking to nucleus
Cytoplasmic trafficking using microtubules, dynein and dynactin
5. Docking at nuclear pore complex
Capsid dissassembly
DNA import
Viral transgene expression
vDNA
αv integrins
CAR

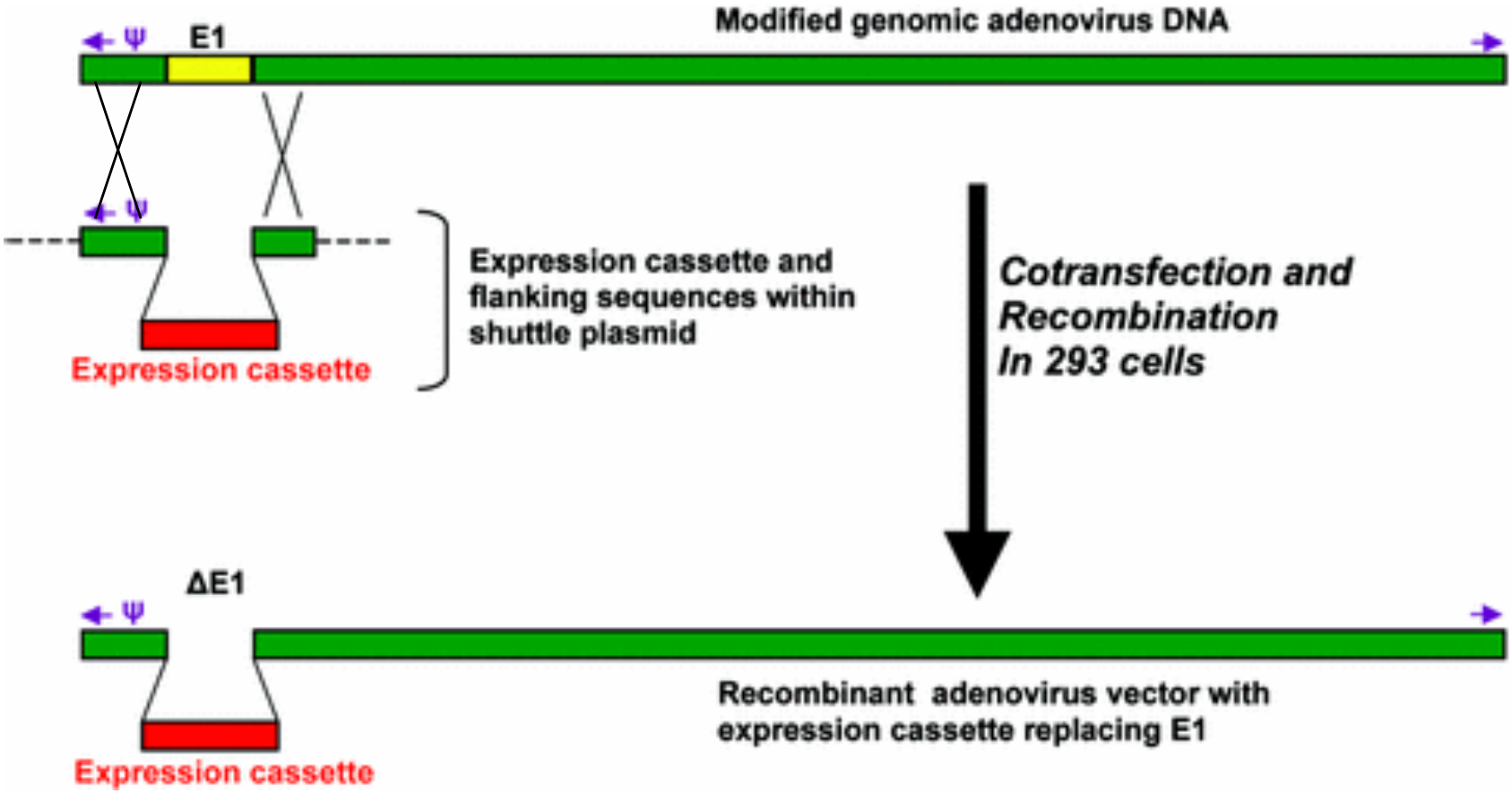
Adenovirus genome



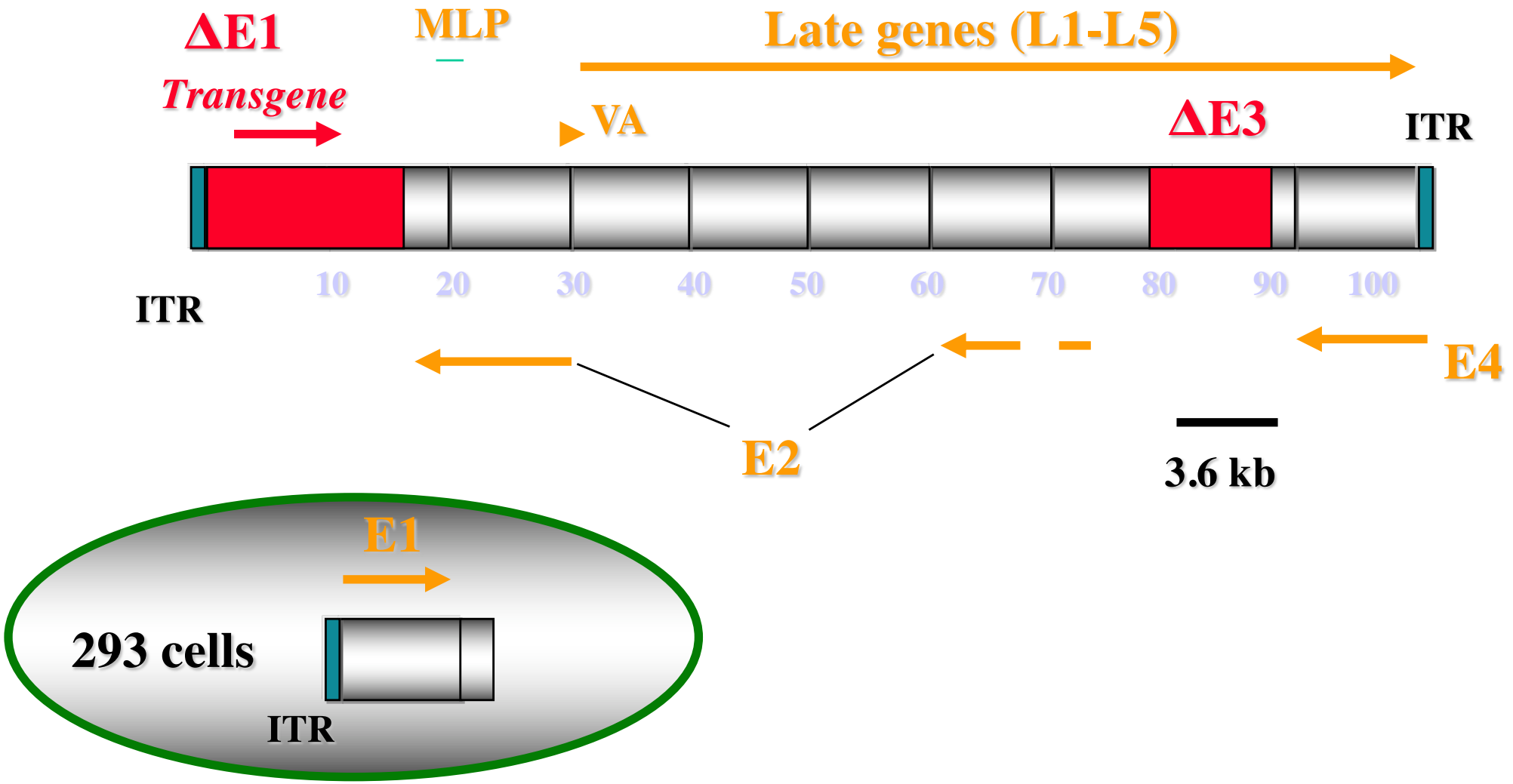
Adenovirus genes



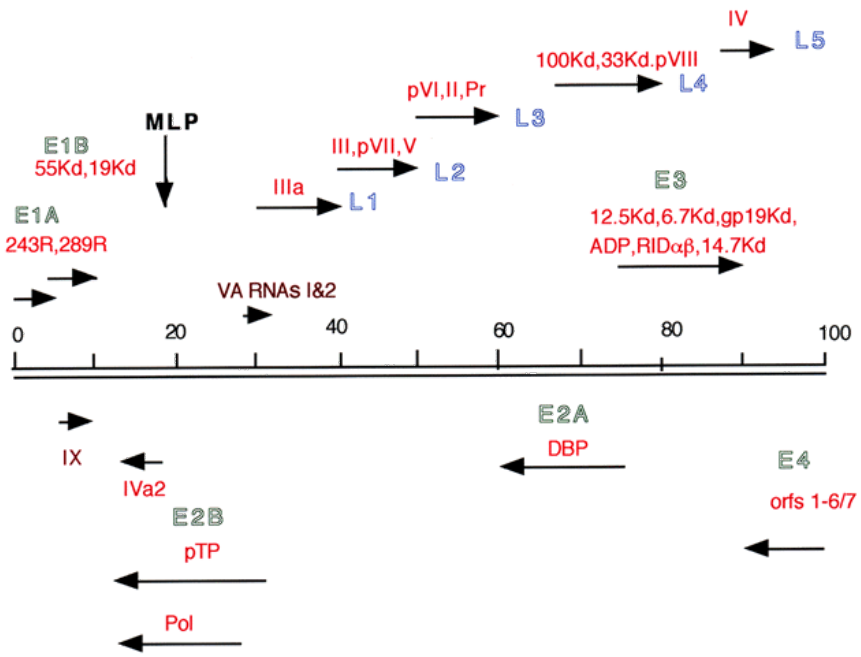
1st generation adenoviral vectors

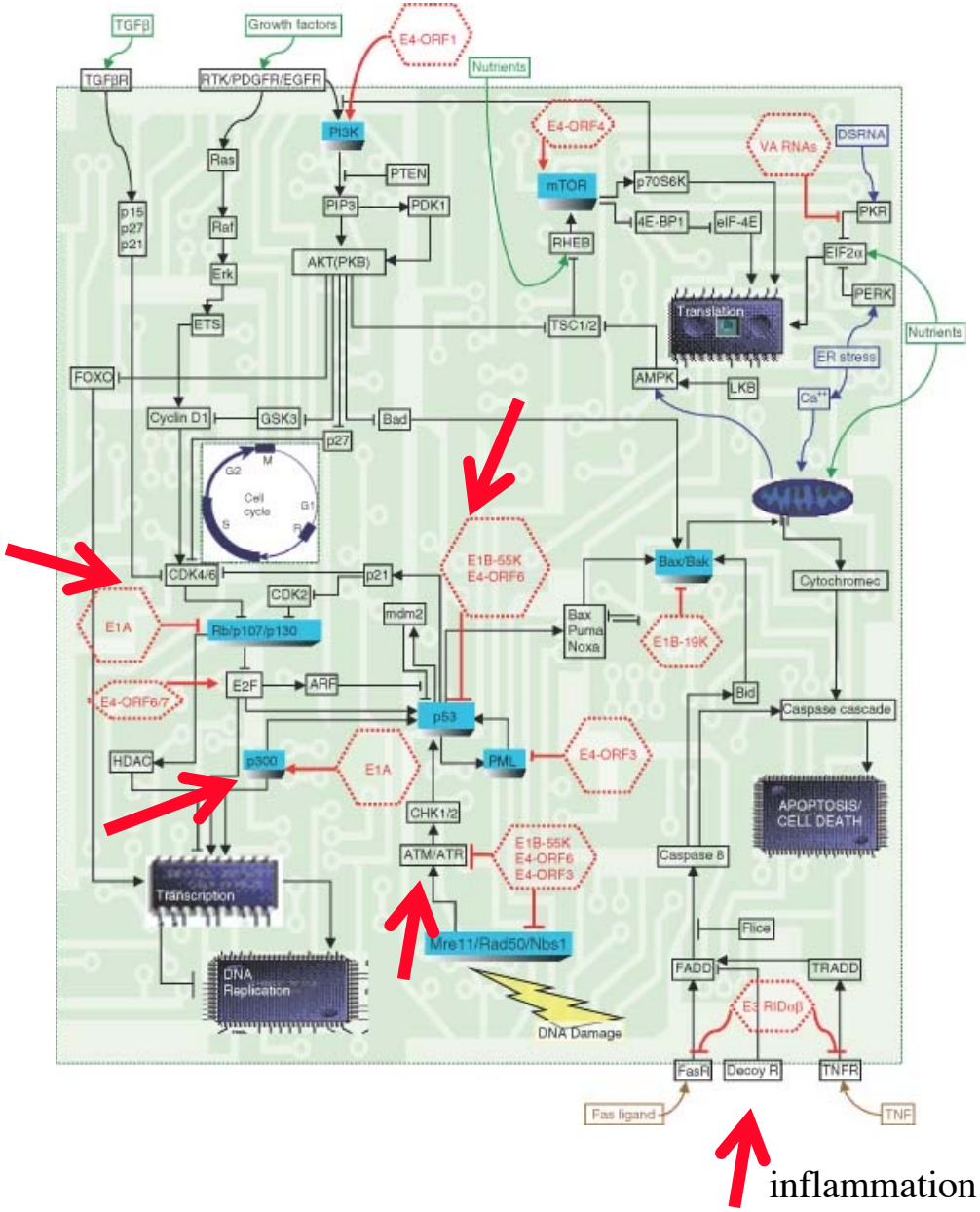


1st generation adenoviral vectors



Ad modulates cell functions



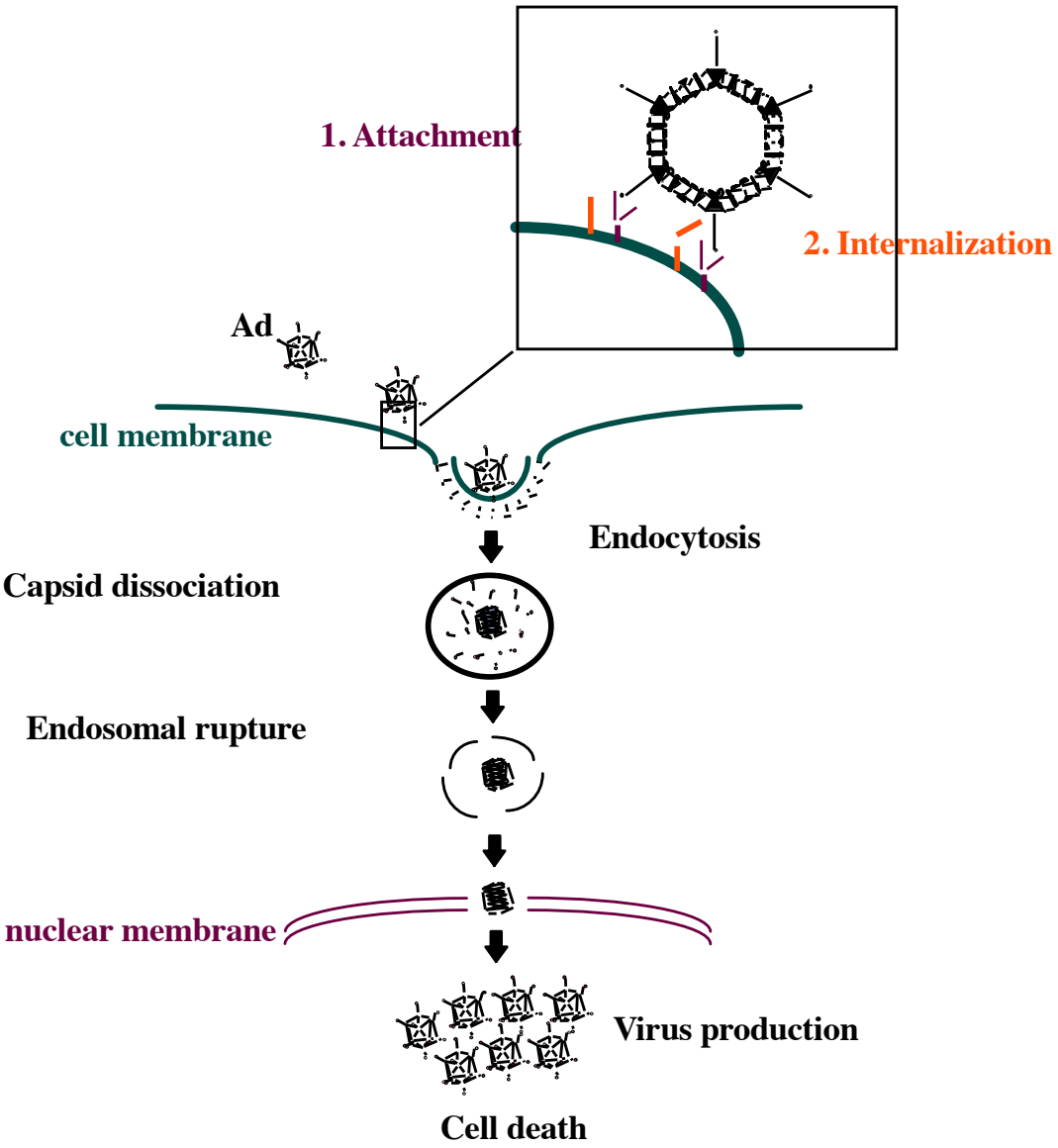


inflammation

Problems and ameliorations of Ad vectors

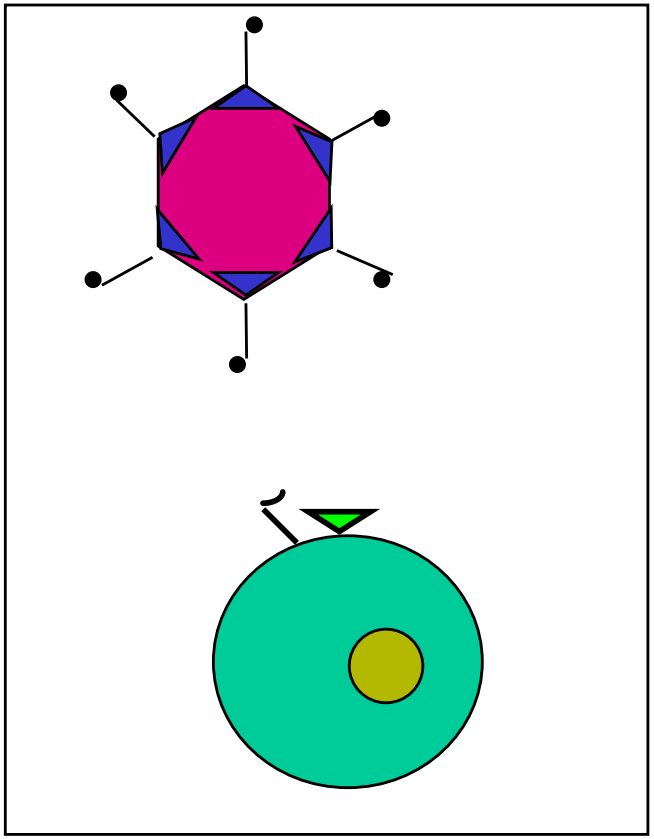
- no integration => chimaeres AAV/ Retro
- seropositivity to Ad => change of serotype, higher doses, immunosuppression
- large tropism => **targeted transduction**, targeted expression
- immunogenicity => **immuno-suppression**, **new vectors**
- size of the insert => new vectors
- short term expr. => chimaeres AAV/Retro, immuno-suppression, new generation vectors
- Replication Competent Adenovirus => new lines, new vectors
- transcomplementation => new vectors

Ad entry into cells

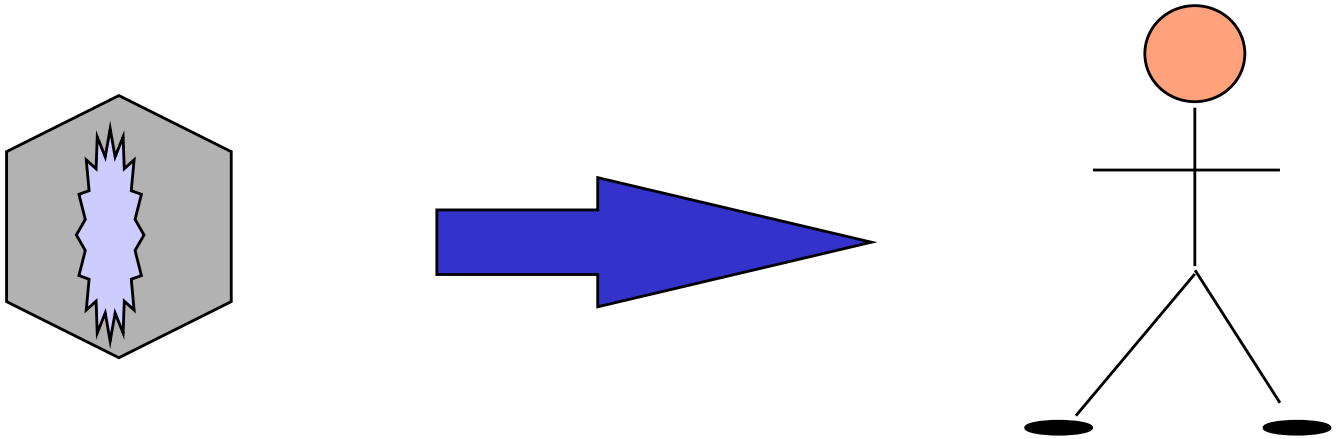


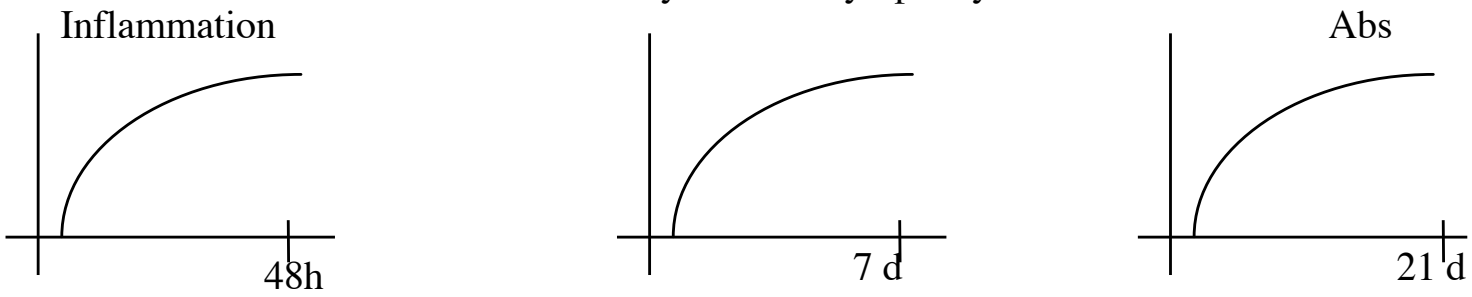
Ad modifications for targeting

- bispecific ABs antifiber/antireceptor (nabs)
- bispecific abs anti fiber insert/antireceptor (antiflag/antireceptor)
- fiber inserts (RGD)
- hexon inserts
- penton base inserts



Immune response to adenoviral vectors



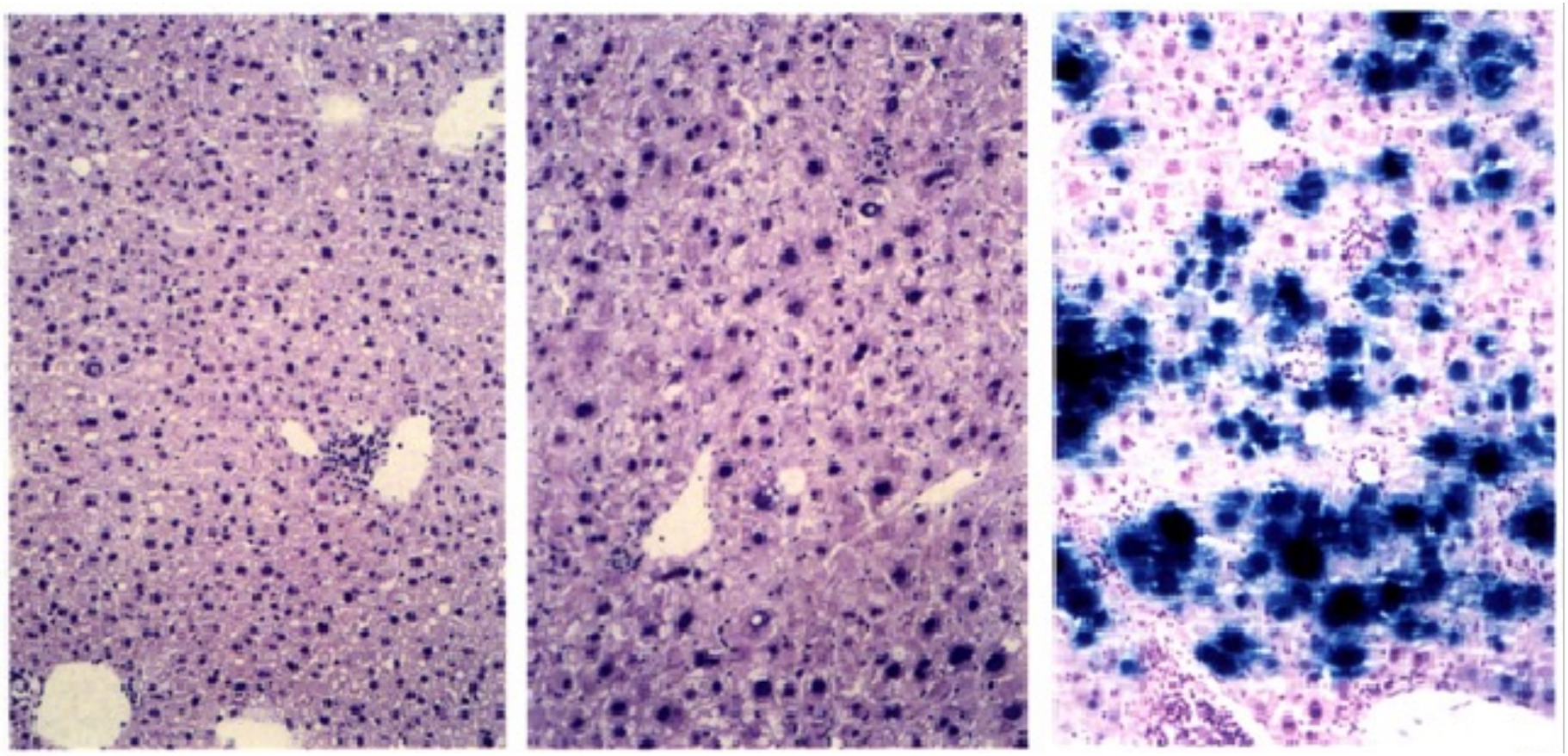


IL-6 and TNF-α in the immune response to Ad

- IL-6 inflammatory cytokine
- IL-6 in rabbit model of Ad induced pneumonia
- IL-6 in Ad injected patients

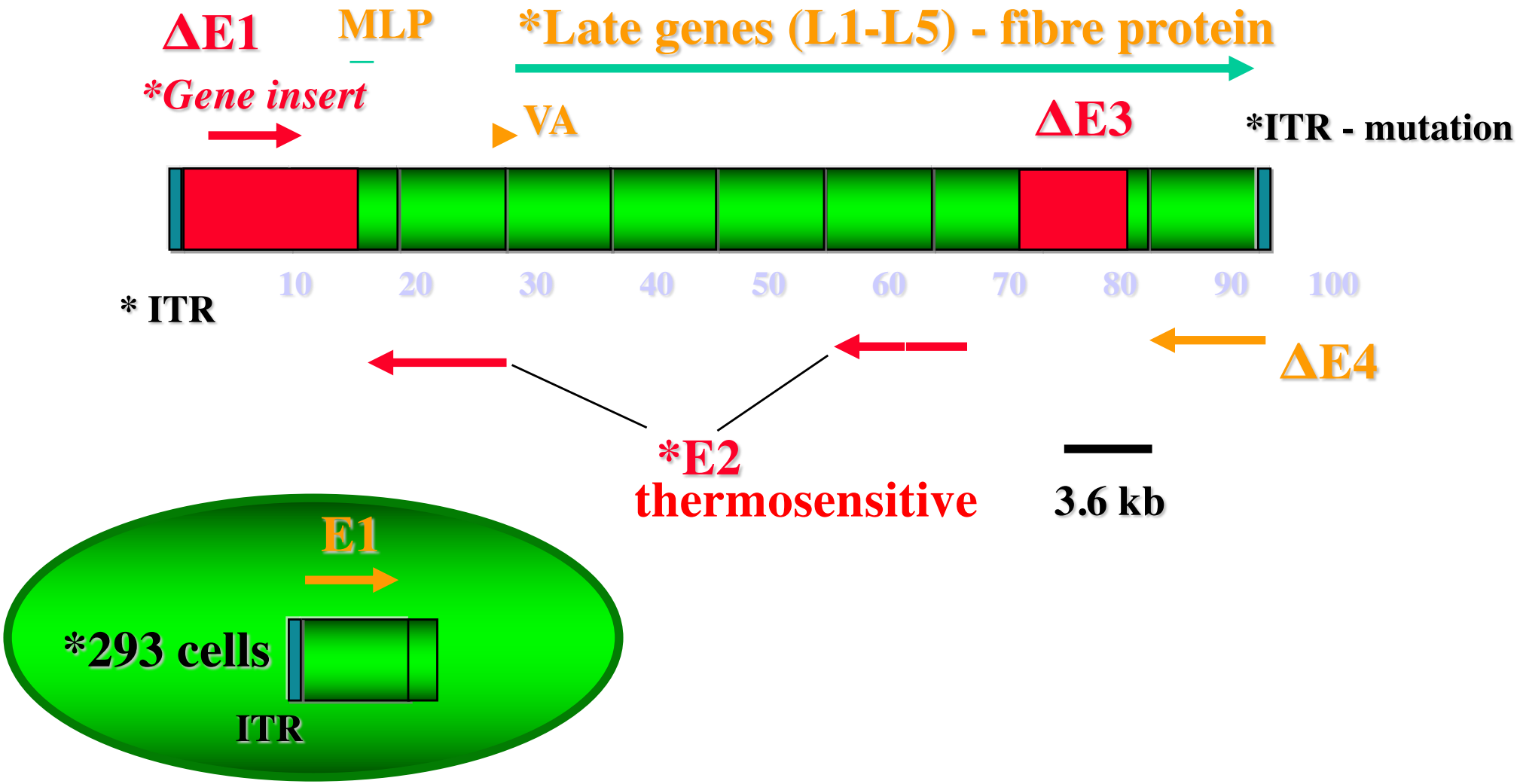
- TNF- α inflammatory cytokine
- anti-TNF genes in Ad

Ad reinjection in TNF-α KO

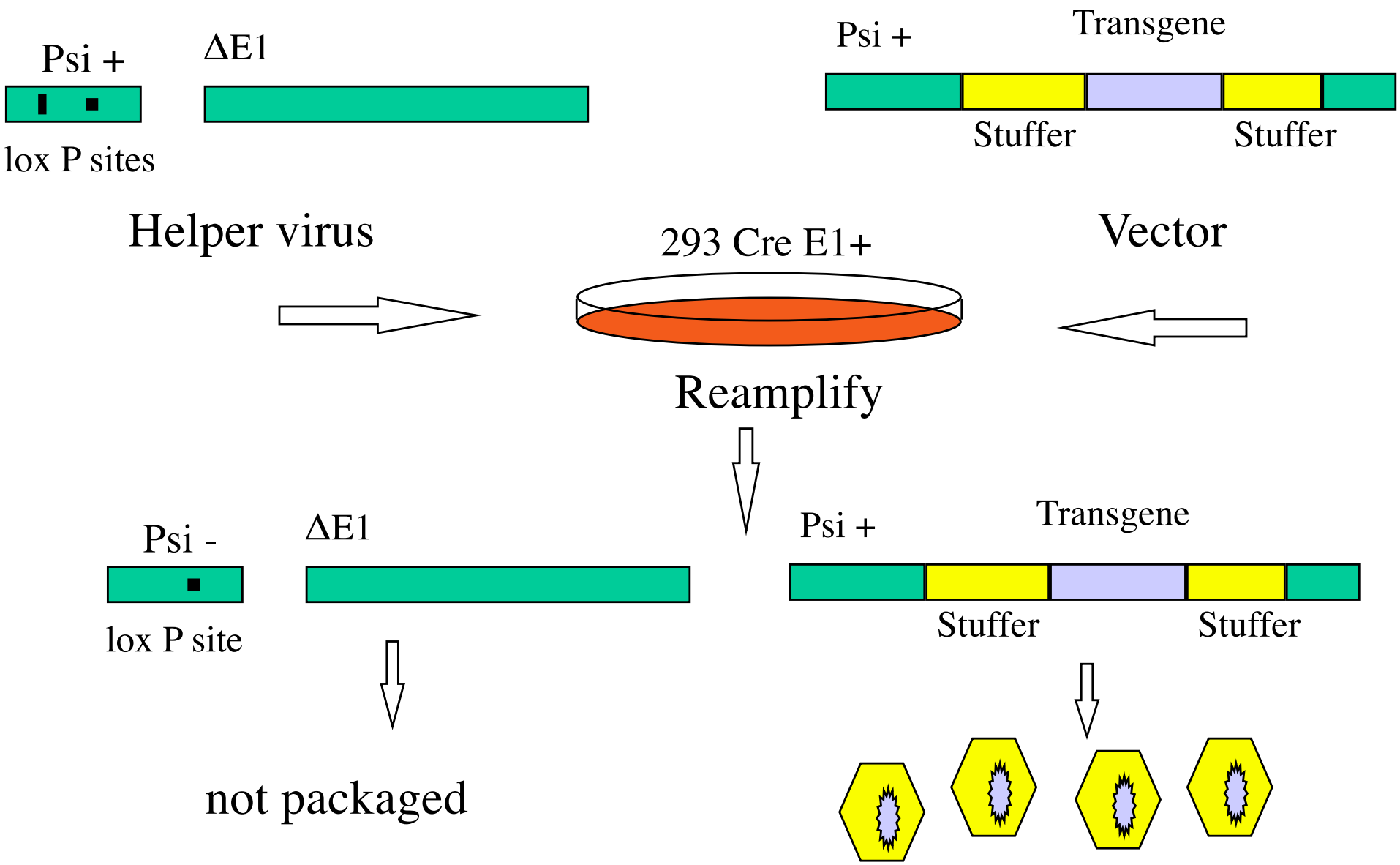


+/+ +/- -/-

2nd generation Adenoviral vectors



3rd generation Adenoviral vectors



3rd generation Ad vectors: advantages

- size of the insert (36kb)
- low immunogenicity (no viral sequences)
- long term expression

3rd generation ad vectors: disadvantages

- titers
- instability
- helper contaminations
- stuffer?

Adenovirus mediated gene therapy: history

- *Welsh Cell 1993* Adenovirus mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis
- *Wilson Nature Genetics1993* Gene therapy in a xenograft model of cystic fibrosis lung corrects chloride transport more effectively than the sodium defect
- *Peschanski Nature Genetics 1993* Transfer of a foreign gene into the rat brain using adenovirus vectors
- *Wilson 1993* Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenoviruses

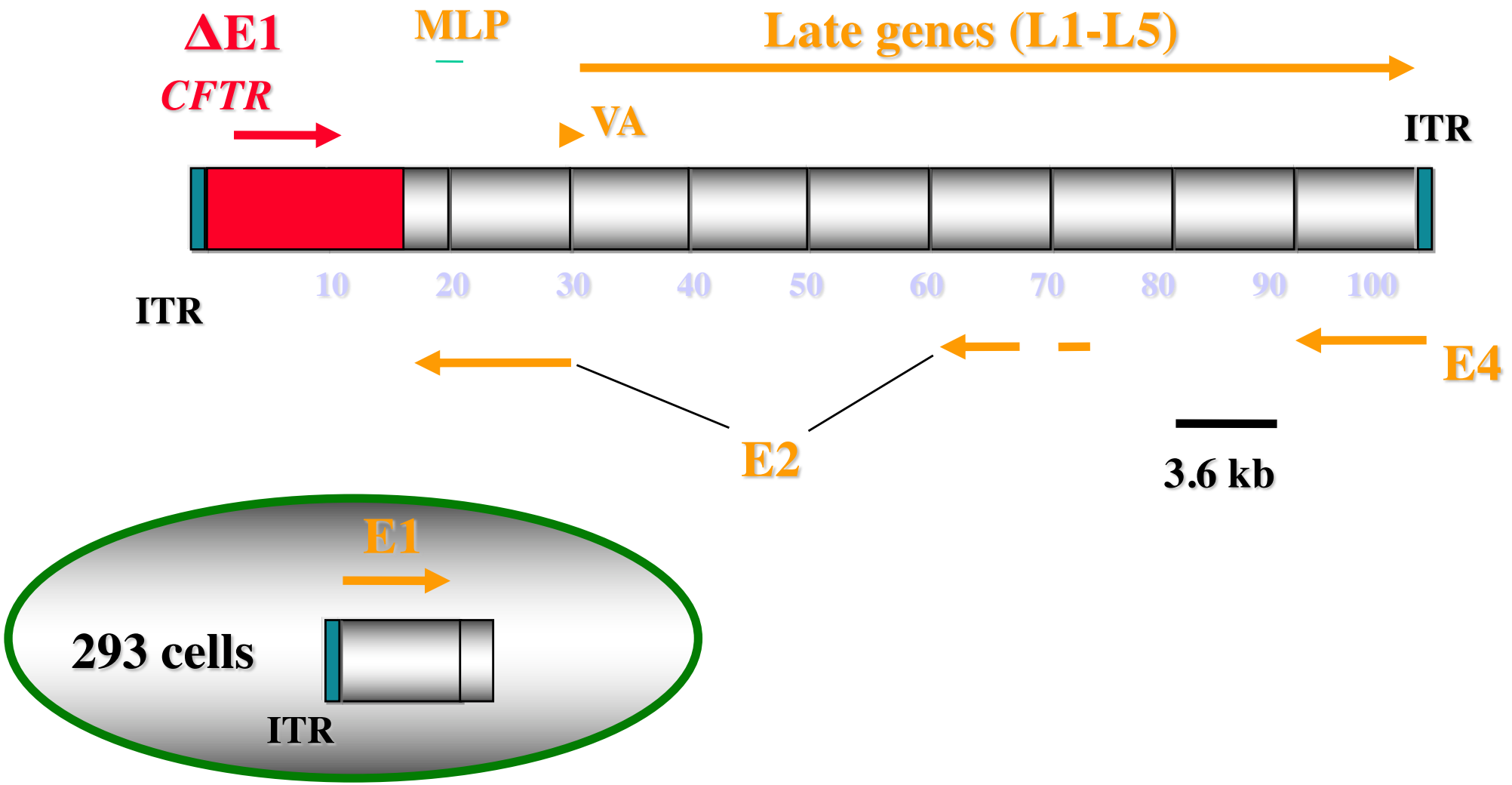
Ad-mediated gene therapy: history (follows)

- *Crystal Nature Genetics 1994* Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis
- *Wilson Nature Genetics 1996* Effective treatment of familial hypercolesterolaemia in the mouse model using adenovirus-mediated transfer of the VLDL receptor gene
- *McCormick Science 1996* An adenovirus mutant that replicates selectively in p53 deficient human tumor cells

Cystic fibrosis gene therapy

- Autosomal recessive disease caused by mutations in the transmembrane conductance regulator (CFTR)
- The Cl- channel is deregulated => defective Cl- transport => lung disease

1st generation Ad-CFTR



Cell 93: AdCFTR in human patients

Check of ion transport:

amiloride creates a gradient and if the channel works, terbutaline makes Cl^- going out

Cl- voltage

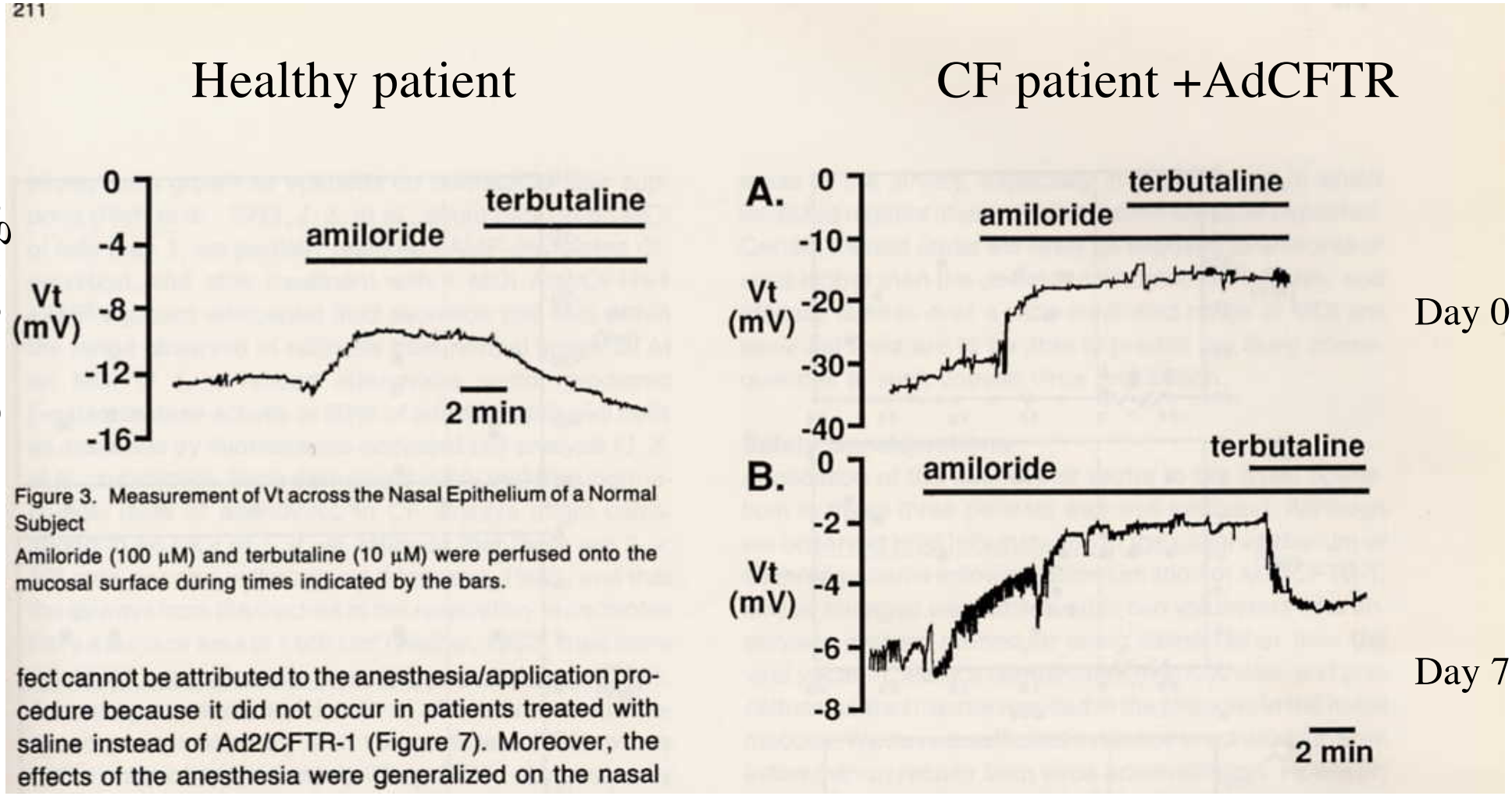


Figure 3. Measurement of Vt across the Nasal Epithelium of a Normal Subject

Amiloride (100 μM) and terbutaline (10 μM) were perfused onto the mucosal surface during times indicated by the bars.

fect cannot be attributed to the anesthesia/application procedure because it did not occur in patients treated with saline instead of Ad2/CFTR-1 (Figure 7). Moreover, the effects of the anesthesia were generalized on the nasal

Ad-CFTR

Nature Genetics 93/94

"S*emi-in vivo*"
AdCFTR in human bronchial xenografts implantation into nu/nu mice

Bronchus ->

Xenograft ->

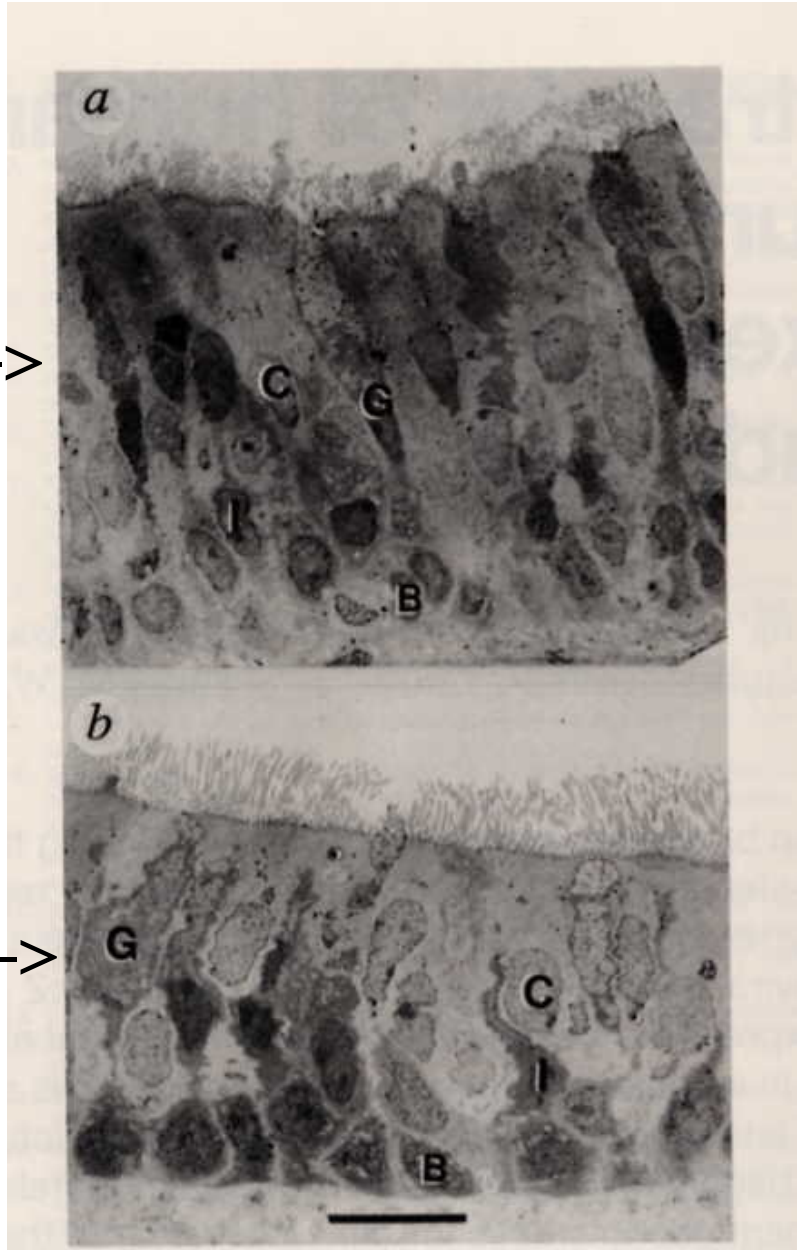
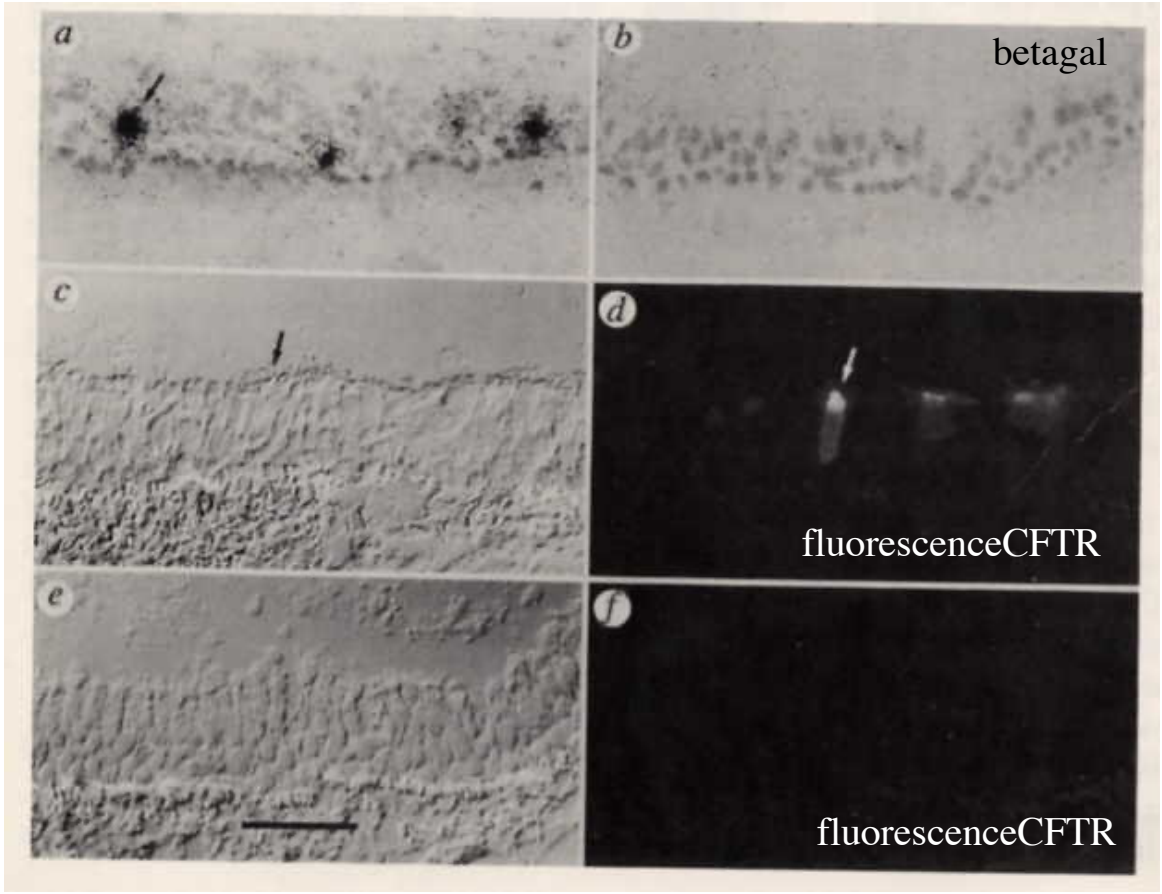


Fig. 1 Electron micrographs of bronchial epithelia from human bronchus and a xenograft. Micrograph of human bronchial epithelium *a*, and epithelium from a xenograft seeded with human bronchial epithelial cells and harvested at 42 days *b*. C, ciliated cell; G, goblet cell; B, basal cell and I, intermediate cell

AdCFTR in human bronchial epithelial xenografts; 1 week after infection



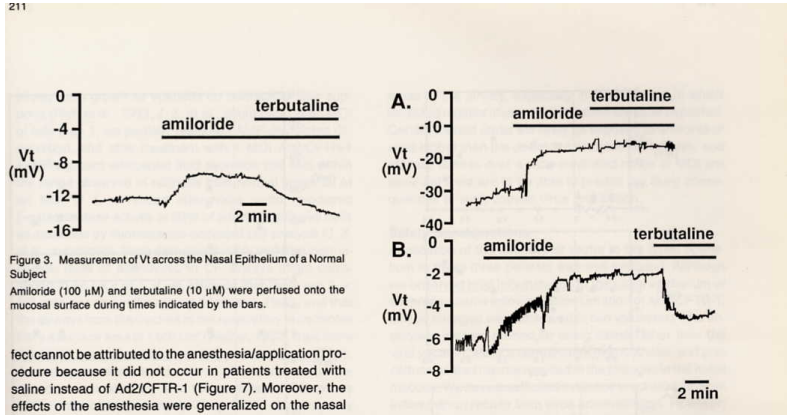
Adbgal

AdCFTR

Adbgal

Nat Gen 93 Wilson/Perricaudet

Ion function in AdCFTR infected human bronchial xenografts



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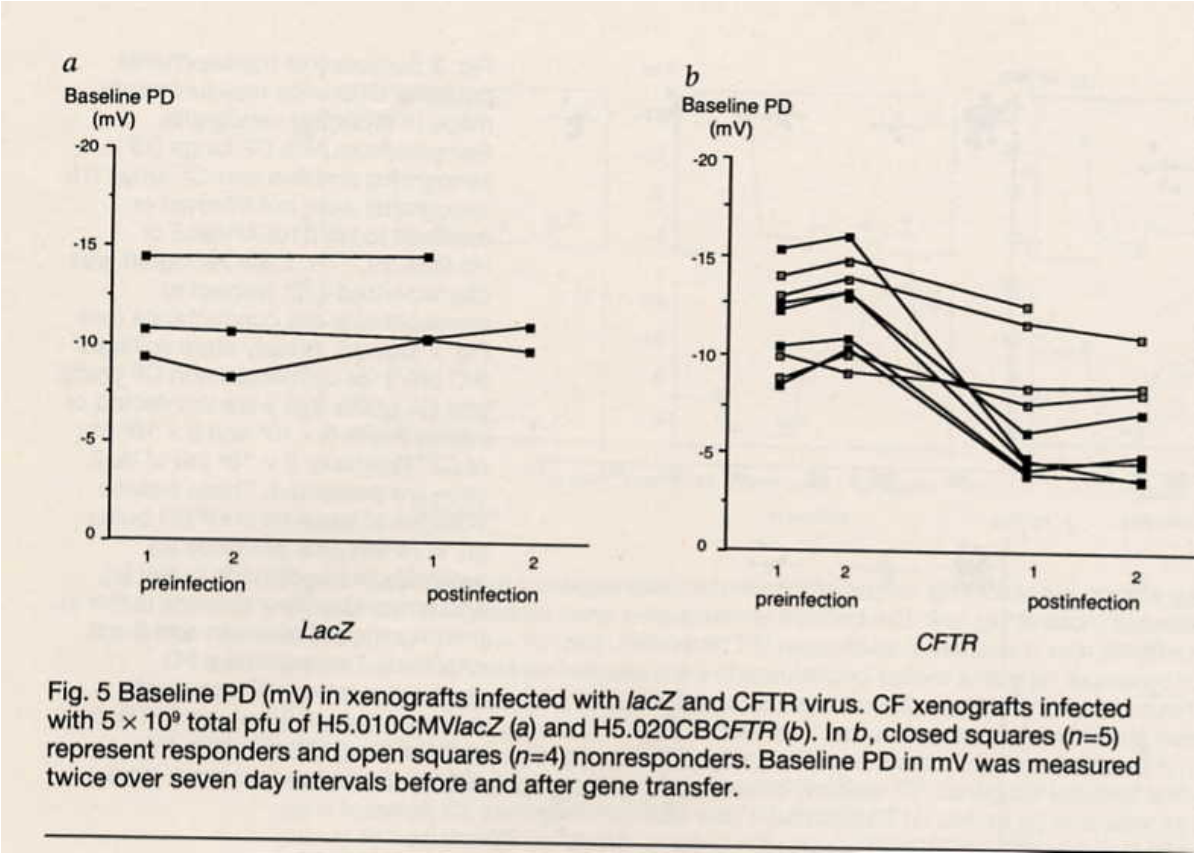


Fig. 5 Baseline PD (mV) in xenografts infected with *lacZ* and CFTR virus. CF xenografts infected with 5×10^9 total pfu of H5.010CMV*lacZ* (*a*) and H5.020CB*CFTR* (*b*). In *b*, closed squares (n=5) represent responders and open squares (n=4) nonresponders. Baseline PD in mV was measured twice over seven day intervals before and after gene transfer.

Nat Gen 93 Wilson/Perricaudet

Ad-residual activity in bronchial xenografts

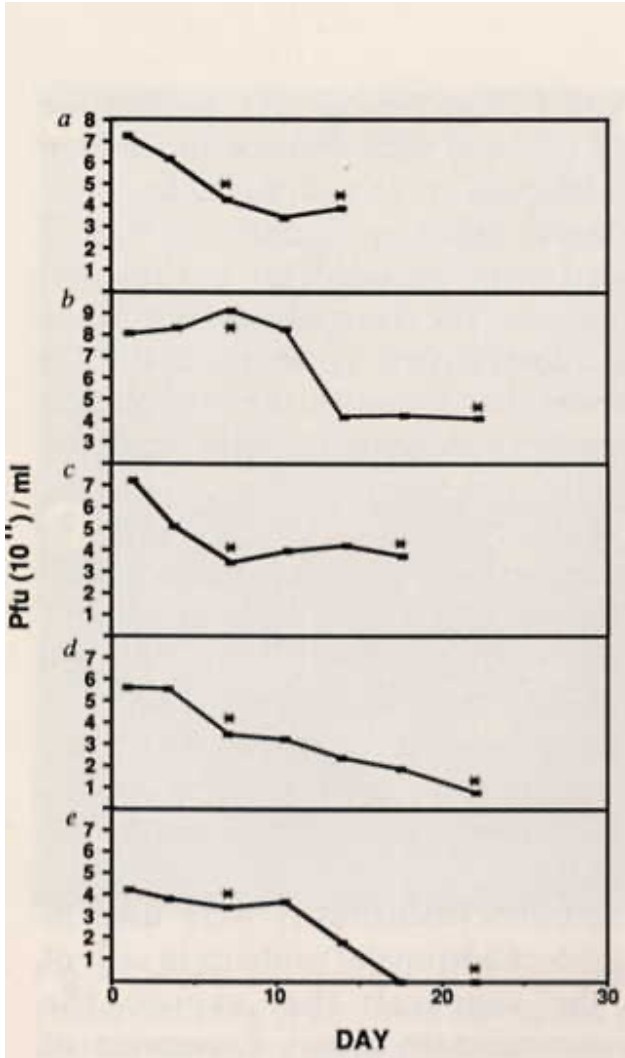


Fig. 7 Recovery of recombinant virus in xenograft effluents. Effluents (1 ml) were collected at 3 1/2 day intervals from xenografts infected with Ad.CMV*lacZ* and were titered by Xgal stained pfu assay on 293 cells. All plaques generated on 293 cells contained β-galactosidase as evident by blue precipitate. Recovered virus is plotted on a log scale versus the time after infusion of virus measured in days. Following the completion of the experiment, the xenografts were harvested, xgal stained and evaluated for % genetic reconstitution in the surface epithelial cells: *a–c*, 5–20% *lacZ* positive cells; *d*, 1% *lacZ* positive cells; and *e*, less than 0.01% *lacZ* positive cells. Asterisks mark effluents that were assayed for wild type adenovirus by the ability to cause cytopathic effects on Hela cells.

Nat Gen 93 Wilson/Perricaudet

AdCFTR in humans: immunocytochemical detection of CFTR in human bronchial cells

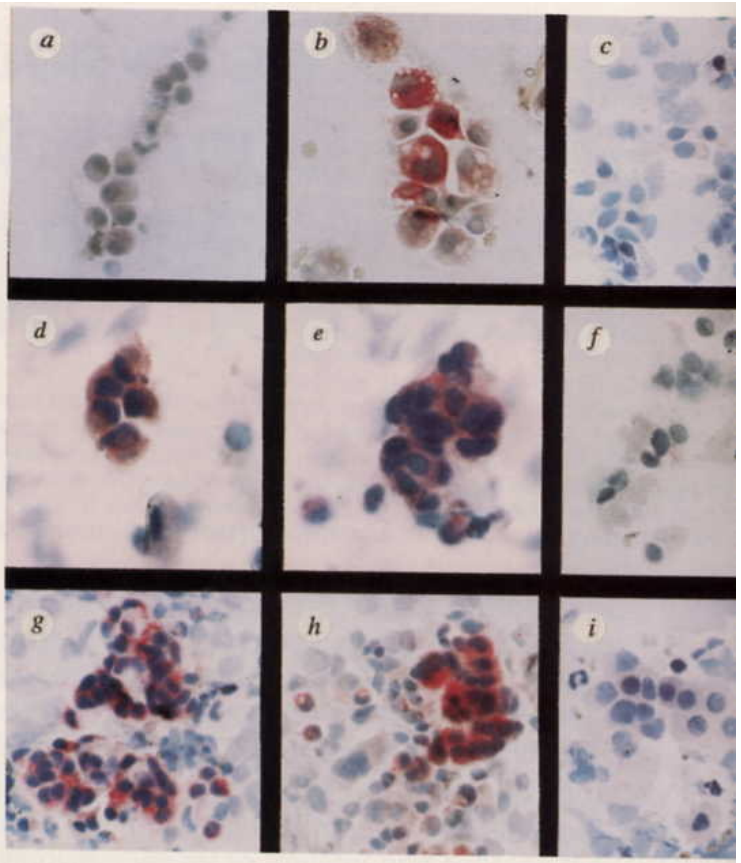


Fig. 3 Immunocytochemical detection of human CFTR in bronchial epithelial cells before and after *in vivo* administration of Ad*CFTR* to the bronchial epithelium. Low level endogenous expression of *CFTR* in the bronchial epithelium pre-therapy (*a–e*) is not detected; for *f–i*, the cells were exposed to the substrate for a longer period, and minimal pink staining is observed in the control (*f*). *a*, Bronchial epithelial cells from individual 3A recovered pre-therapy and maintained in culture under conditions identical to *b*. *b*, as (*a*), but infected with Ad*CFTR in vitro* as a positive control. *c*, Fresh (not cultured) bronchial epithelial cells of the same individual obtained pre-therapy immediately prior to intrabronchial administration of Ad*CFTR*. *d* and *e*, Left bronchial epithelium 4 d after intrabronchial administration of Ad*CFTR* (2×10^6 pfu) to the airway epithelium of the left lower lobe. *f* and *g*, same individual as in (*c–e*), but with the cells exposed to the colorimetric substrate for a longer time to increase the sensitivity of the assay. *f*, Bronchial epithelial cells obtained prior to intrabronchial administration of Ad*CFTR*. *g* and *h*, Evaluation similar to (*f*) of the left bronchial epithelium 4 d after intrabronchial administration of Ad*CFTR*. In (*h*), the morphology of one "positive" cell (at 9 o'clock, far left) is indeterminate. *i*, as (*g*) and (*h*), but with an irrelevant isotype control antibody. The positive epithelial cells following therapy include ciliated, non-ciliated columnar or basal cells. For *a–e*, the time of exposure to the alkaline phosphatase substrate was 9 min; for *f–i*, the time was 17 min. For the short exposure to the substrate, quantification of the "% positive cells" demonstrated in the pre-therapy period 0% epithelial cells were positive (n=800 cells), and 0% of inflammatory cells were positive (n=200), while in the post-therapy period 5.0% of epithelial cells were positive (n=500) and 0% inflammatory cells were positive (n=500). For the longer exposure, in the pre-therapy period 0% epithelial cells were positive (n=500), and 0% inflammatory cells were positive (n=200), while in the post-therapy period 14.0% epithelia were positive (n=500), and 0.4% inflammatory cells were positive (n=500). In all panels, the samples were counterstained with hematoxy panels 630× except *d* and *e*, 1,000×.

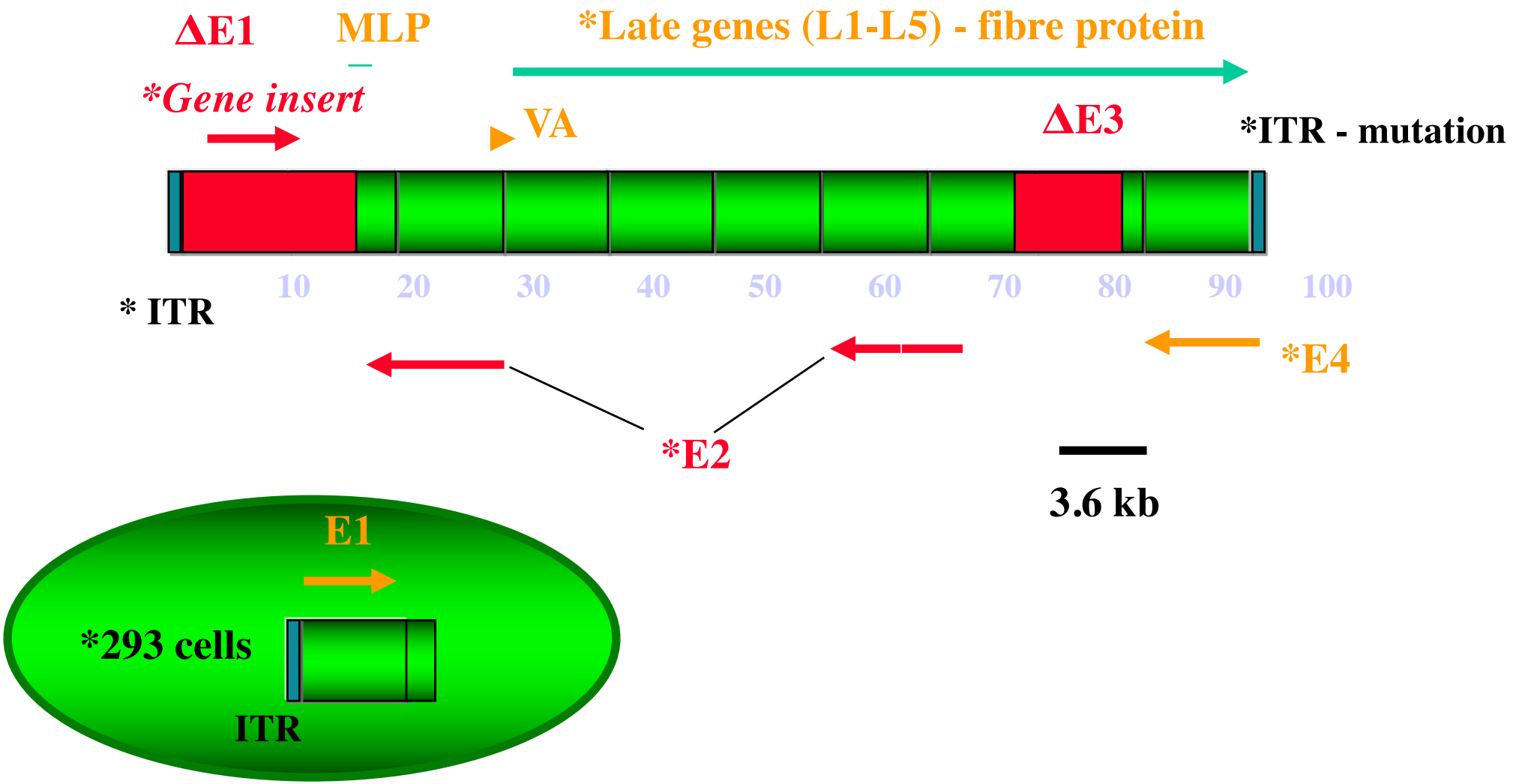
A: pretherapy
B: prether inf in vitro
C: pretherapy
D: 4 d after AdCFTR
E: 4 d after AdCFTR
F: pretherapy
G: 4d after AdCFTR
H: 4d after AdCFTR
I : control ab

Crystal Nat Gen 94

Problems and ameliorations of Ad vectors

- no integration => chimaeres AAV/ Retro
- seropositivity to Ad => change of serotype, higher doses, immunosuppression
- large tropism => **targeted transduction**, targeted expression
- immunogenicity => **immuno-suppression**, **new vectors**
- size of the insert => new vectors
- short term expr. => chimaeres AAV/Retro, immuno-suppression, new generation vectors
- RCA => new lines, new vectors
- transcomplementation => new vectors

2nd generation adenoviral vectors



Adeno-death
(clinical trials Wilson)

- 18 year old boy
- To correct ornithyne transcarbamylase deficiency (OCT), a metabolic disease that can induce ammonia accumulation in the body
- Ad-OCT 3.8 x10e13 2nd generation vector (E1-deleted, E2A-temperature-sensitive) in the hepatic artery
- Patient dyes 4 days after injection

Why?

CLINICAL TRIALS

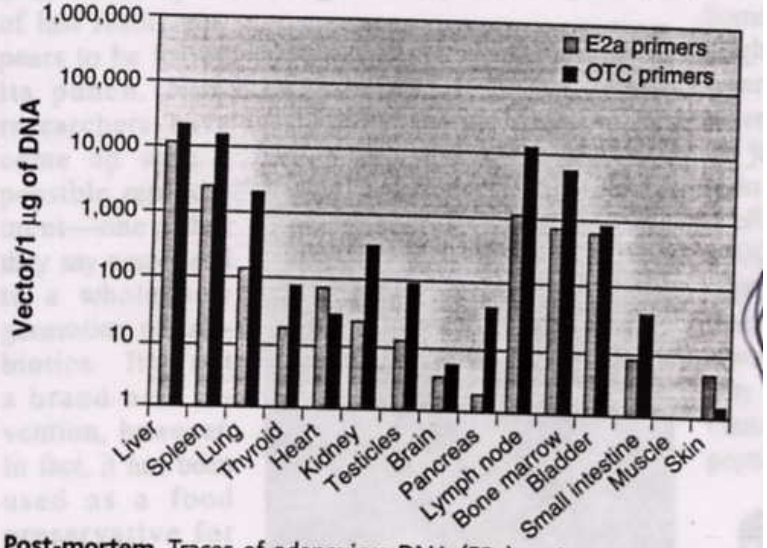
Gene Therapy Death Prompts Review of Adenovirus Vector

For the past 3 months, one-third of the 250 faculty and staff members connected with the University of Pennsylvania's Institute for Human Gene Therapy have been studying a single case. They've been trying to understand why Jesse Gelsinger, a relatively fit 18-year-old with an inherited enzyme deficiency, died on 17 September, 4 days after doctors at Penn injected a genetically altered virus into his liver.

Gelsinger was the first patient in a gene therapy trial to die of the therapy itself, as James Wilson, who heads the Penn institute, confirmed at a public meeting last week. His death is the latest blow to a field that has been struggling to live up to the promise and hype surrounding the first gene therapy trials a decade ago. And Penn isn't the only one investigat-

Gelsinger had died. It was a tense session.

After releasing stacks of clinical data and answering questions for 2 days, however, Wilson and colleagues said that they didn't fully understand what had gone amiss. They report-



Post-mortem. Traces of adenovirus DNA (E2a) and a curative gene (OTC) it carried turned up in many tissues outside the patient's target organ, the liver.

Science 1999 Marschall

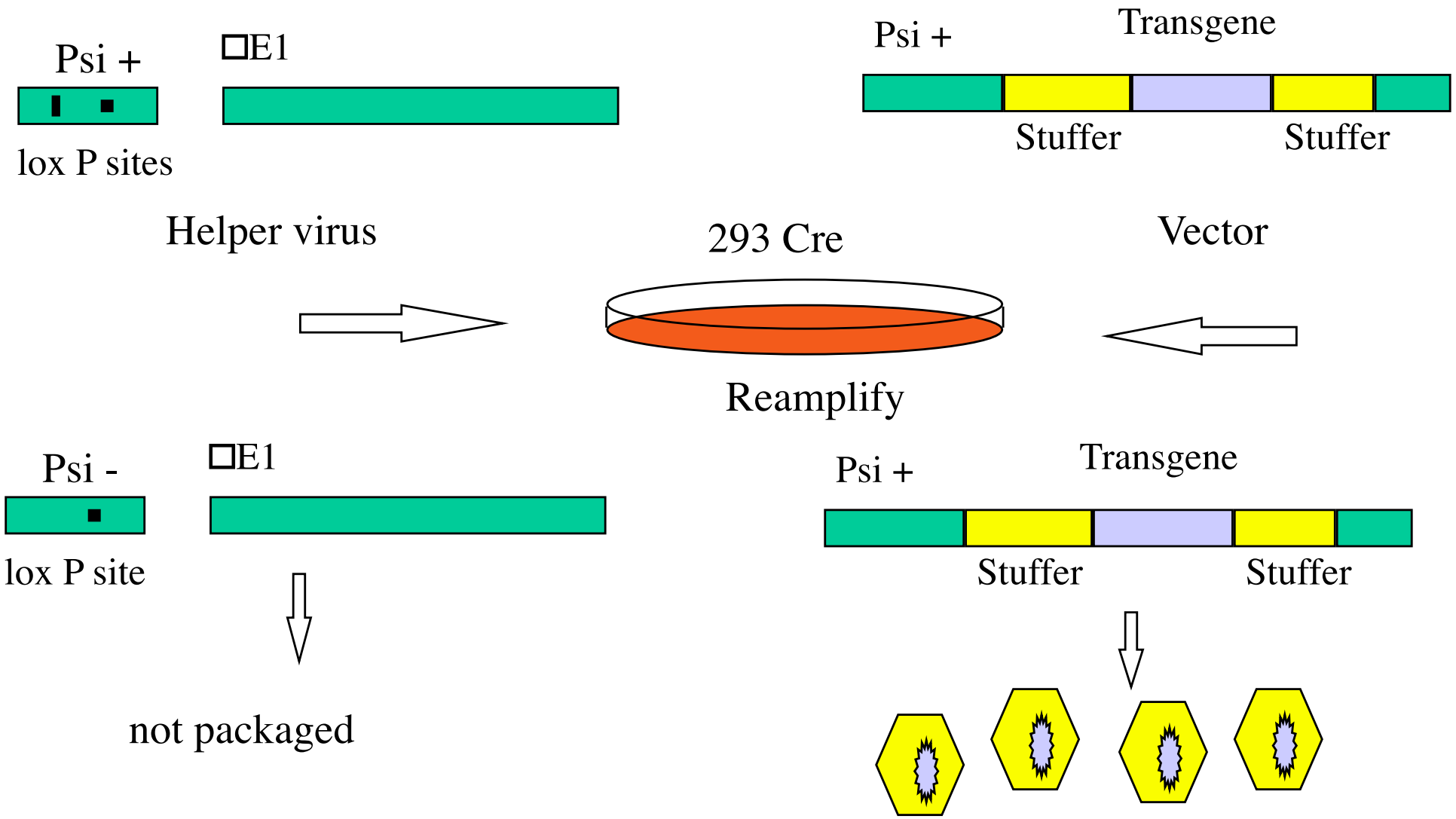
Sanctions agreed over teenager's gene therapy death

- 5 year investigation
- According to an investigation by the university, Gelsinger died from an immune reaction to the adenovirus vector.
- The justice department alleged that the researchers and their institutions made false statements regarding the safety of the trial to the National Institutes of Health, the Food and Drug Administration, and the institutional review board that oversaw the research.
- 3 researchers will pay 1 million $
- The terms of the settlement state that a monitor will supervise Wilson's work in humans for three years, and he will be allowed to conduct only one trial at a time. Any of Wilson's animal research that could affect patient safety will also be supervised
- Wilson : retraining for clinical trial, clinical trials in 2010

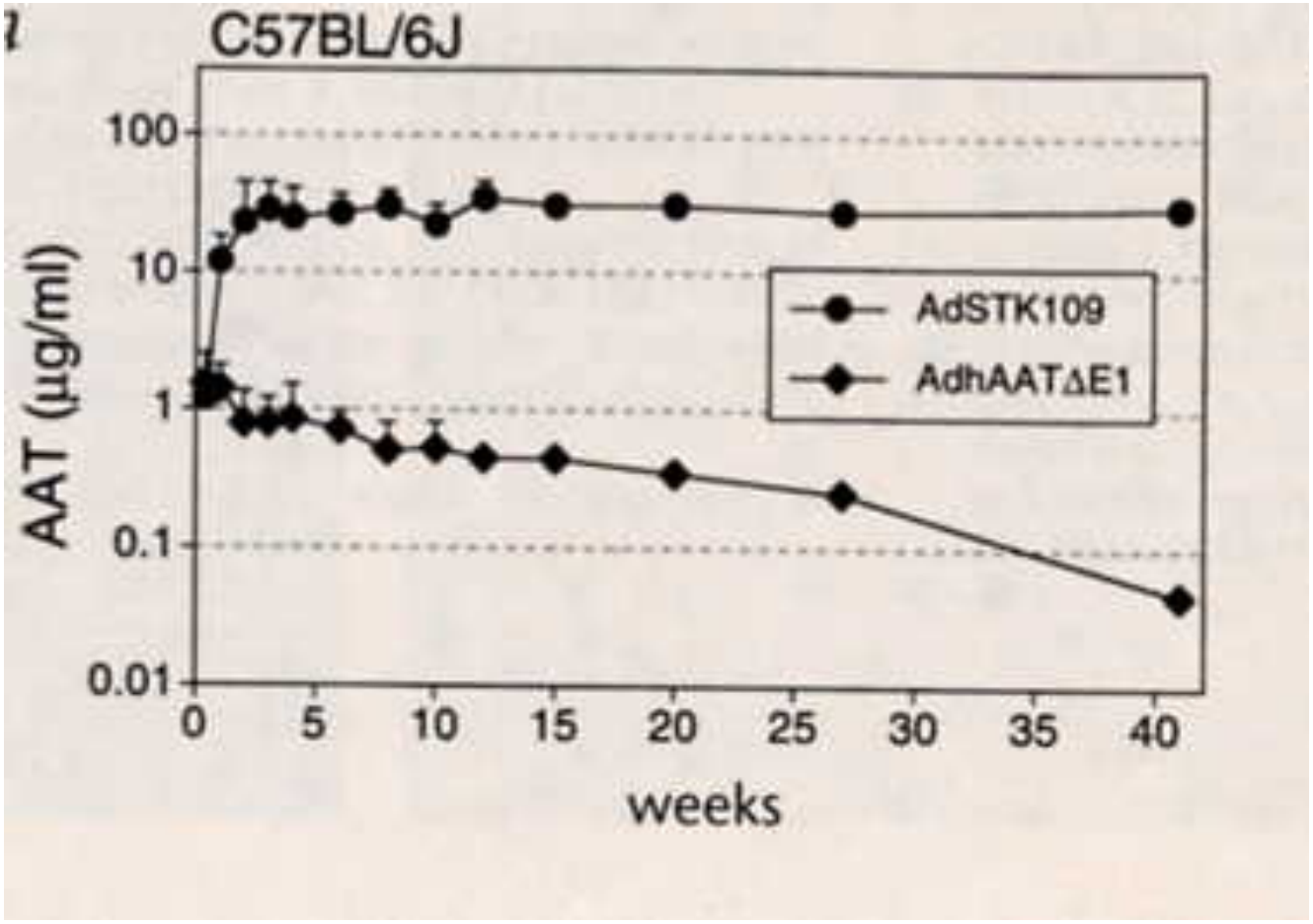
Nature, 2005

http://www.nih.gov/catalyst/2000/00.01.01/page1.html

3rd generation Ad- vectors

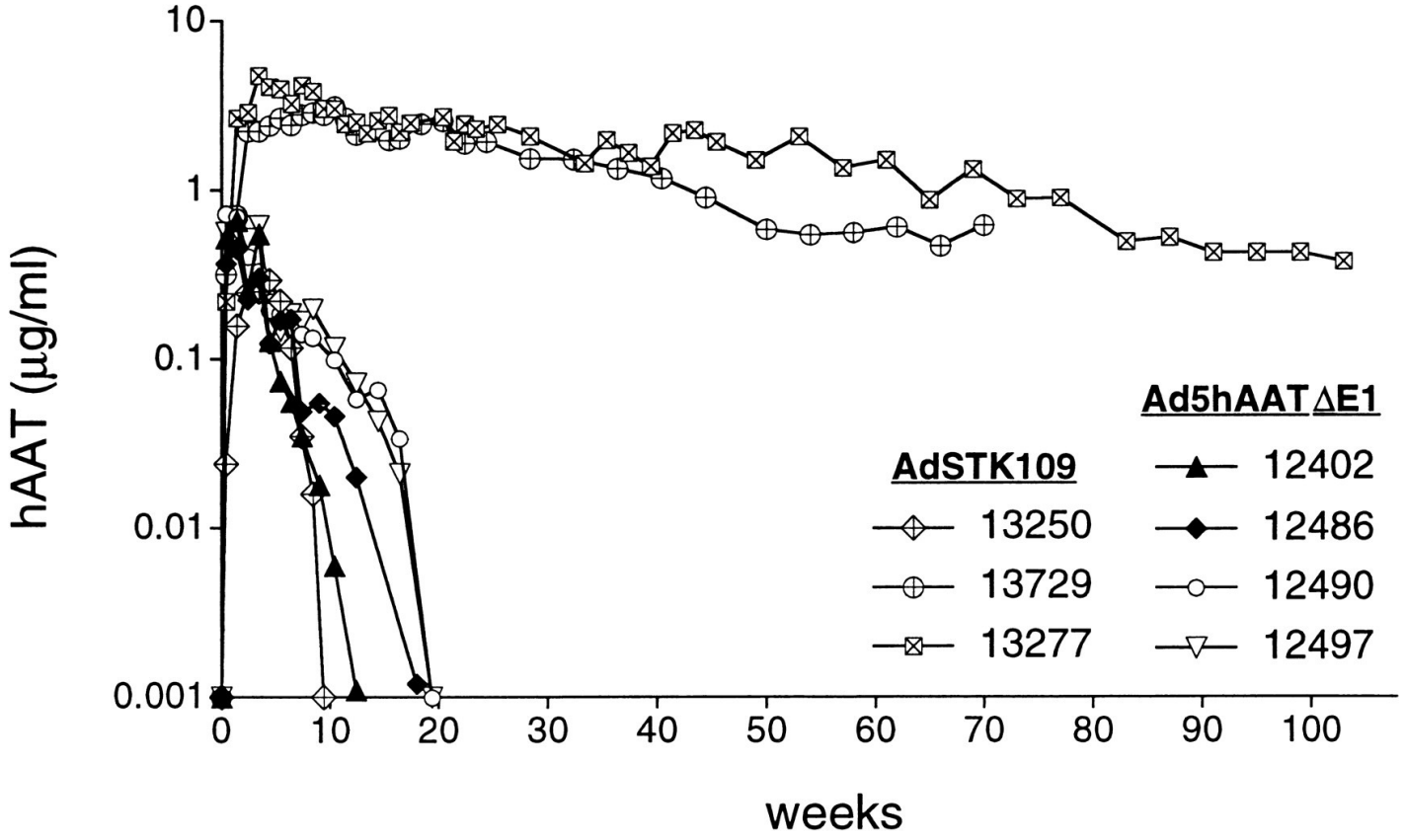


Ad gutless in mice



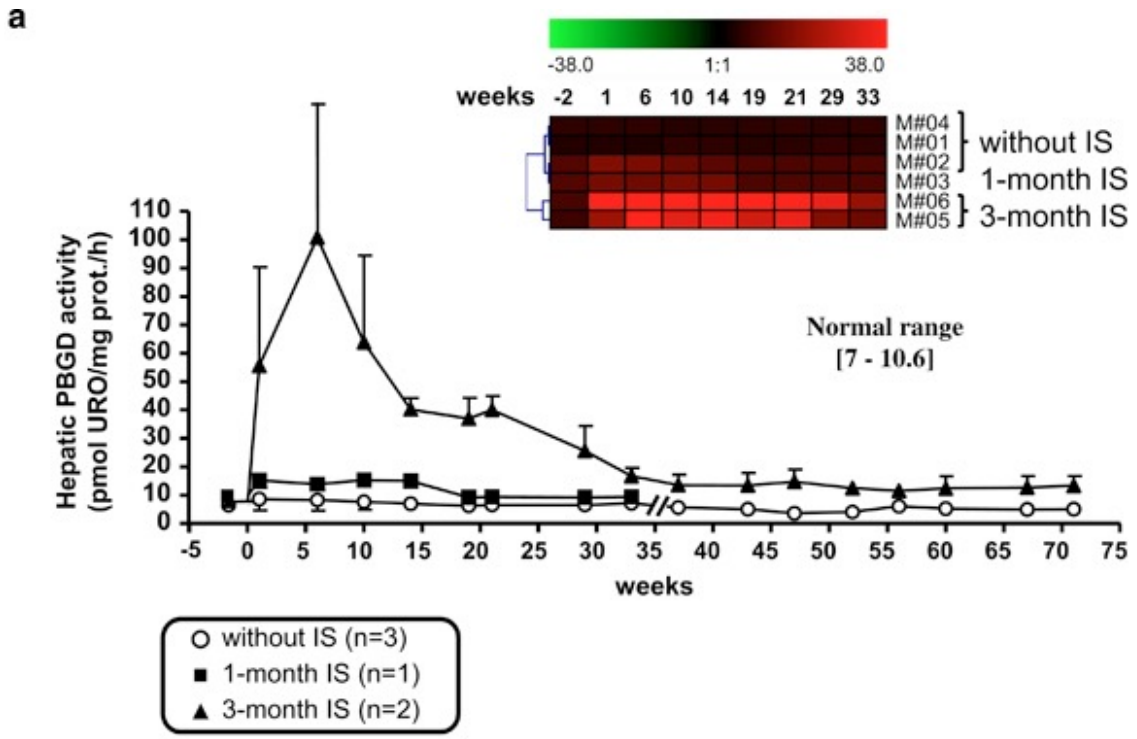
Shiedner et al Nat Gen 1998

Ad gutless in baboons



Morral et al PNAS 99

Ad gutless in primates – porphyria disease



Helper-dependent adenovirus achieve more efficient and persistent liver transgene expression in non-human primates under immunosuppression

Gen Ther Unzu et al 2015

Adenovirus and vaccination

Attenuated adenovirus expressing
Gag, nef, pol immunogens.

Ongoing Trials: Phase II

Protocol Number	Status as of December 2007	Prime			
		Class	Producer	Product	Adjuvant
HVTN 502/Merck 023 (Step) (n=3000)	Closed to accrual	Nonreplicating adenoviral vectors (clade B Gag-Pol-Nef)	Merck	MRKAd5 trivalent	

Adenovirus and vaccination

Higher infection

anti-Ad5 antibody titer	HIV incidence rate (%) vaccine	placebo
<18	4.0	4.0
19 – 200	4.4	2.2
201 – 1000	6.1	3.0
>1000	4.4	1.2

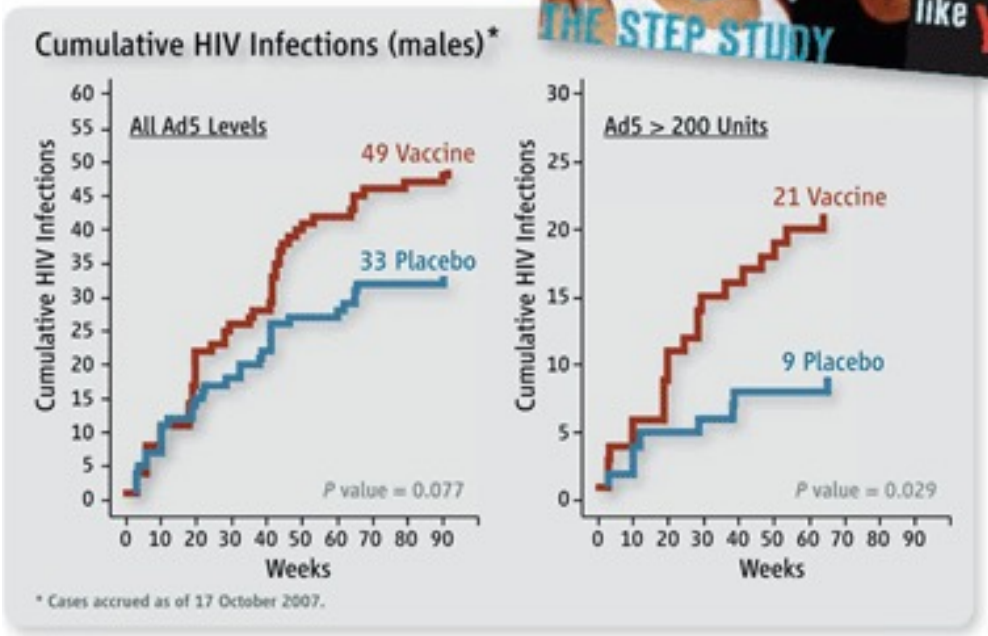
Table 3. HIV incidence rates during STEP trial. *This table shows the HIV incidence observed in vaccine and placebo recipients during the STEP trial, according to Ad5 antibody titer.*

	Ad5 antibody titer			
	<18	<18<Ad5≤200	200<Ad5≤1,000	Ad5>1,000
Vaccine	20/382	8/140	14/229	7/163
Placebo	20/394	4/142	7/229	2/157

Table 1. Number of HIV infections according to Ad5 antibody titer. *Number of HIV-infected individuals, out of the total number of vaccine and placebo recipients, according to increasing Ad5 antibody titer. This data, from the post-hoc analysis of the STEP trial, was presented at the HVTN meeting by Mike Robertson of Merck.*

Adenovirus and vaccination

science



Two prominent hypotheses have emerged to explain the observed trend of increased HIV infections among some vaccinated Step participants: the first suggests that rAd5 activates memory Ad5-specific CD4 T cells in Ad5-seropositive individuals, expanding the potential targets for incoming HIV virions; the second suggests that preexisting nAb to Ad5 can form immune complexes with an rAd5 vaccine vector and promote infection of target CD4 T cells with HIV.

2010

Adenovirus and vaccination

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LIVE BBC News 24

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Last Updated: Friday, 21 September 2007, 21:52 GMT 22:52 UK

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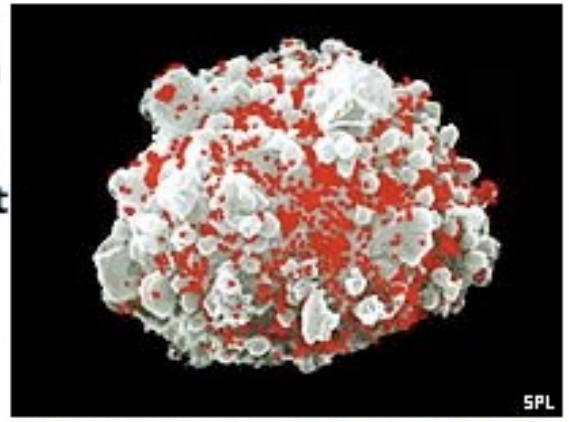
Merck abandons HIV vaccine trials

International drug company Merck has halted trials on an HIV vaccine that was regarded as one of the most promising in the fight against Aids.

Merck stopped testing the vaccine after it was judged to be ineffective.

In trials, the vaccine failed to



The vaccine was loaded with copies of three HIV genes

Simian Adenovirus

Science Translational Medicine Integrating Medicine and Science

2012

GENE THERAPY

Vaccine Vectors Derived from a Large Collection of Simian Adenoviruses Induce Potent Cellular Immunity Across Multiple Species

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EMERGENZA SANITARIA

Ebola, dall'Italia 10 mila dosi di vaccino per la sperimentazione in Usa

Falsa la notizia che l'Oms sarebbe intenzionata a chiedere una commessa di un milione di vaccini alla Okairos (che ha laboratori a Napoli) e all'Irbm di Pomezia

di Redazione Online Roma

ROMA — Le prime notizie su uno dei vaccini contro il virus Ebola si erano diffuse alla vigilia dell'estate e allora i riflettori si erano accesi su Okairos, con sede in Svizzera e laboratori a Napoli (presso Ceinge) e a Pomezia (in joint venture con l'Irbm Science Park). Circa 10mila dosi del prodotto saranno

Simian Adenovirus

Table 1.

Simian adenoviral vectors used in clinical trials.

Vector (species isolated from)	Classification (group)	Trial (phase)	Pathogen/disease	Ref.
PanAd3 (*Pan paniscus*)	C	I	RSV	[53,54]
ChAd3 (*Pan troglodytes*)	C	I, II	Ebola, HCV	[25,55–58]
ChAd63 (*Pan troglodytes*)	E	I, II	Malaria, HIV	[43,52]
ChAdOx1 (modified from *Pan troglodytes* Y25)	E	I	Influenza A, prostate cancer, tuberculosis	[53,59–60]

Morris et al, Future Virology, 2016

Adenovectors for COVID-19 vaccination

Viral Vector Vaccines	Another viral vector containing genes coding SARS-CoV-2 spike antigens	• Safe and effective • Suitable for multi-valent vaccine development • Can induce cellular immunity	• Effectiveness might be compromised by pre-existing immunity to adenoviruses	Ad26.COV2.S- Janssen (Johnson & Johnson)
				AZD1222- Oxford/AstraZeneca
				Covishield- Serum Institute of India
				Ad5-nCoV- CanSino
				Sputnik V, Sputnik Light- Gamaleya