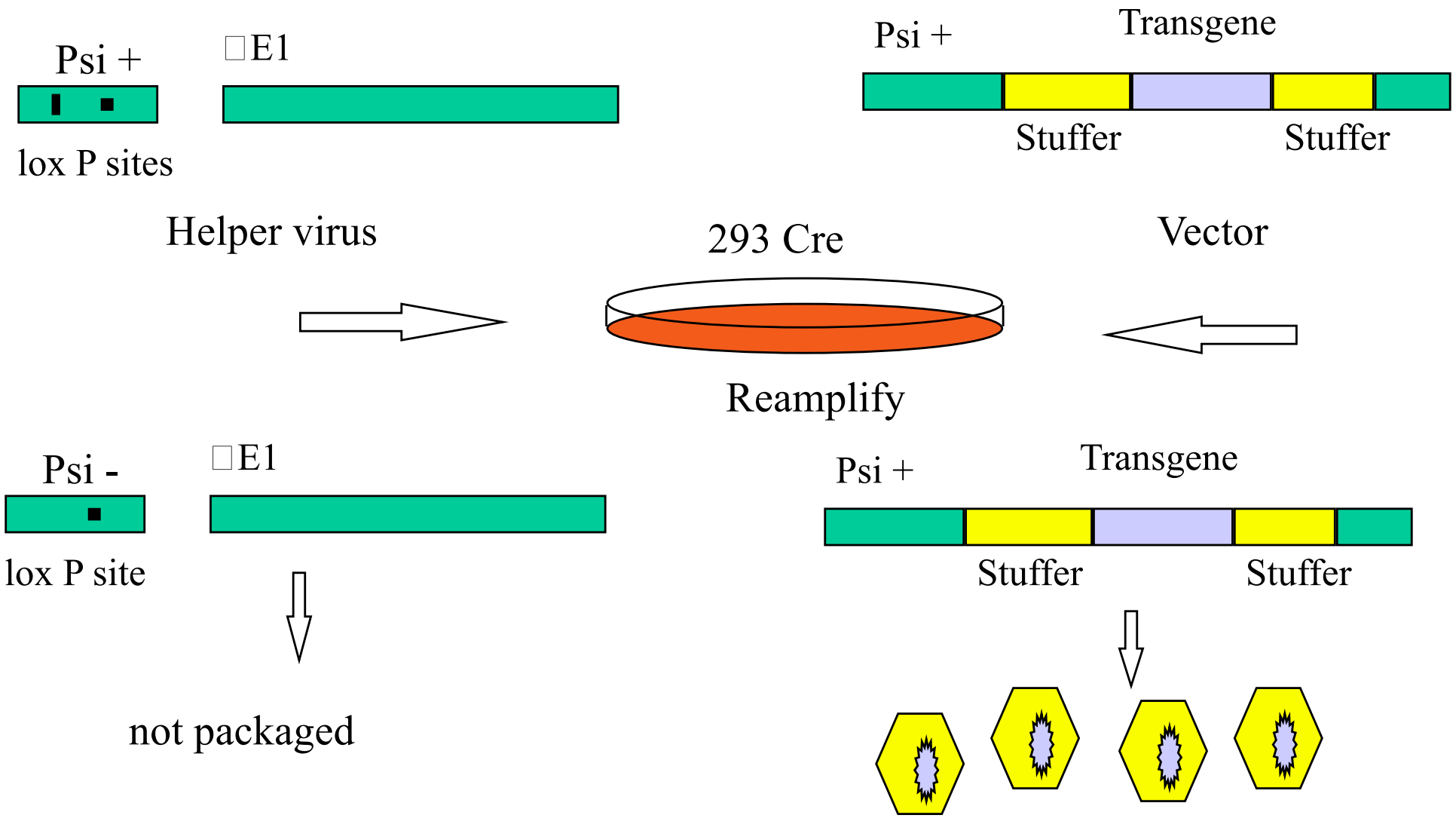
ascoltai a fondo le lezioni. Mi accorsi di com'erano importanti le cose che imparavo. Era bello che un uomo le metteva davanti a un'assemblea di giovani seduti, che avevano uno slancio nell'ascolto, nell'afferrare al volo. Bella un'aula in cui stare per conoscere. Bello l'ossigeno che si legava al sangue e che portava in fondo al corpo il sangue e le parole. Belli i nomi delle lune intorno a Giove, bello il grido di "Mare, mare" dei greci alla fine della ritirata, bello il gesto di Senofonte di scriverlo per non farlo smettere. Bello pure il racconto di Plinio sul Vesuvio esploso. Le loro scritture assorbivano le tragedie, le trasformavano in materia narrativa per trasmetterle e così superarle. Entrava luce in testa come ne entrava in aula. Fuori era un giorno lucente, uno di maggio finito nel mazzo di dicembre.

Erri de Luca Il giorno prima della felicita' Feltrinelli 2009

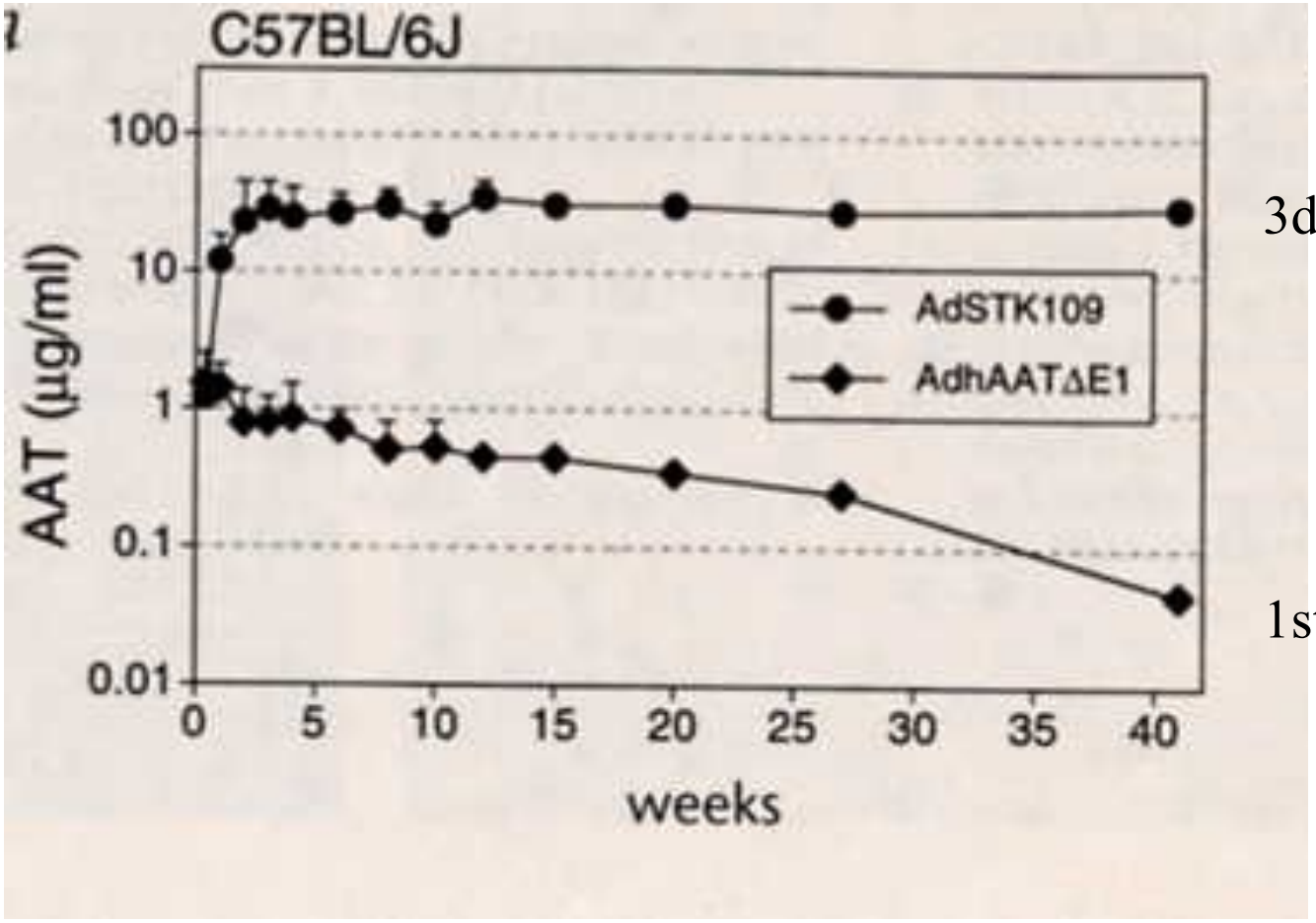
Problems and ameliorations of Ad vectors

- 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

3rd generation Ad- vectors

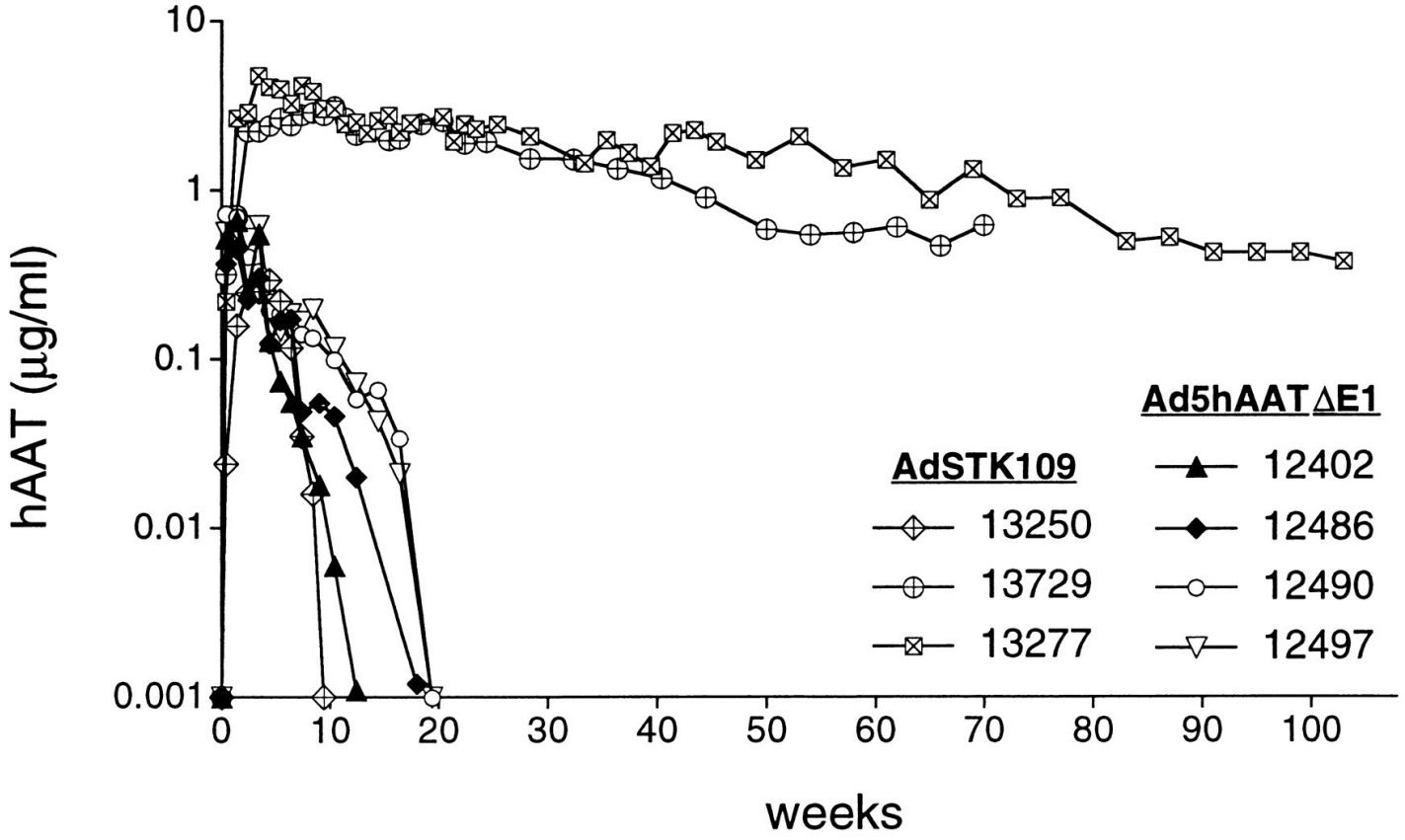


Ad gutless in mice



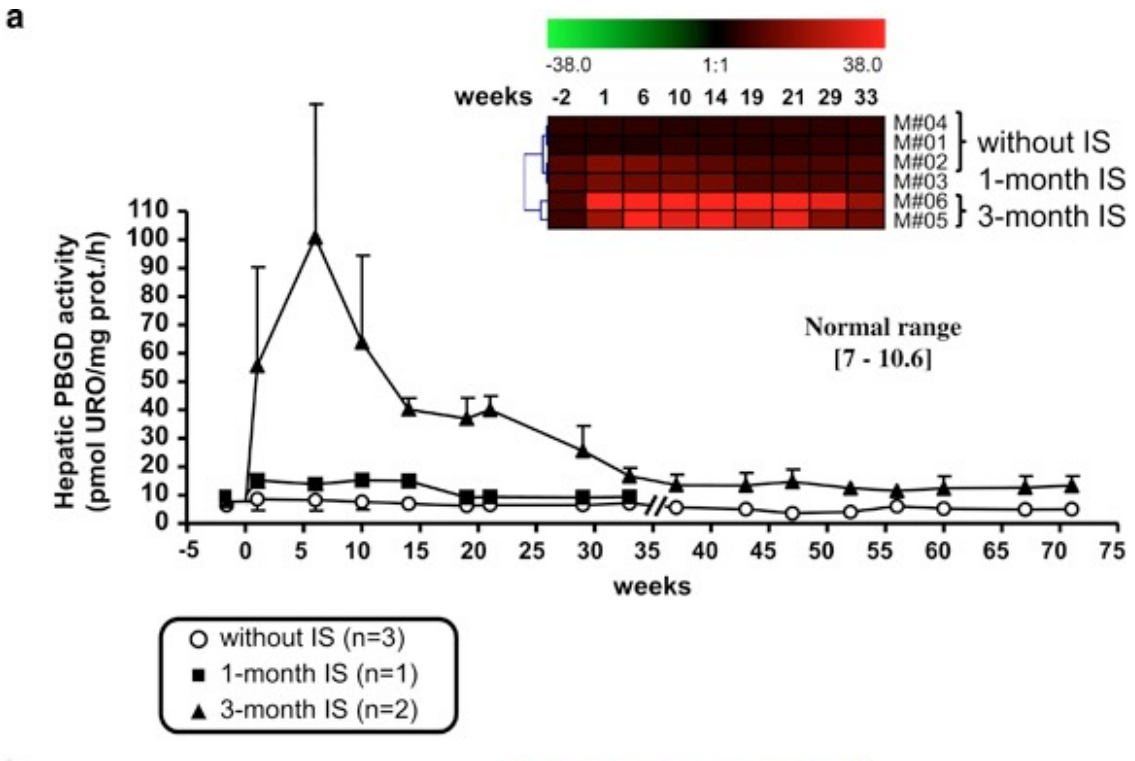
Shiedner et al Nat Gen 1998

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

PBGD deficiency
Intrahepatic administration of 5 × 10(12) viral particles kg(-1)
immunosuppressive regimen (tacrolimus, mycophenolate, rituximab and steroids

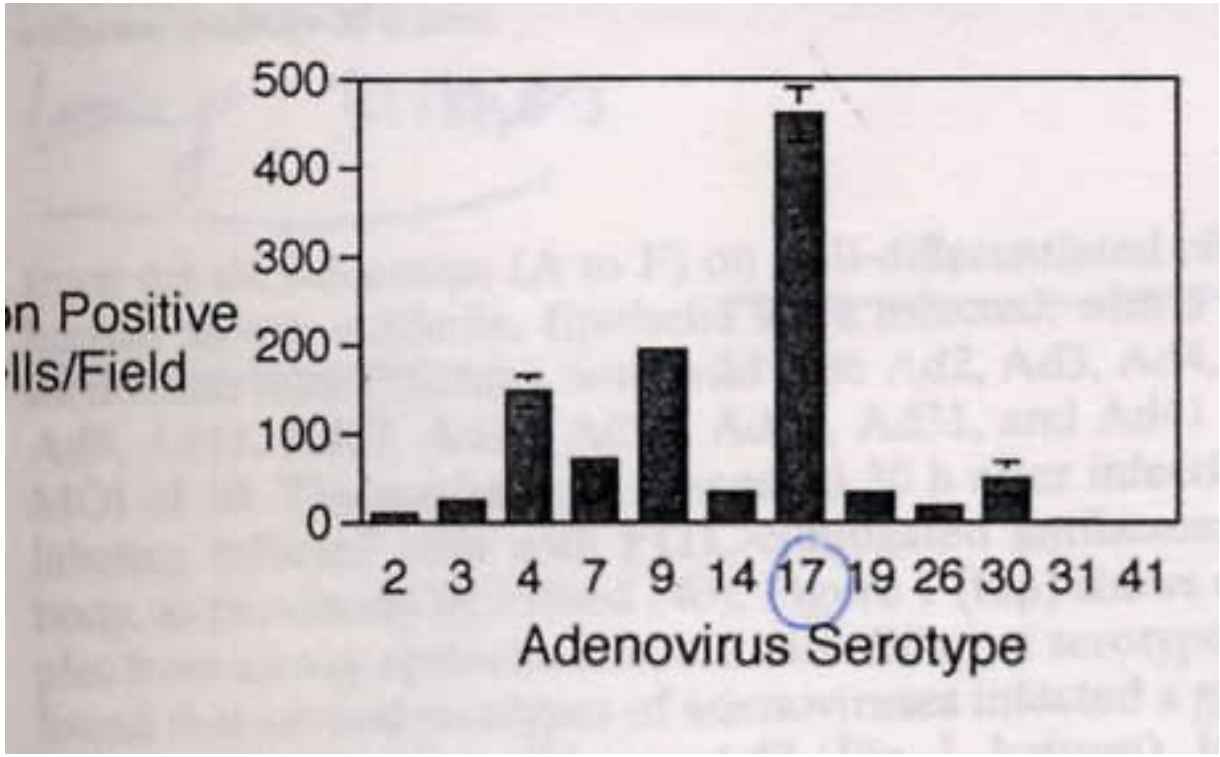
Gen Ther Unzu et al 2015

Ad: other improvement attempts

- Better transduction of specific tissues => Ad-heparan binding, Ad17- Ad2 chimerae
- Better transduction => Ad+liposomes

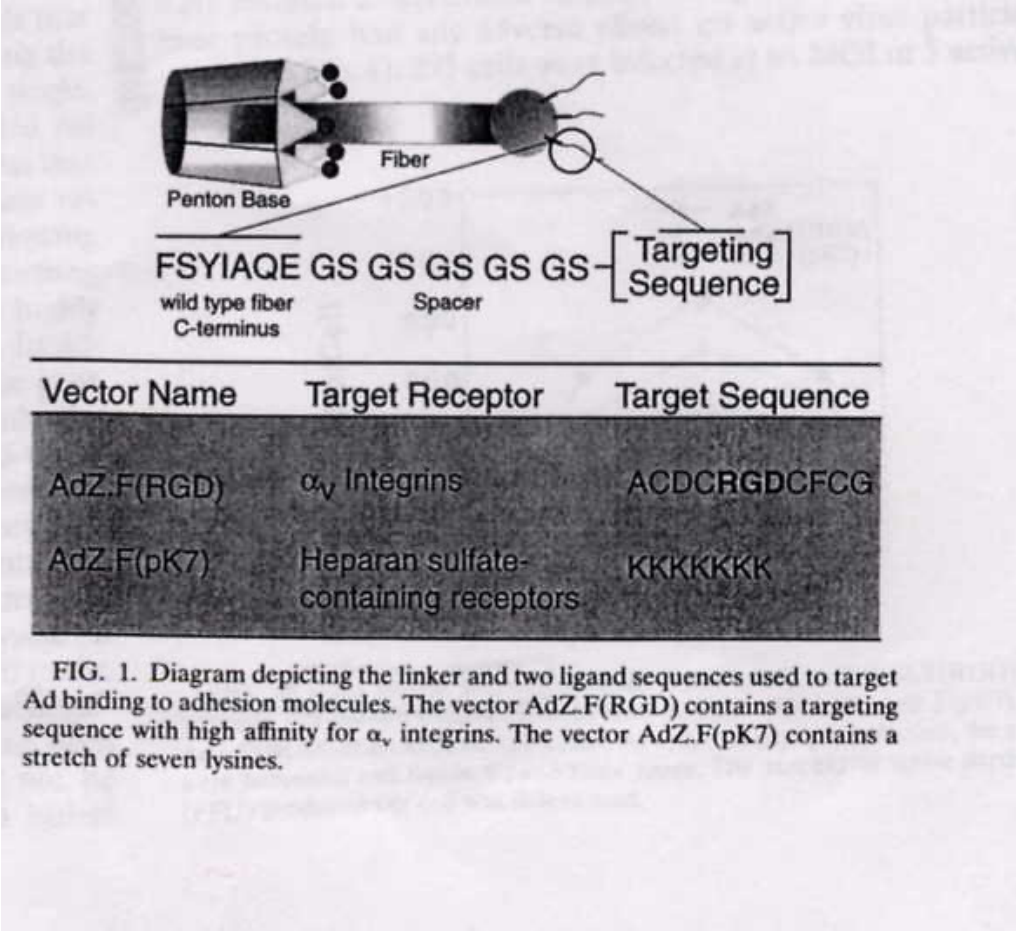
Ad2-Ad17 (fiber) chimerae

Airway epithelia



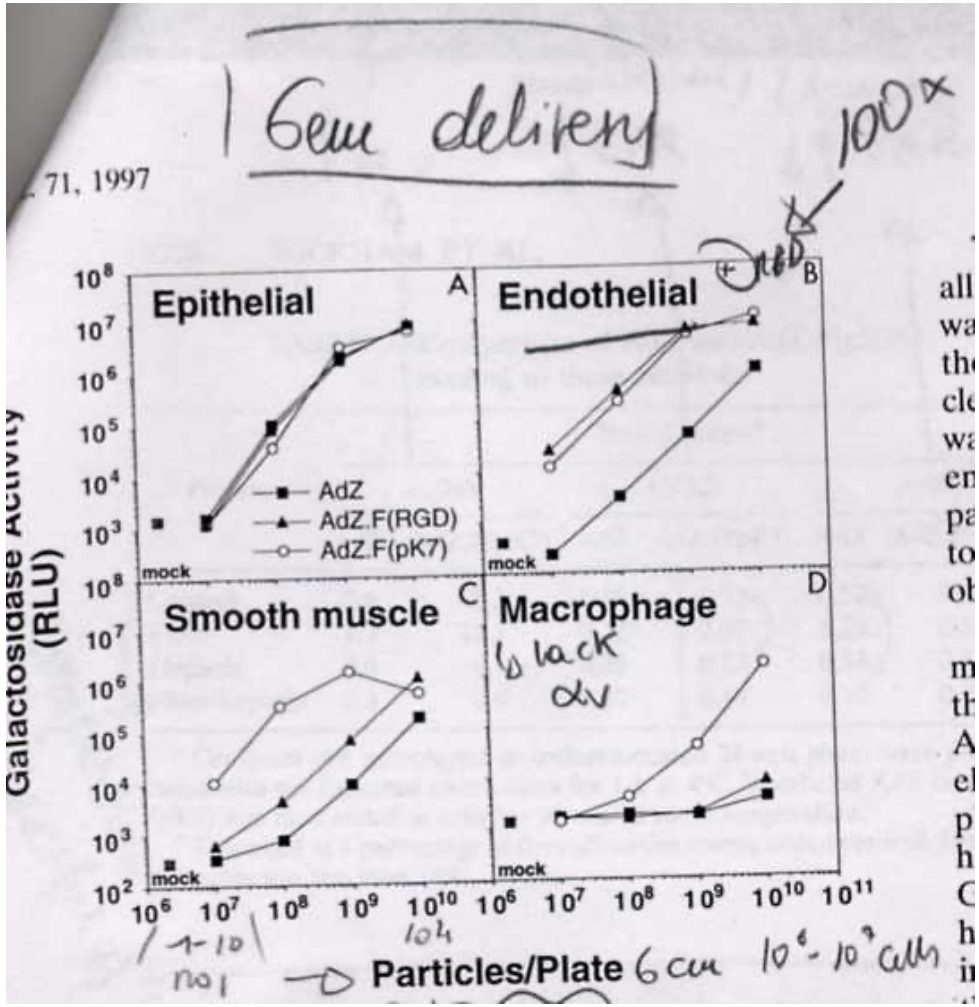
J Virology 99, Zabner

Ad binding heparan sulfate or integrins



Vector Name	Target Receptor	Target Sequence
AdZ.F(RGD)	α_v Integrins	ACDCRGDCFCG
AdZ.F(pK7)	Heparan sulfate-containing receptors	KKKKKKK

FIG. 1. Diagram depicting the linker and two ligand sequences used to target Ad binding to adhesion molecules. The vector AdZ.F(RGD) contains a targeting sequence with high affinity for α_v integrins. The vector AdZ.F(pK7) contains a stretch of seven lysines.



J Virol 97, Wickham

Ad+liposomes

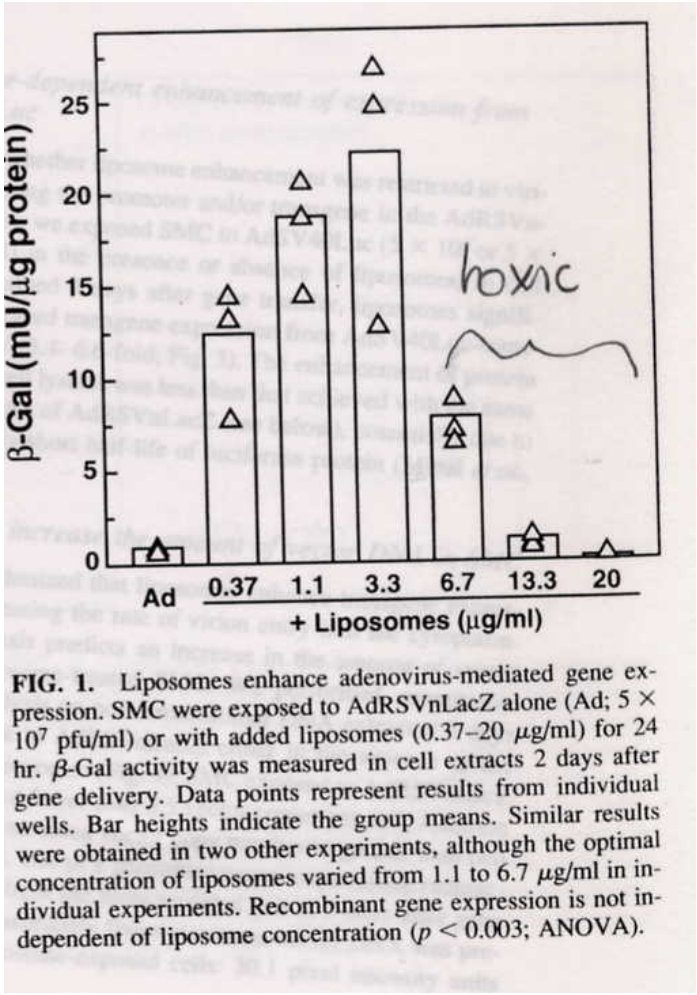


FIG. 1. Liposomes enhance adenovirus-mediated gene expression. SMC were exposed to AdRSVnLacZ alone (Ad; 5×10^7 pfu/ml) or with added liposomes (0.37–20 μg/ml) for 24 hr. β-Gal activity was measured in cell extracts 2 days after gene delivery. Data points represent results from individual wells. Bar heights indicate the group means. Similar results were obtained in two other experiments, although the optimal concentration of liposomes varied from 1.1 to 6.7 μg/ml in individual experiments. Recombinant gene expression is not independent of liposome concentration ($p < 0.003$; ANOVA).

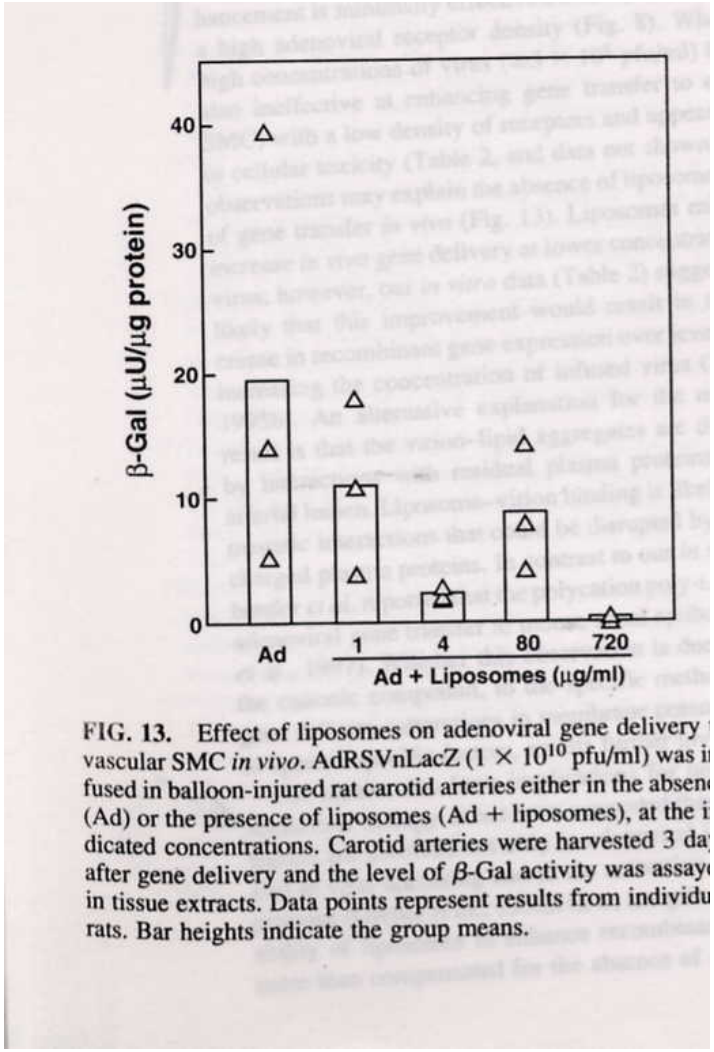


FIG. 13. Effect of liposomes on adenoviral gene delivery vascular SMC *in vivo*. AdRSVnLacZ (1×10^{10} pfu/ml) was i fused in balloon-injured rat carotid arteries either in the absen (Ad) or the presence of liposomes (Ad + liposomes), at the i dicated concentrations. Carotid arteries were harvested 3 da after gene delivery and the level of β-Gal activity was assay in tissue extracts. Data points represent results from individu rats. Bar heights indicate the group means.

HGT 98, Qiu

Clinical trials

https://clinicaltrials.gov/ct2/results?term=adenoviral+vectors&pg=1

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

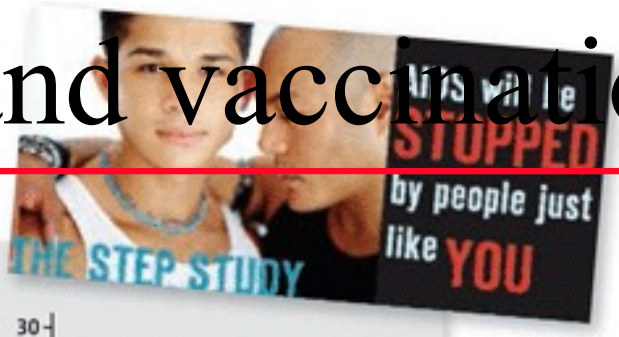
Table 3. HIV incidence rates during STEP trial. *This table shows the HIV incidence observed in vaccine and placebo recipients during the STEP trial, according to Ad5 antibody titer.*

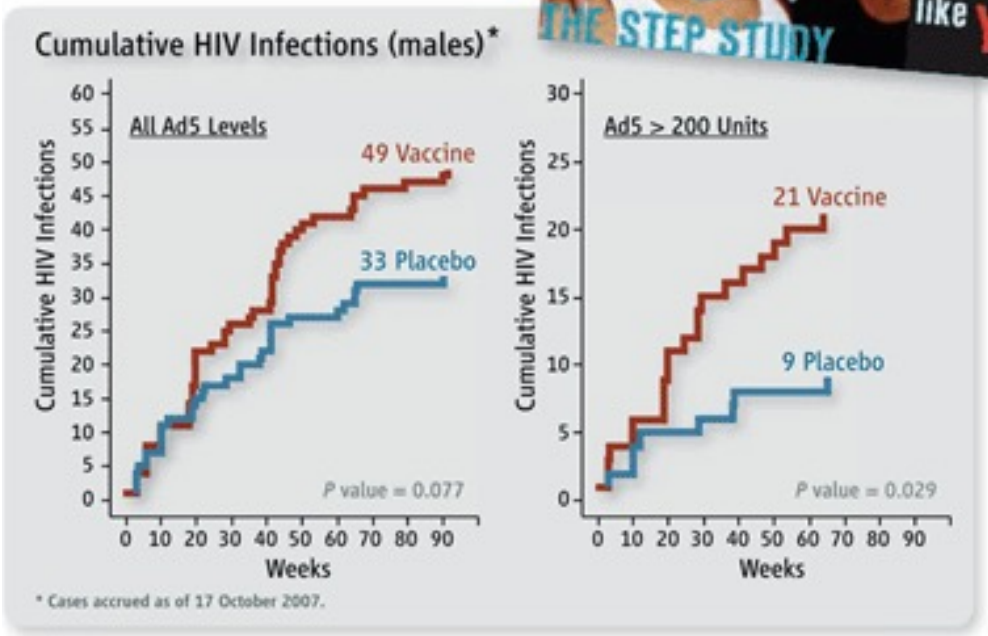
	Ad5 antibody titer			
	<18	<18<Ad5≤200	200<Ad5≤1,000	Ad5>1,000
Vaccine	20/382	8/140	14/229	7/163
Placebo	20/394	4/142	7/229	2/157

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

BBC NEWS

LIVE BBC News 24

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

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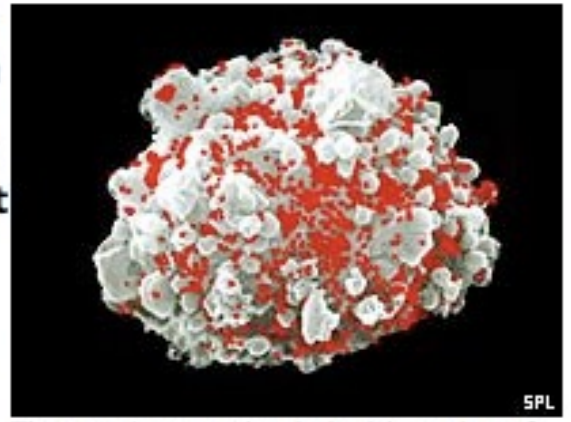
Merck abandons HIV vaccine trials

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

Stefano Colloca[1,*], Eleanor Barnes[2,3,*], Antonella Folgori[1], Virginia Ammendola[1], Stefania Capone[1], Agostino Cirillo[4,†], Loredana Siani[1], Mariarosaria Naddeo[1], Fabiana Grazioli[1], Maria Luisa Esposito[1], Maria Ambrosio[1], Angela Sparacino[1], Marta Bartiromo[1], Annalisa Meola[4], Kira Smith[2], Ayako Kurioka[2], Geraldine A. O'Hara[5], Katie J. Ewer[5], Nicholas Anagnostou[5], Carly Bliss[5], Adrian V. S. Hill[5], Cinzia Traboni[1], Paul Klenerman[2], Riccardo Cortese[1,6] and Alfredo Nicosia[1,6,‡]

EMERGENZA SANITARIA

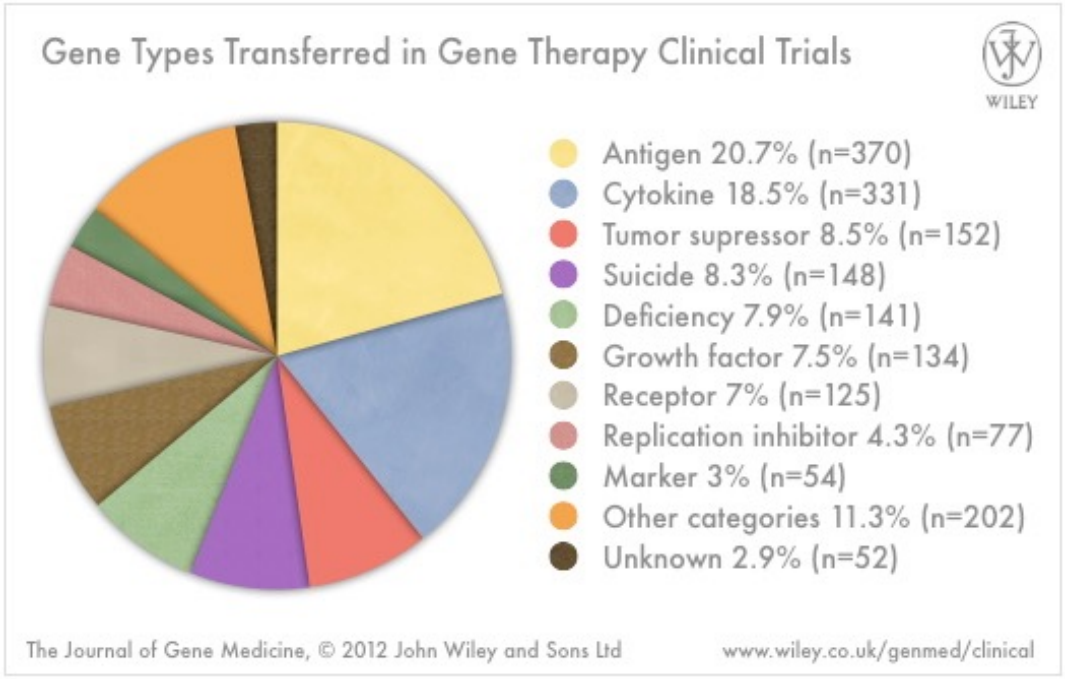
Ebola, dall'Italia 10 mila dosi di vaccino per la sperimentazione in Usa

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

Cancer gene therapy



J Gene Med 2013

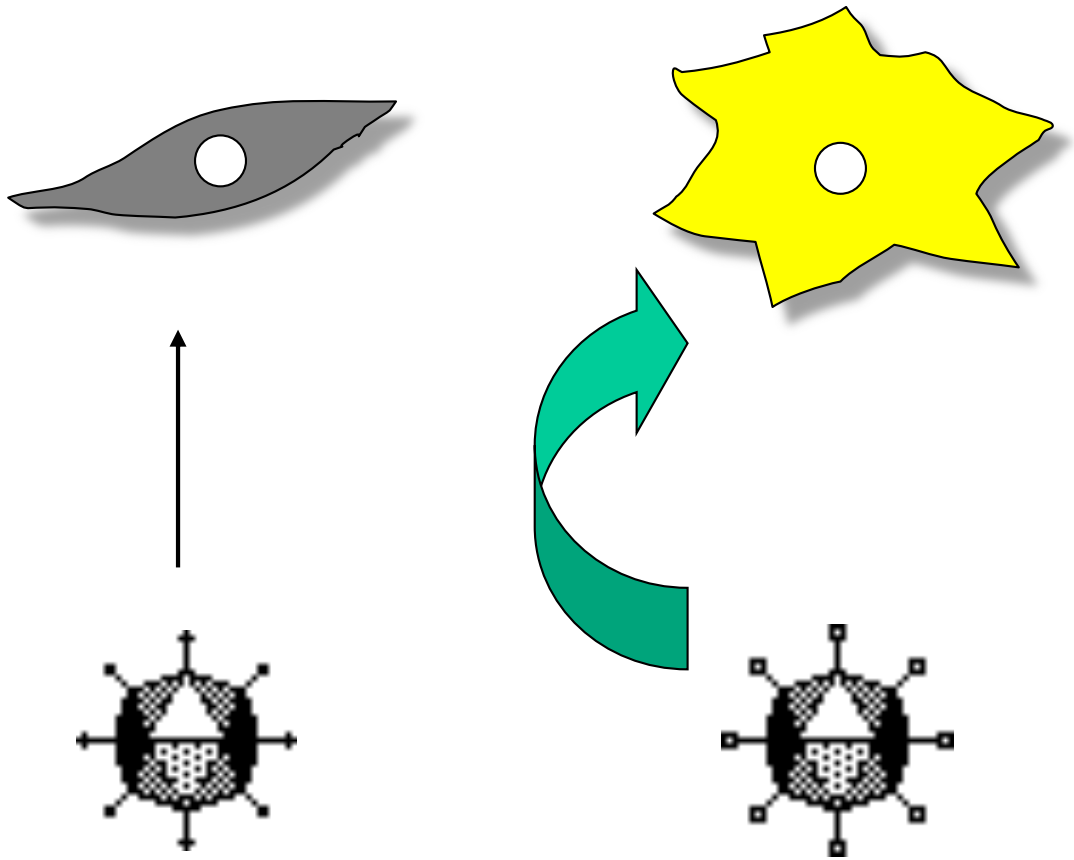
Gene type	Gene Therapy Clinical Trials	
	Number	%
Adhesion molecule	10	0.5
Antigen	417	21.2
Antisense	15	0.8
Cell cycle	8	0.4
Cell protection/Drug resistance	20	1
Cytokine	349	17.7
Deficiency	156	7.9
Growth factor	143	7.3
Hormone	9	0.5
Marker	54	2.7
Oncogene regulator	12	0.6
Oncolytic virus	52	2.6
Porins, ion channels, transporters	16	0.8
Receptor	149	7.6
Replication inhibitor	87	4.4
Ribozyme	6	0.3
siRNA	12	0.6
Suicide	156	7.9
Transcription factor	32	1.6
Tumor suppressor	158	8
Others	58	2.9
Unknown	51	2.6
Total	1970	

Tumor suppressor for cancer gene therapy

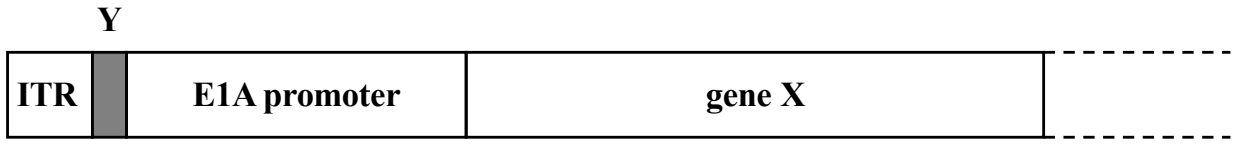
p53

Rb

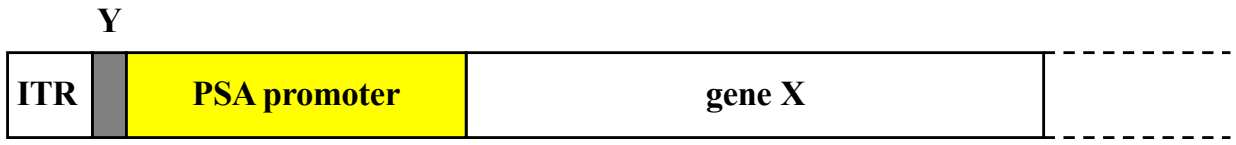
Receptor mediated targeting



Promoter mediated targeting



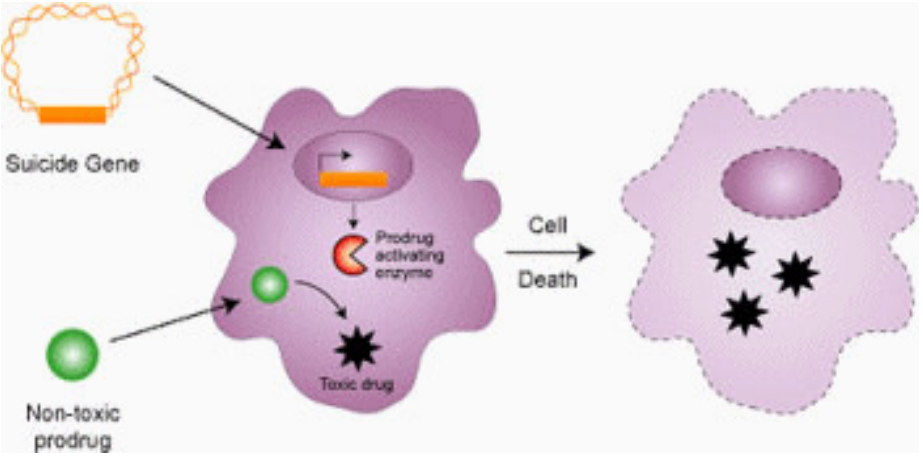
Ampia espressione

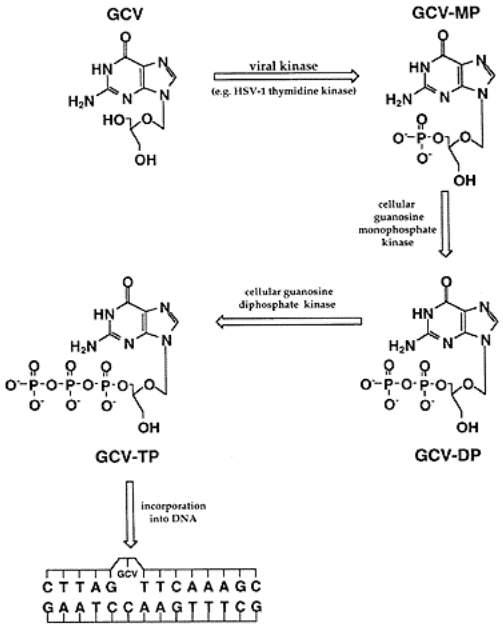


Suicide genes and prodrugs

Table 3 | **Enzyme-prodrug combinations for suicide gene therapy***

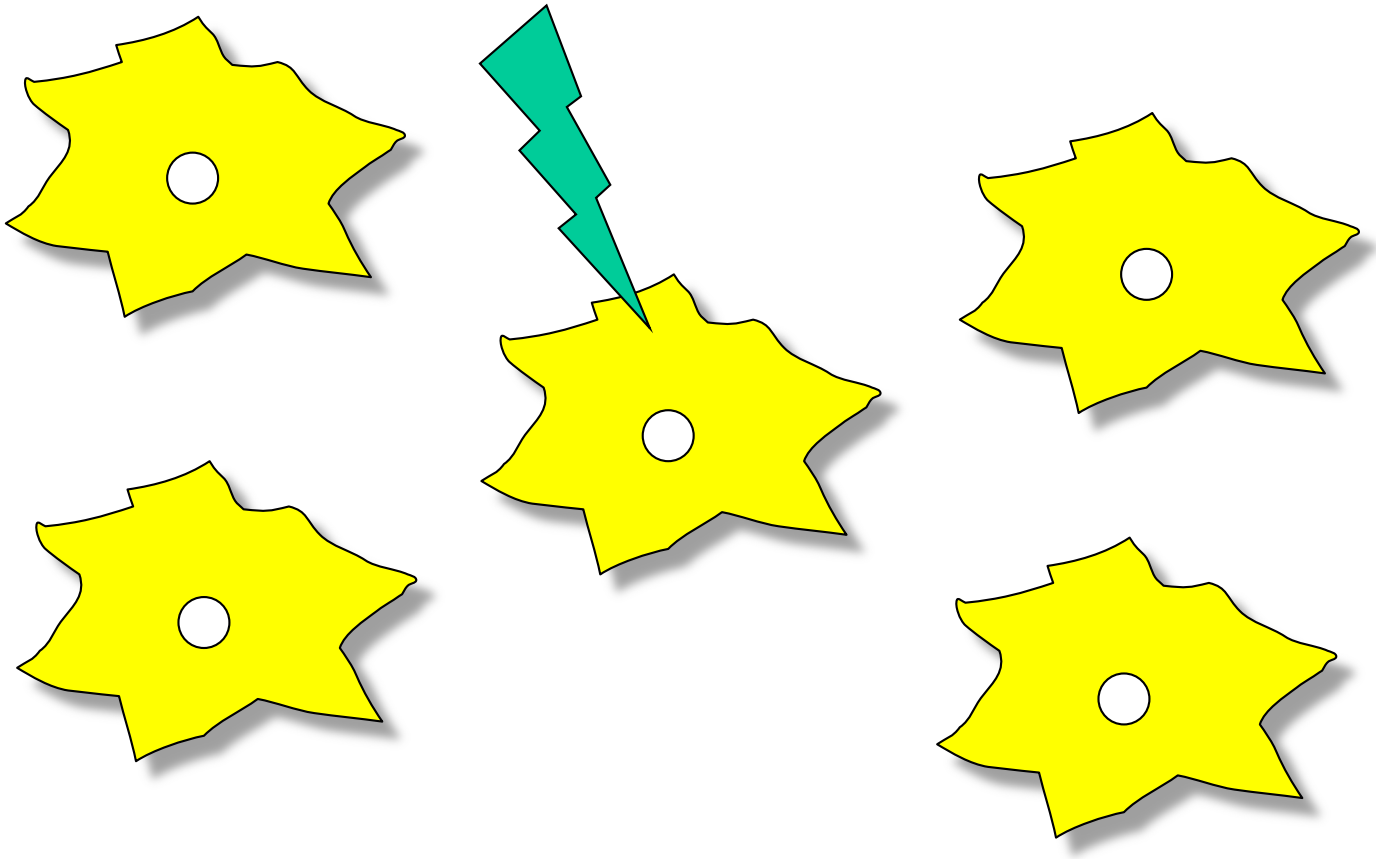
Enzyme	Prodrug	Product	Mechanism
HSV-tk	Ganciclovir	Gancicolvir triphosphate	Blocks DNA synthesis
Cytosine deaminase	5-Fluorocytosine	5-Fluorouracil (5-FU)	Pyrimidine antagonist: blocks DNA and RNA synthesis
Nitroreductase	Nitrobenzyloxycarbonyl anthracyclines	Anthracyclines	DNA crosslinking
Carboxylesterase	CPT-11	SN38	Topoisomerase inhibitor
Cytochrome P450	Cyclophosphamide	Phosphoramide mustard	DNA alkylating agent: blocks DNA synthesis
Purine nucleoside phosphorylase	6-Mercaptopurine-DR	6-Mercaptopurine	Purine antagonist: blocks DNA synthesis



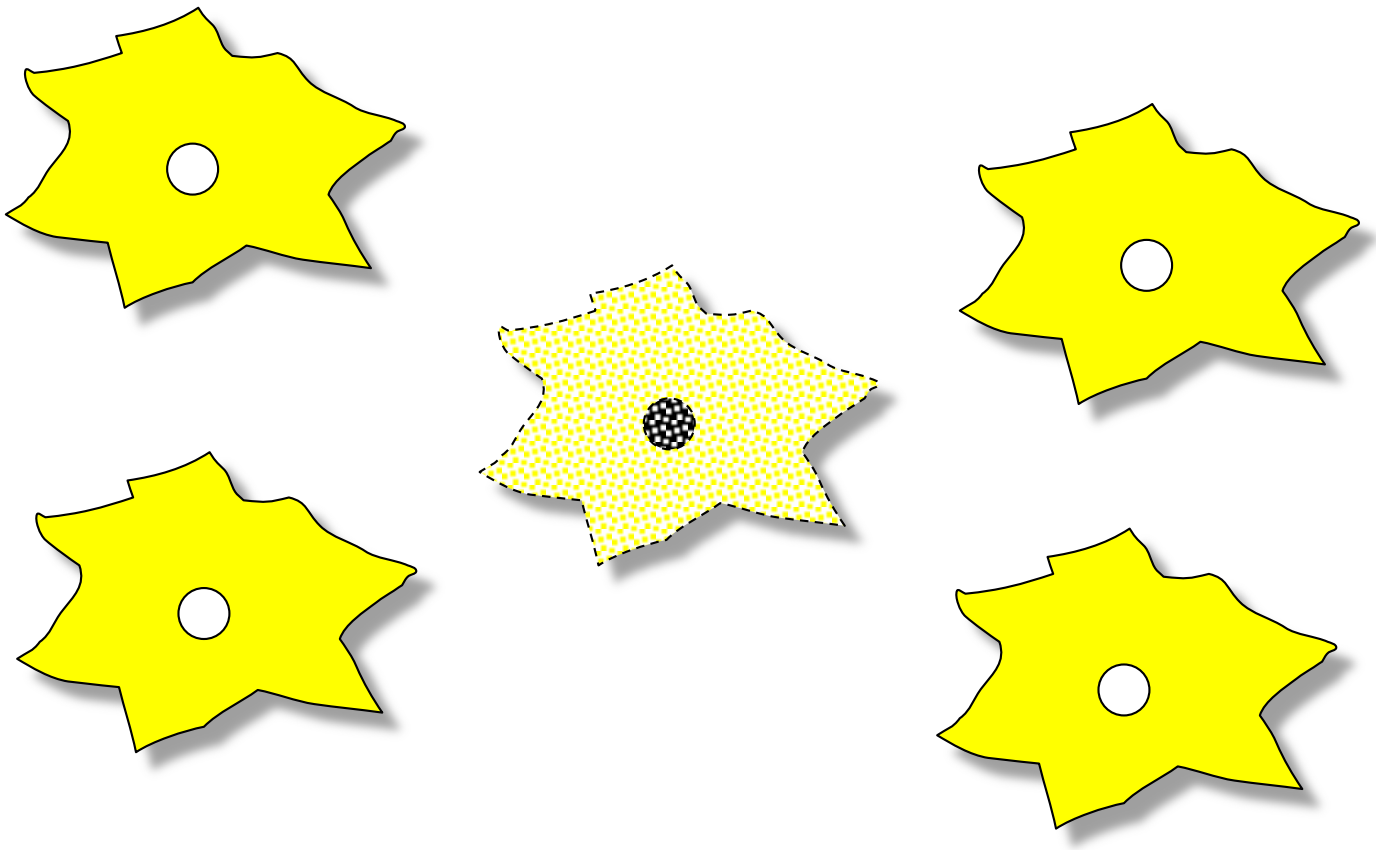


F. McCormick - Nature Reviews on Cancer 1:130, 2001

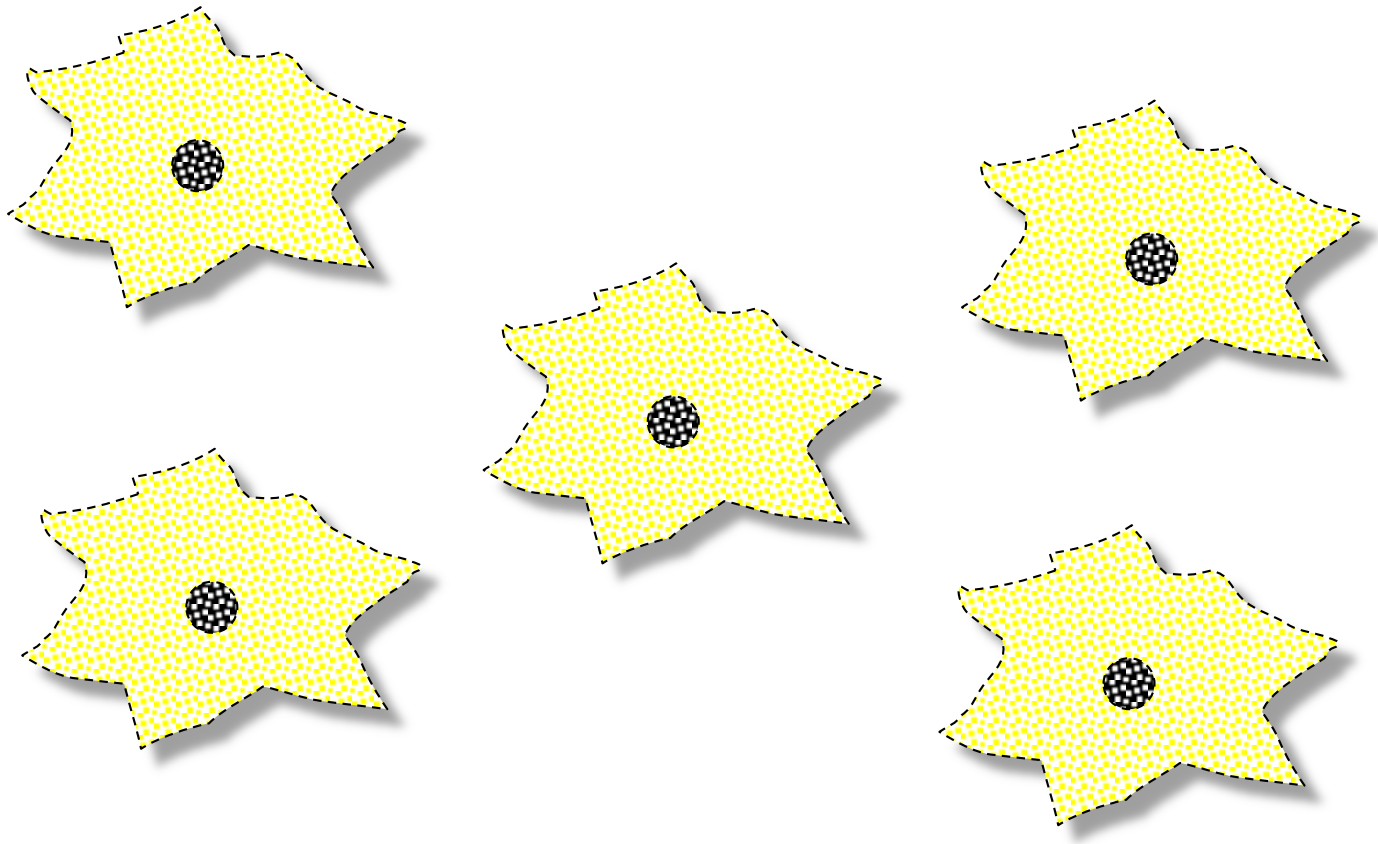
Bystander effect



Bystander effect

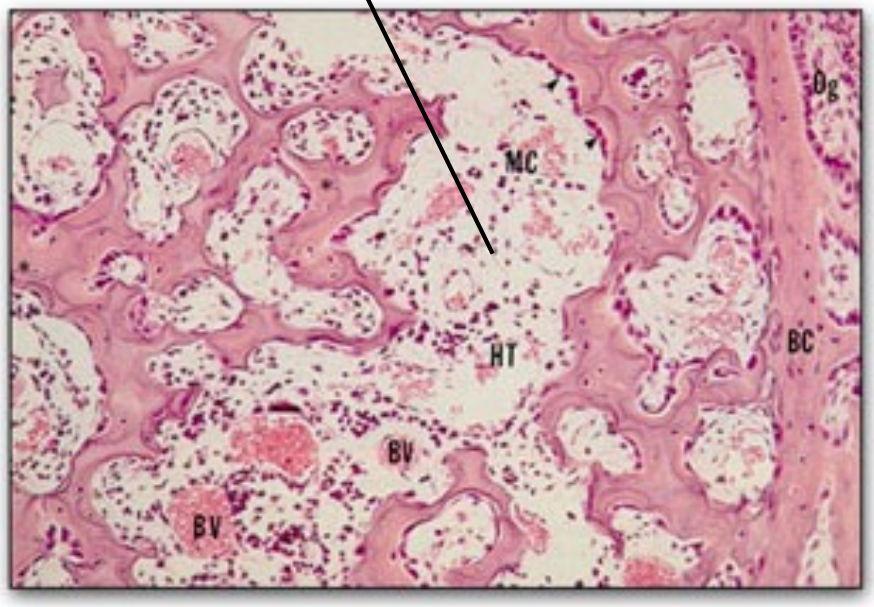


Bystander effect

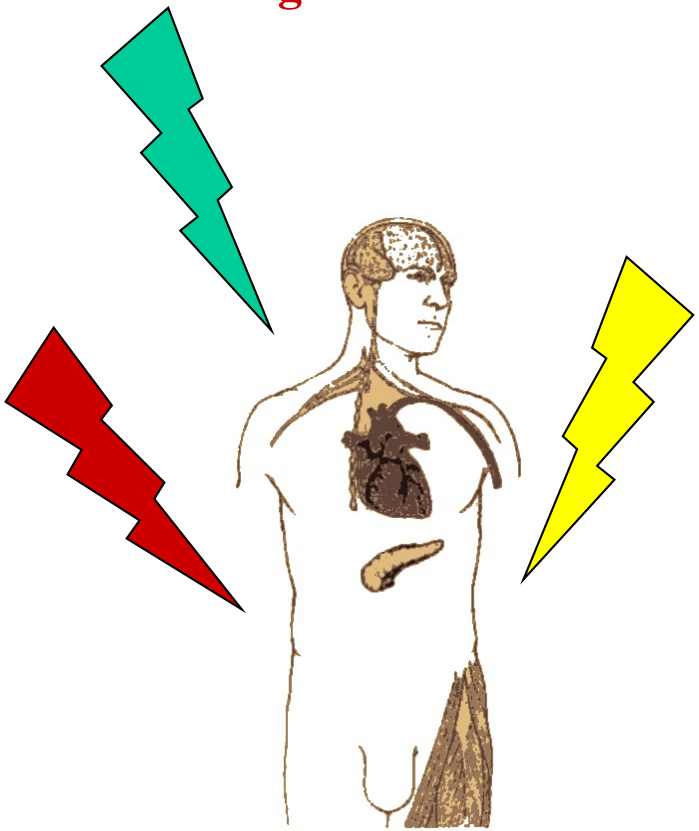


Bone marrow protection for chemotherapy

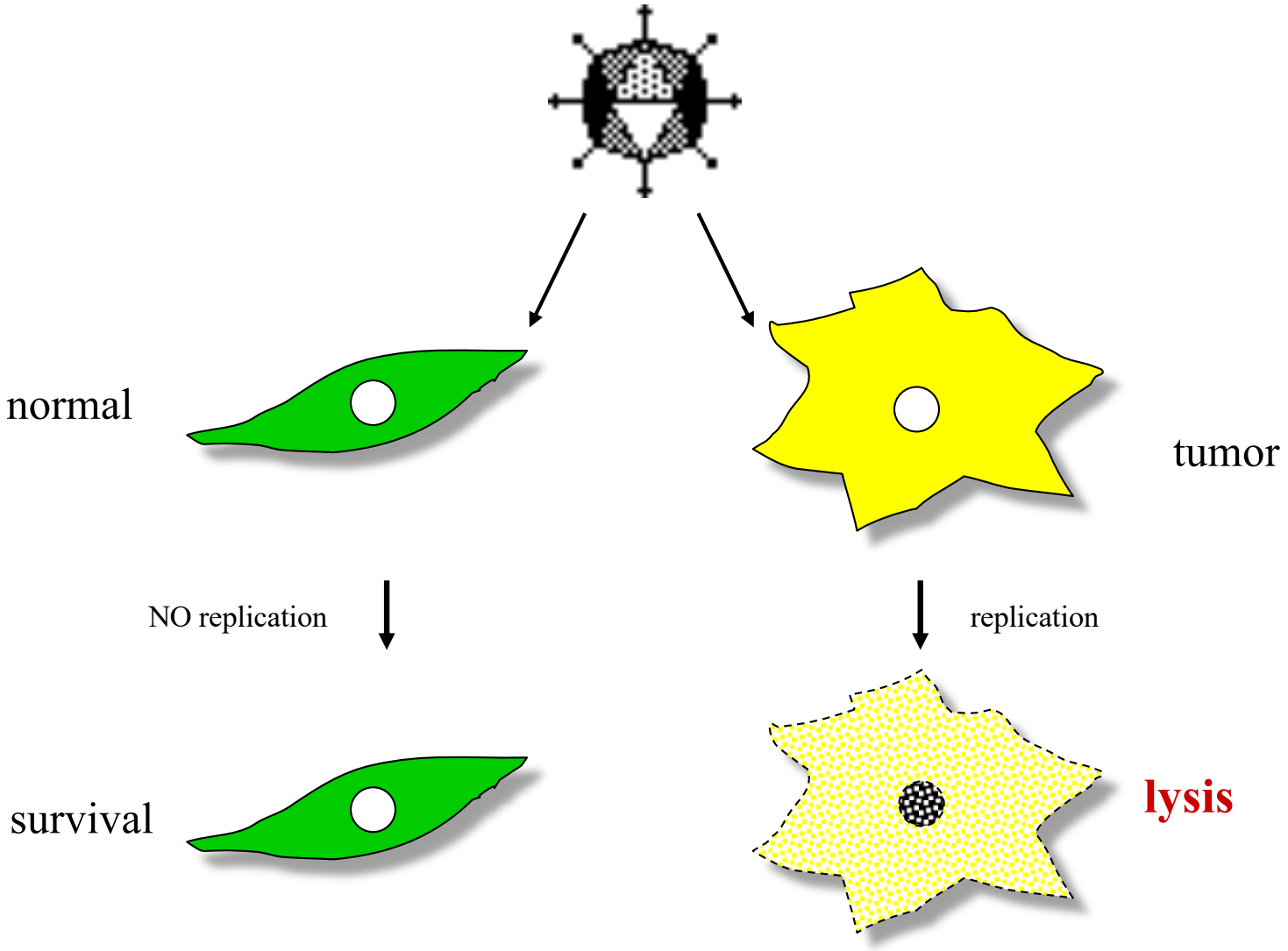
Multiple Drug Resistance



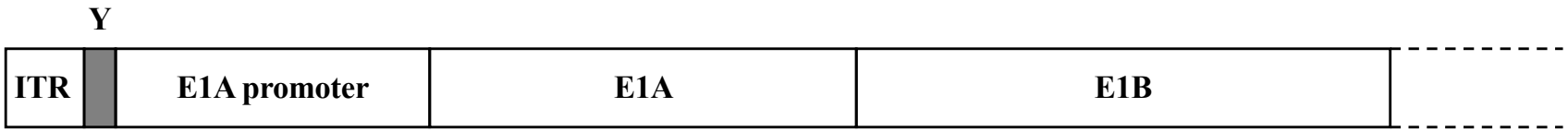
High dose chemotherapy



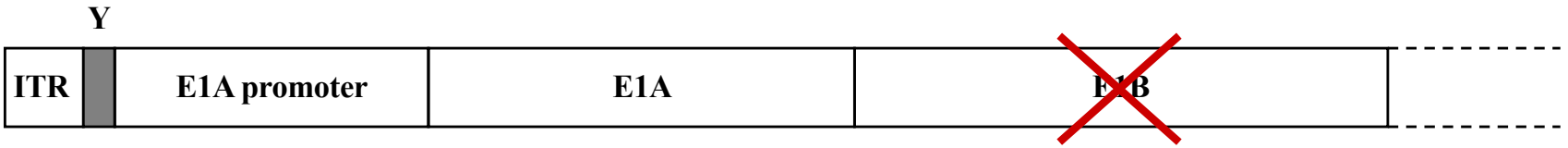
Oncolytic viruses



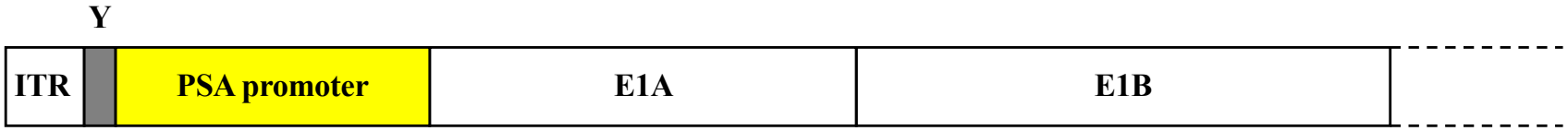
Tumor selectivity



Wt virus



E1 deleted virus tumor targetes

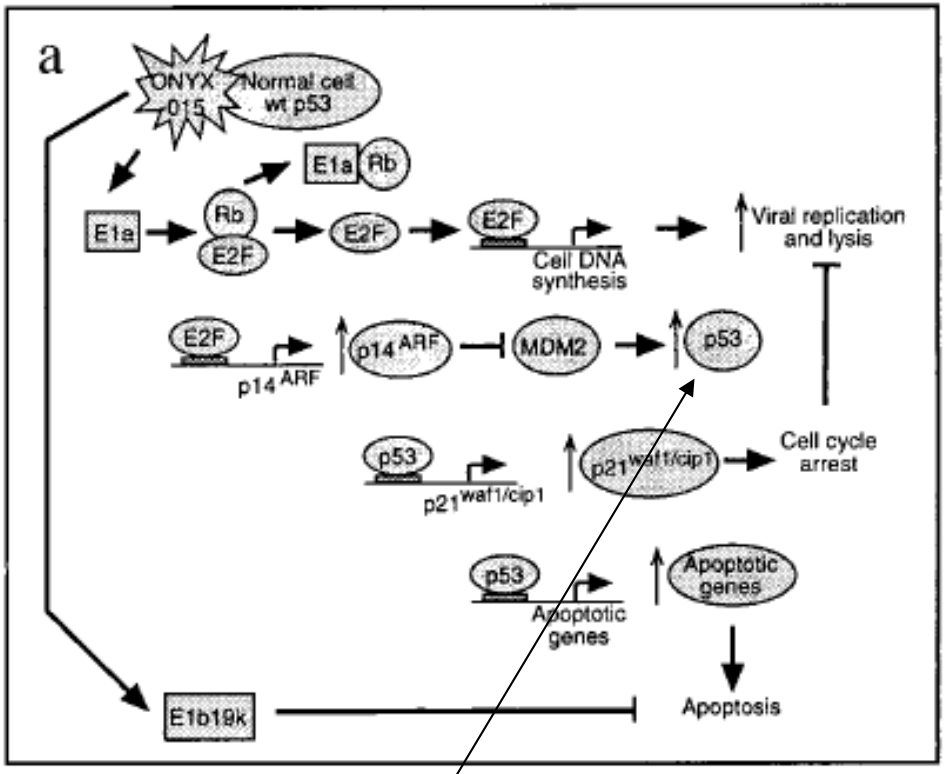


Prostate specific promoter, tumor targeted

ONYX-015

- A E1B-55K DELETED ADENOVIRUS, FOR THE TREATMENT OF TUMORS p53-
- CURRENTLY THE MOST USED ONCOLYTIC VIRUS
- PHASE III CLINICAL TRIALS ARE UNGOING

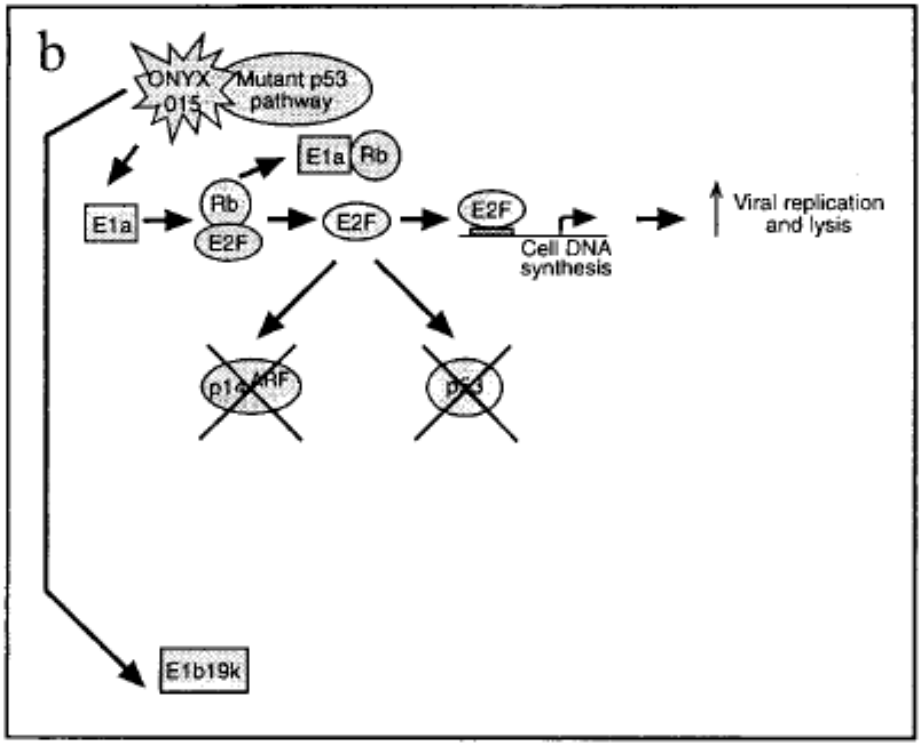
ONYX-015 ON NORMAL CELLS



E1B55K

- Infects normal cells that have functional p53 gene
- p53 gene increases the production of antiviral protein
- Virus does not replicate
- Normal cell is not killed

ONYX-015 ON CANCER CELLS



- Infects cancer cells that lack a functional p53 gene
- Uses cell's machinery to replicate and make more copies of itself
- Kills the cancer cells through lysis
- New viruses can infect more cancer cells

An Ad mutant that selectively replicates in p53-deficient human tumor cells

- E1B => vp55 protein that inactivates hp53 (whose function would block cell and DNA replication)
- Viral mutant => vp55 can replicate and kill p53ko tumors => good for selective therapy

Science 1996

Replicative p55- Ad for therapy of p53- tumors

Non selective lysis

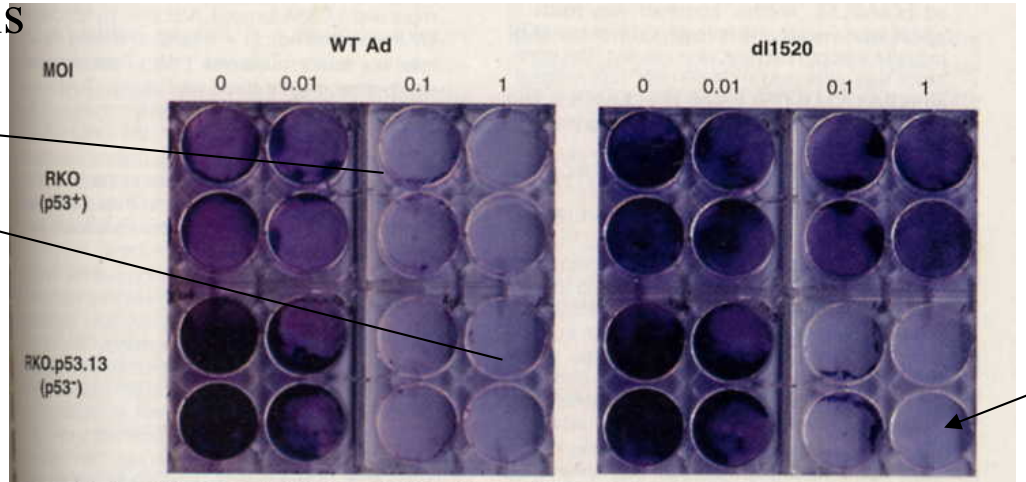


Fig. 4. Effects of dl1520 and WT Ad on RKO cells and RKO.p53.13 cells lacking functional p53. Cells were infected at the MOIs shown and stained for viability 8 days later.

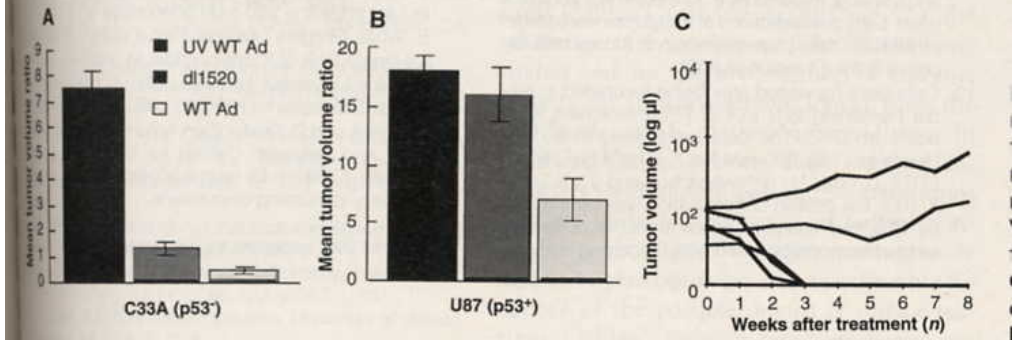


Fig. 5. Effects of UV-inactivated WT Ad, dl1520, or WT Ad on p53⁻ and p53⁺ human tumor xenograft growth. (**A**) C33A cells (p53⁻) or (**B**) U87 human glioblastoma multiforme cells (p53⁺) were injected subcutaneously into the flanks of *nu/nu* mice. Five weeks later, the tumor volume ratio was calculated [2]. (**C**) C33A cells were injected subcutaneously as above. Once tumors reached about 80 µl in volume, they were injected with 10^8 PFU of dl1520 (solid lines) or buffer (dashed lines) for 5 days consecutively. The daily dose was divided equally into each tumor quadrant (15 µl per quadrant).

Selective lysis

Science 96
McCormick

Evaluation in cell culture

- BISCHOFF et al. 1996:
  ONYX replicates in *p53*-, not in *p53*+;
  rescue if I add 55k

- HEISE et al, 1997:
  ONYX does not replicate in primary cells , 100-1000fold attenuated vs wt

Preclinical trials

Preclinical studies in mice xenograft models

- ONYX induces 50% regression of tumor mass at 6 months
- ONYX acts synergically with chemotherapy
  HEISE et al.1997

1996: PHASE I CLINICAL TESTING

Phase II clinical trial

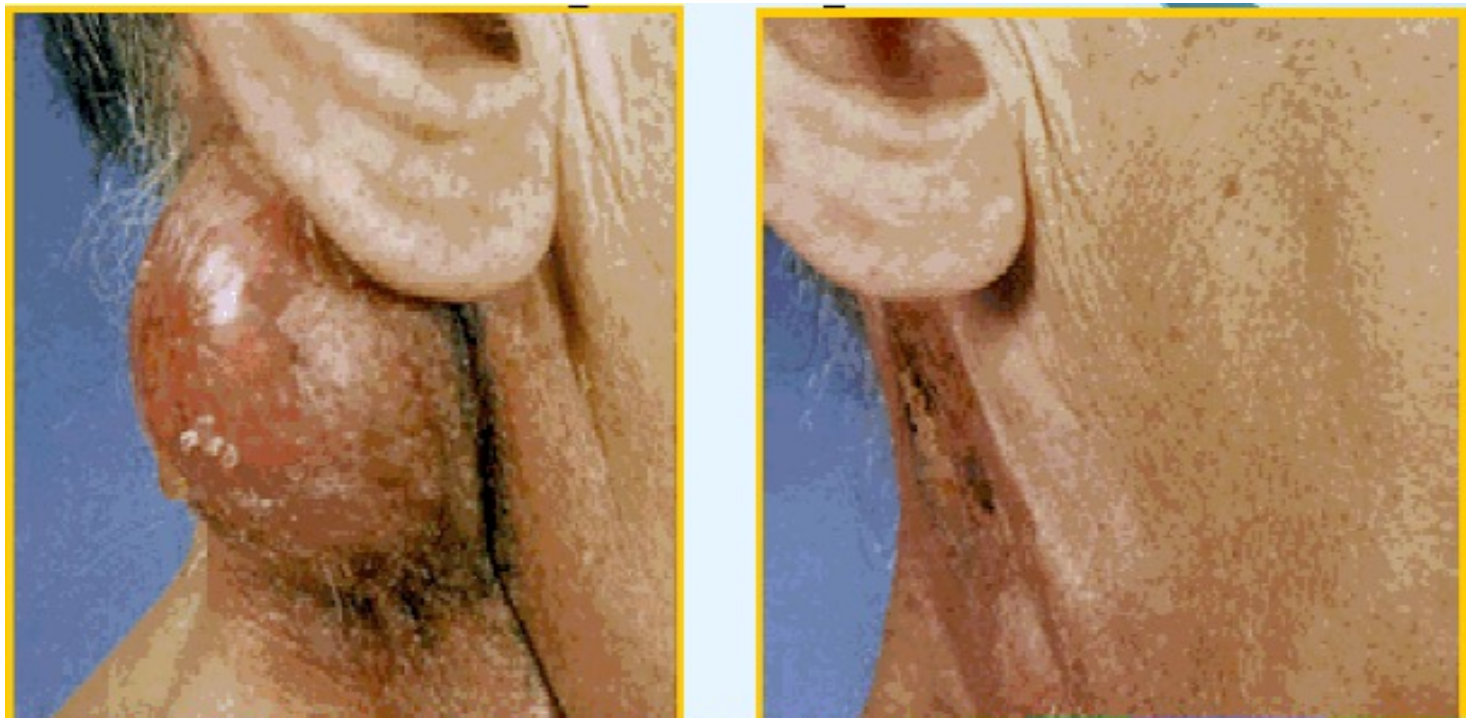
- **ONYX ALONE**:
- 50% TUMOR DESTRUCTION IN 14% PATIENTS ENROLLED
- SIGNIFICANT CORRELATION BETWEEN ANTITUMORAL ACTIVITY (COMPLETE, PARTIAL AND MINOR RESPONSES) AND PRESENCE OF A P53 MUTATION

NEMUNAITIS et al. 2000 e 2001

- **ONYX-015 ADMINISTERED IN COMBINATION WITH CISPLATIN AND 5-FLUOROURACIL**
- 19 (63%) OF THE 30 PATIENTS EXPERIENCED REGRESSION OF 50% OR MORE IN THEIR INJECTED TUMORS
- 8 (27%) OF THE PATIENTS EXPERIENCED A 100% REGRESSION IN THE SIZE OF THEIR INJECTED TUMORS
- SIX MONTHS AFTER THE END OF THE STUDY, NO TUMOR HAD PROGRESSED

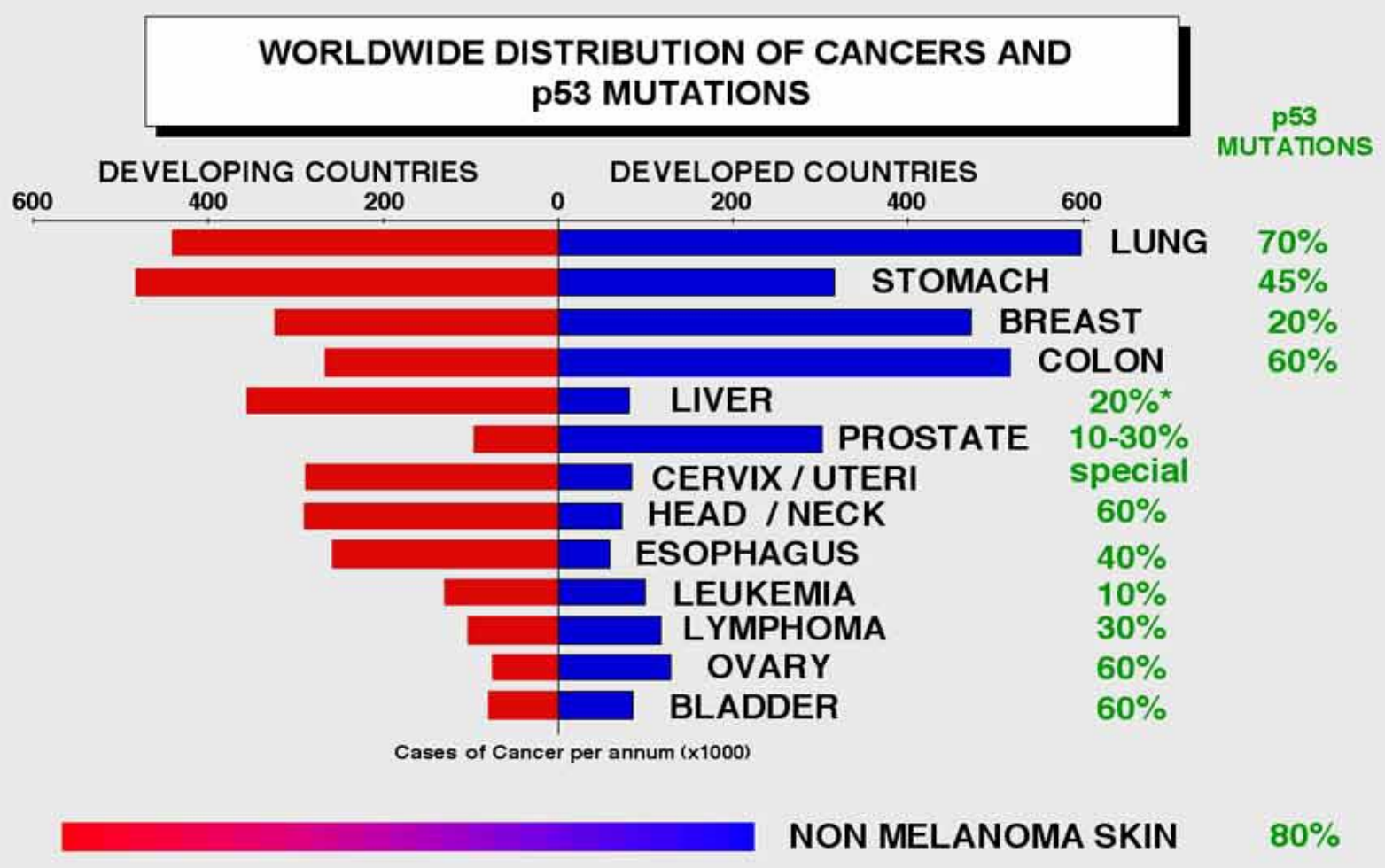
KHURI et al. 2000

ONYX-015 & Chemotherapy: complete response



2005 China FDA approves

Why p53 as target?



virus	administration	cancer type	clinical phase I	II	III
ONYX 015	intratumoral injection + chemotherapy	head and neck	➡		
	intratumoral injection + chemotherapy	pancreatic cancer	➡		
	hepatic artery infusion + chemotherapy	liver metastases colorectal cancer	➡		
	intratumoral injection + chemotherapy	sarcoma	➡		
	intratumoral injection	malignant glioma	➡		
	mouthwash	oral leukoplakia	➡		

virus	administration	cancer type	clinical phase I	II	III
CV706	intratumoral injection	prostate cancer	➡		
CV787	intratumoral injection	prostate cancer	➡		
G207	intratumoral injection	glioblastoma multiforme	➡		
Reovirus	intratumoral injection	advanced tumors	➡		

2008

Onyx ongoing ameliorations

- ARMED ADENOVIRUS:
- SUICIDE GENES
- IMMUNOSTIMULATORY CYTOKINES

- UNDERSTANDING THE BIOLOGICAL MECHANISMS DEFINING
  - INTERACTION WITH HUMAN HOST
  - SYNERGY BETWEEN VIRUS THERAPY AND CHEMOTHERAPY

QUESTIONS?
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