Viral gene therapy

Lecture 25

Biology W3310/4310

Virology

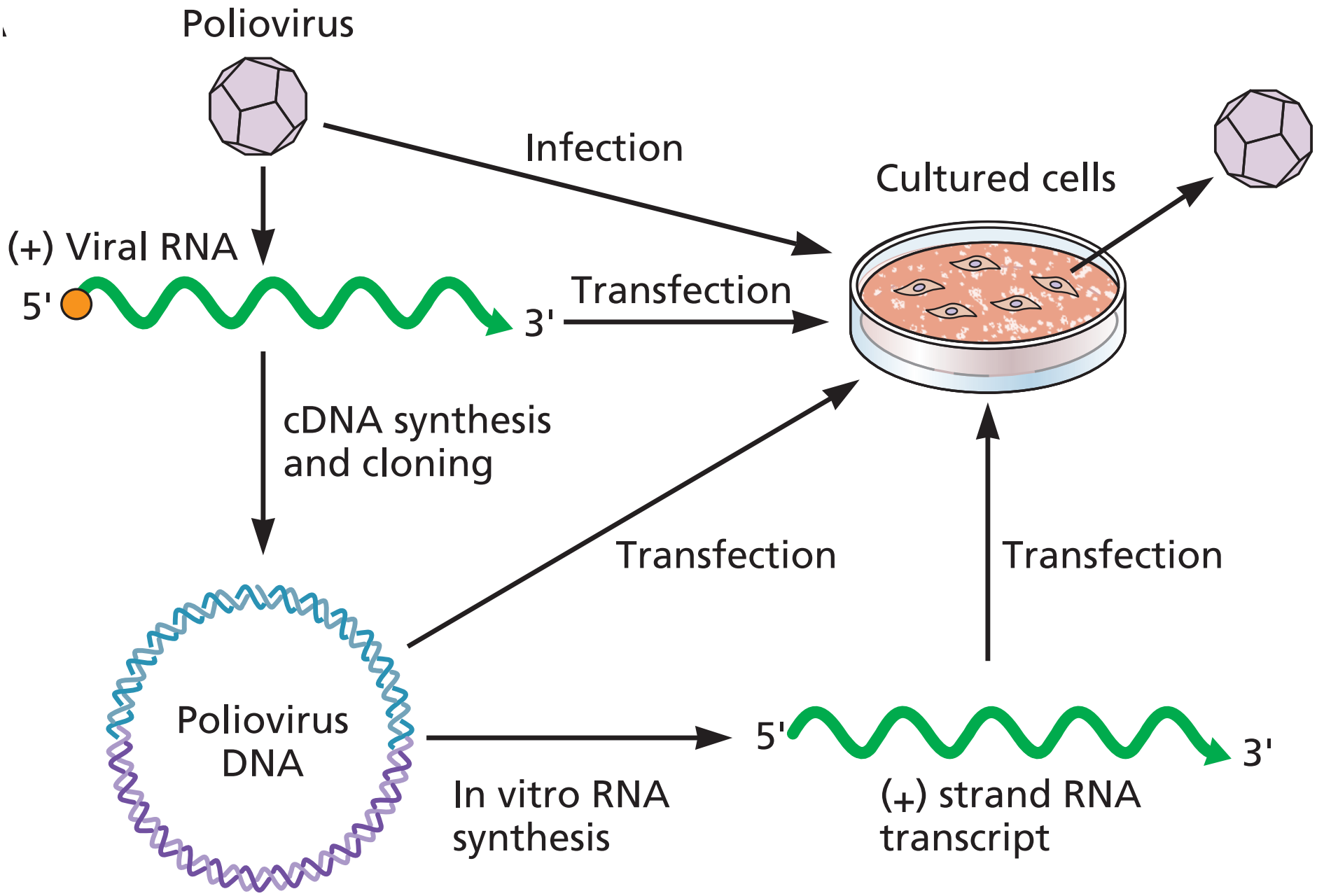
Spring 2016

"Trust science, not scientists"

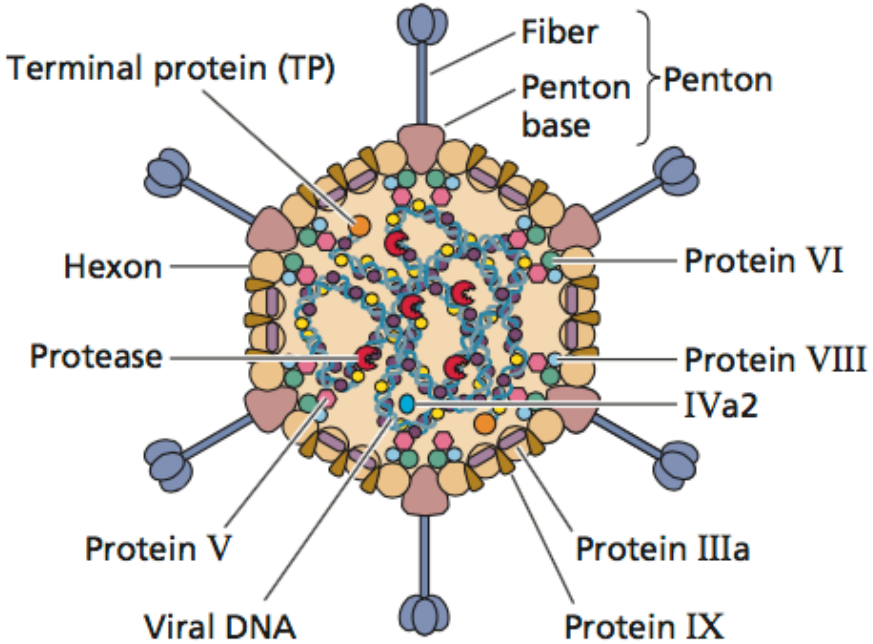
--Dickson Despommier

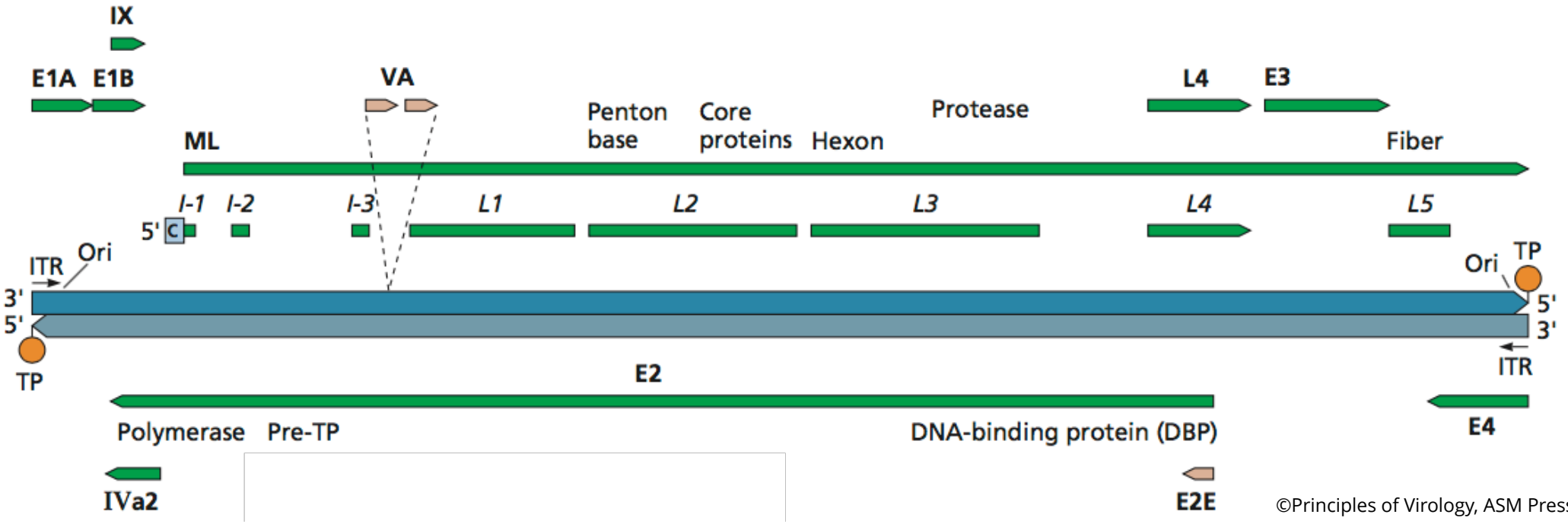
Virus vectors

- Gene therapy: deliver a gene to patients who lack the gene or carry defective versions
- To deliver antigens (viral vaccines)
- Viral oncotherapy
- Research uses



Adenovirus vectors





Adenovirus vectors

- Efficiently infect post-mitotic cells
- Fast (48 h) onset of gene expression
- Episomal, minimal risk of insertion mutagenesis
- Up to 37 kb capacity
- Pure, concentrated preps routine
- >50 human serotypes, animal serotypes
- Drawback: immunity

Adenovirus vectors

- First generation vectors: E1, E3 deleted
- E1: encodes T antigens (Rb, p53)
- E3: not essential, immunomodulatory proteins

http://edoc.ub.uni-muenchen.de/13826/

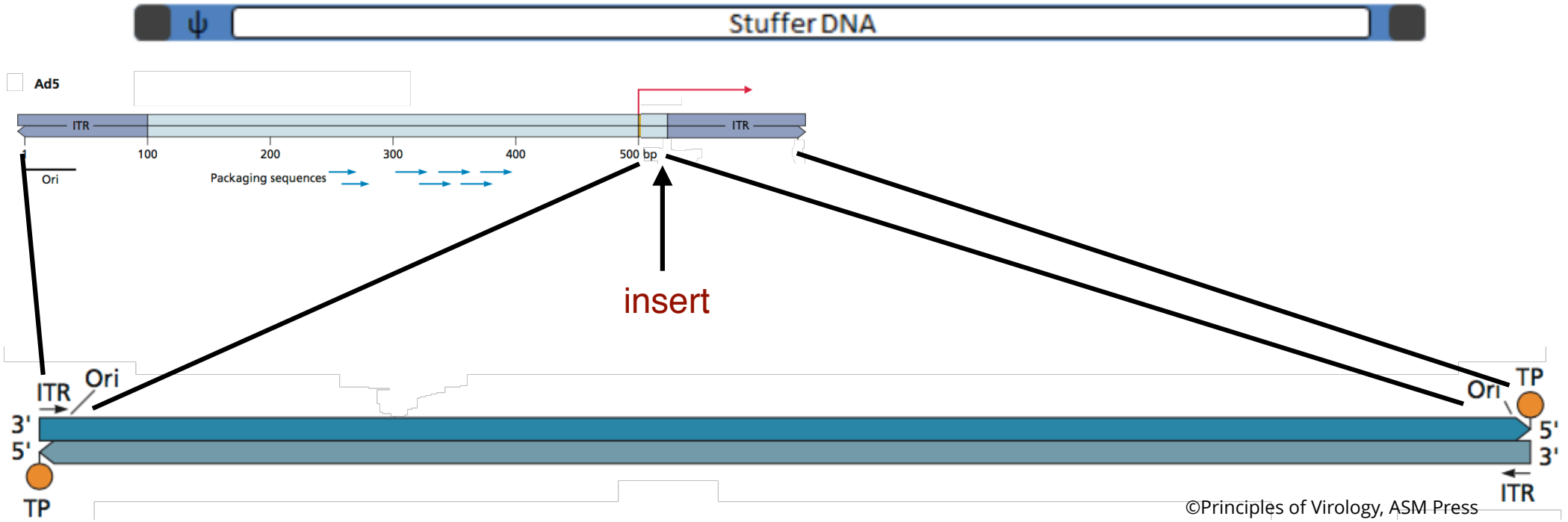
Adenovirus vectors

- Second generation vectors: E1, E3 deleted, plus deletions in E2 or E4
- More space for transgene

http://edoc.ub.uni-muenchen.de/13826/

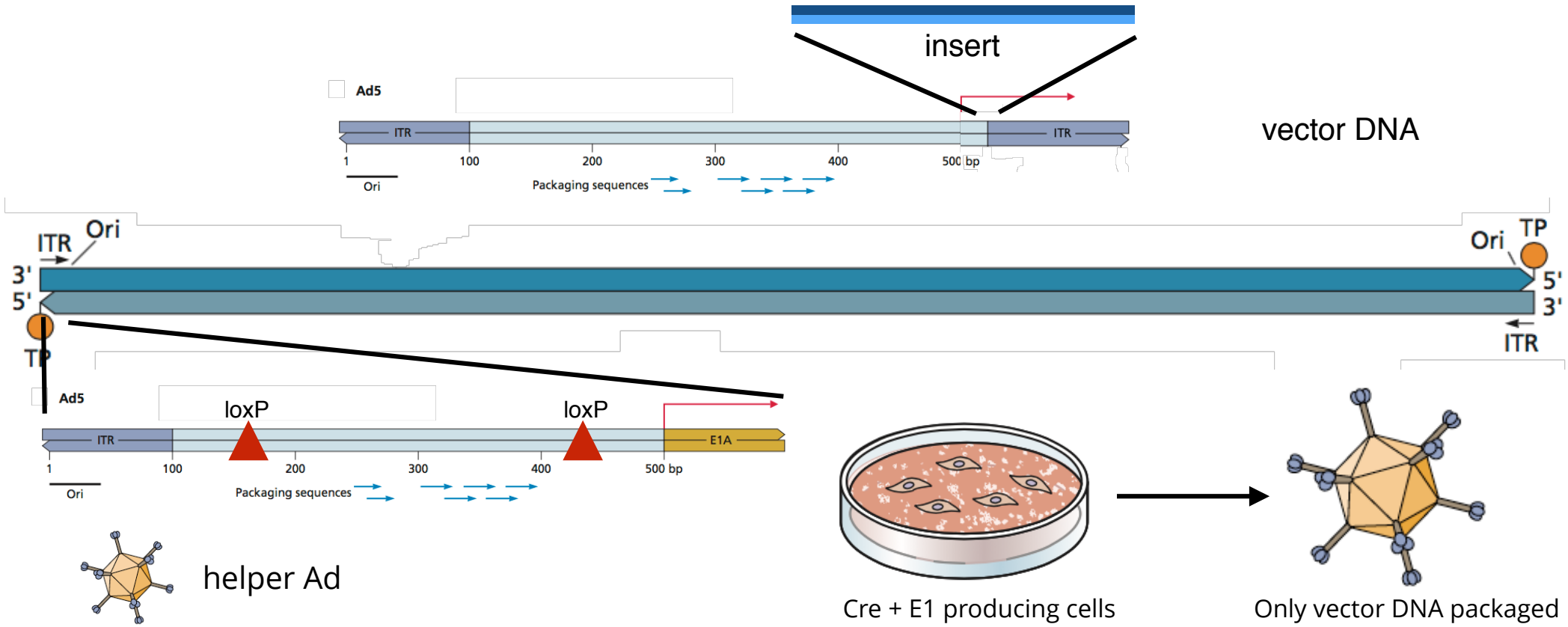
Adenovirus vectors

- Third generation vectors: all genes deleted, contain only two ITRs and *psi*
- Require helper virus, which is E1-deleted

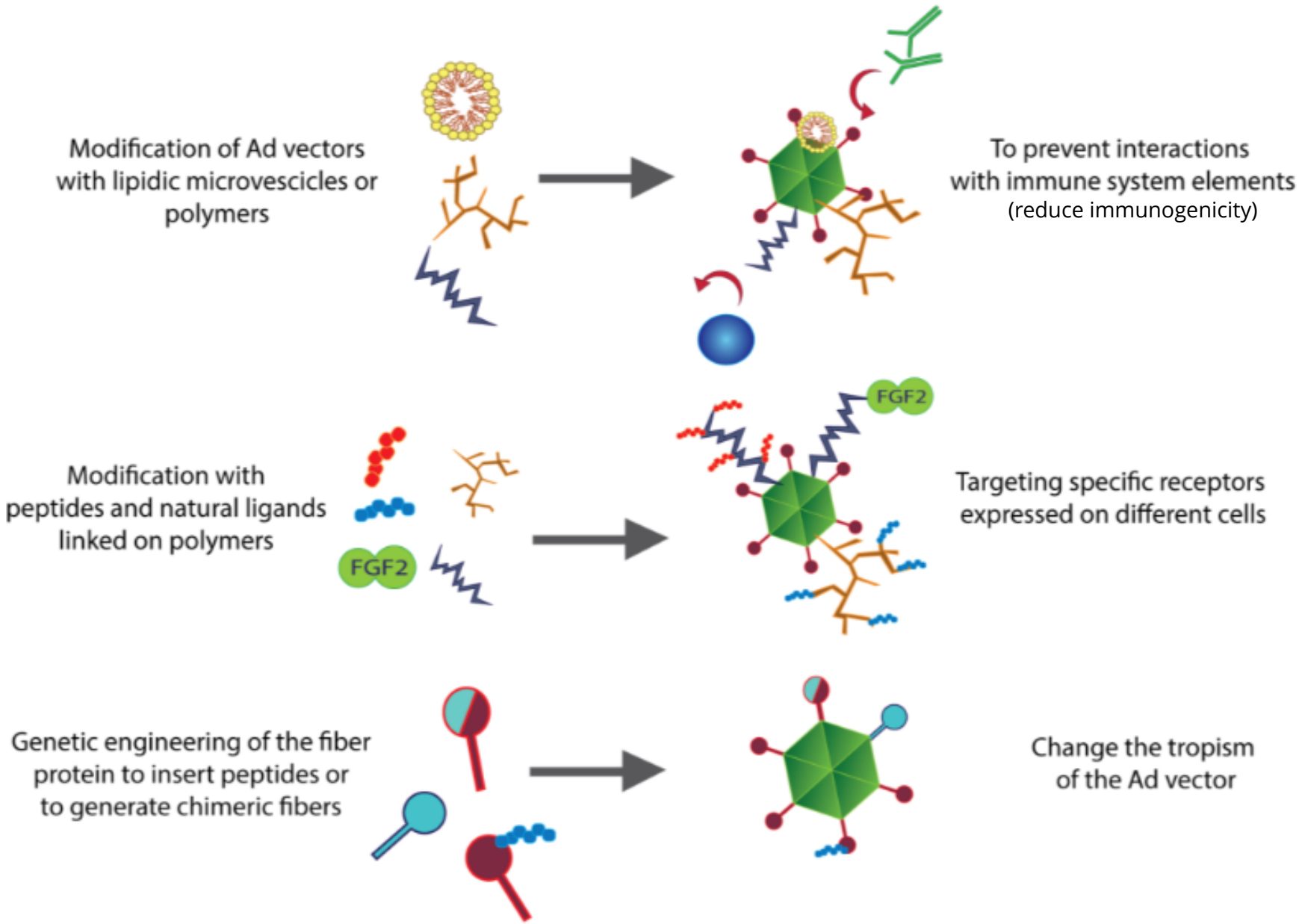


Adenovirus vectors

- Helper Ad has loxP flanking *psi*
- Propagation in Cre producing cells yields helper that cannot be packaged

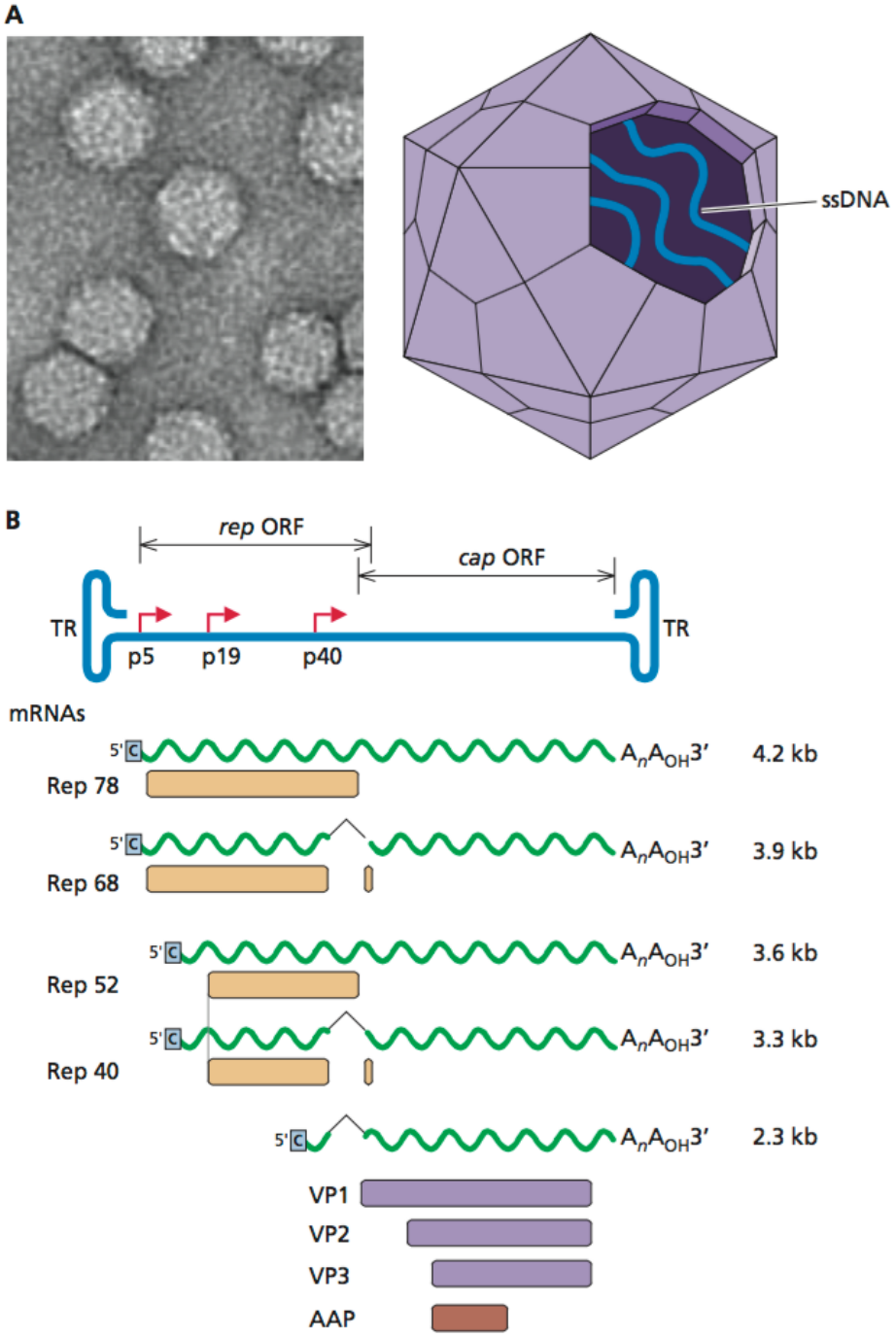


Vector modification



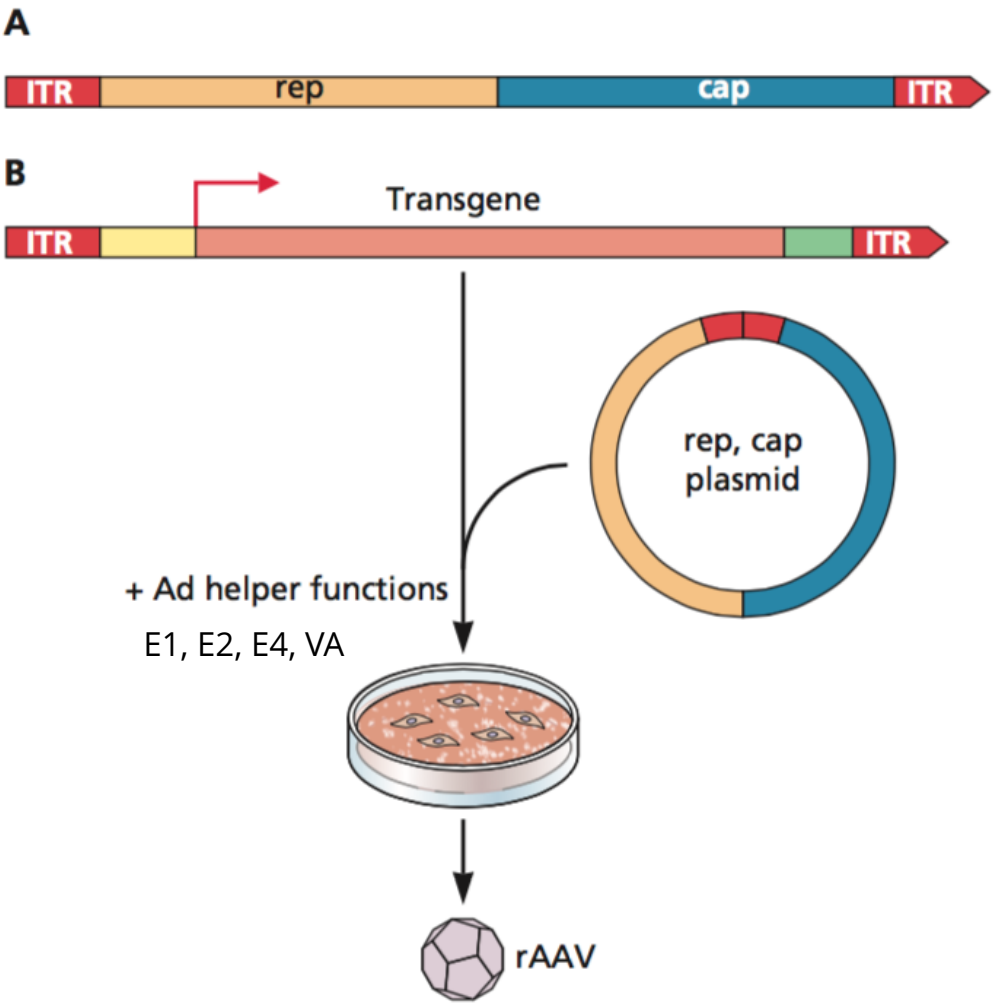
Viruses 2014, 6, 832-855

Adenovirus-associated virus

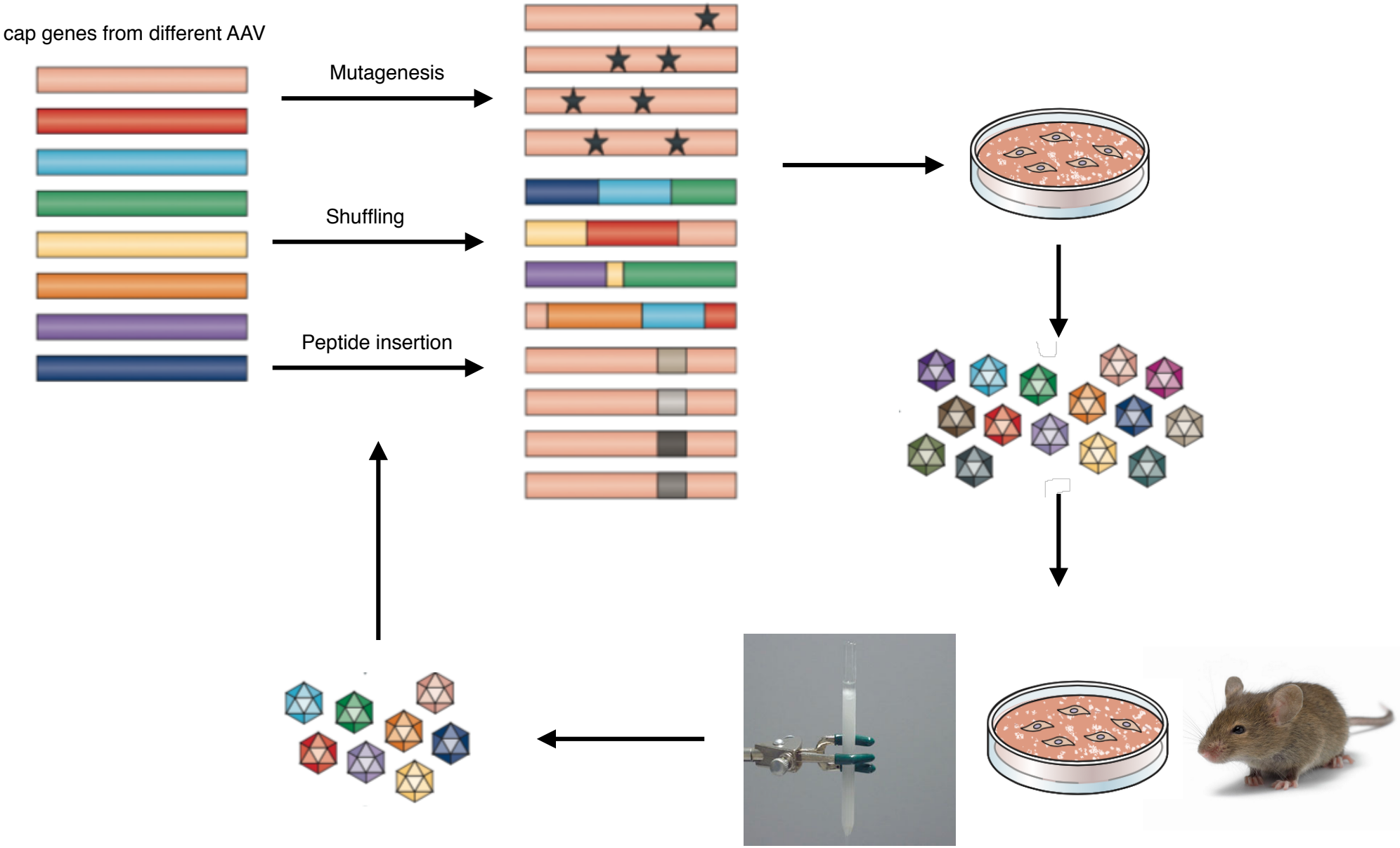


Adenovirus-associated virus vectors

- Long-term gene expression
- Multiple serotypes

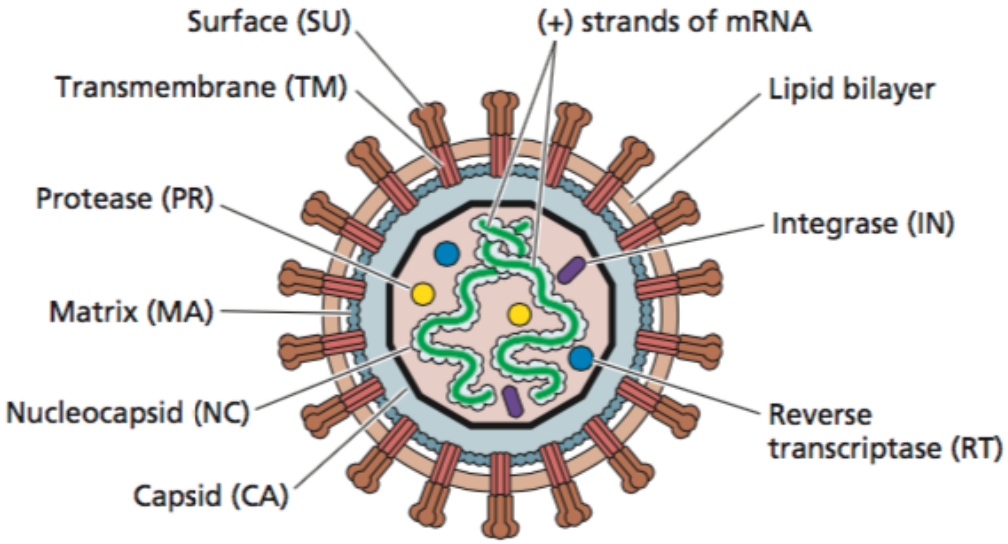


Vector modifications



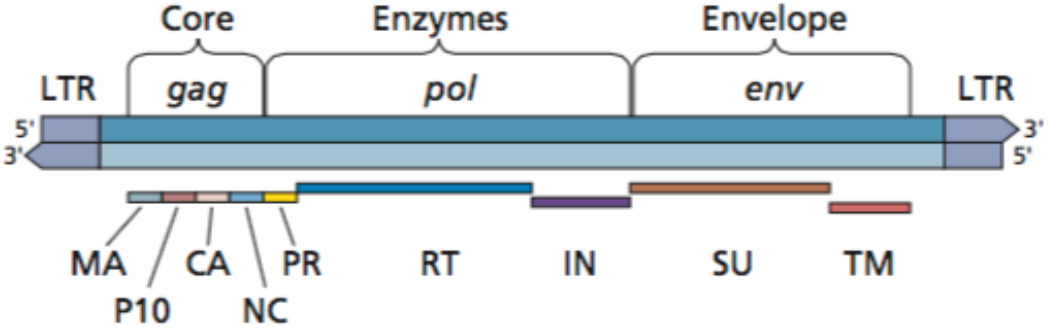
doi:10.1038/nrg3742

Retrovirus vectors



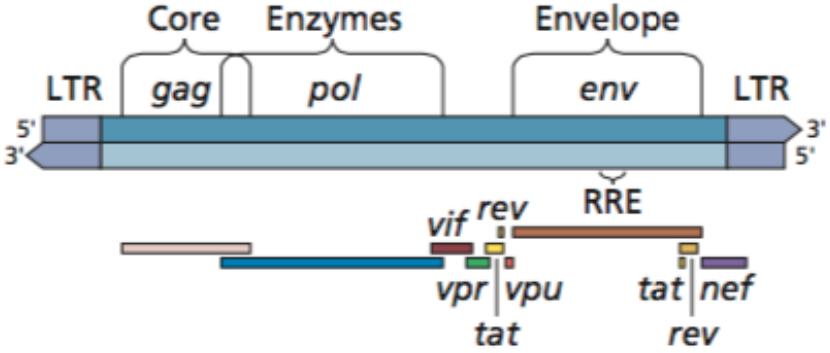
Simple genome (ALV)

Proviral DNA

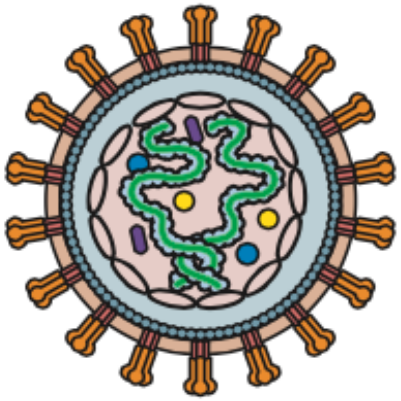


Complex genome (HIV-1)

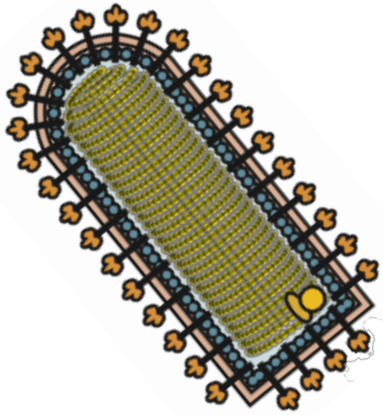
Proviral DNA



Retrovirus vectors

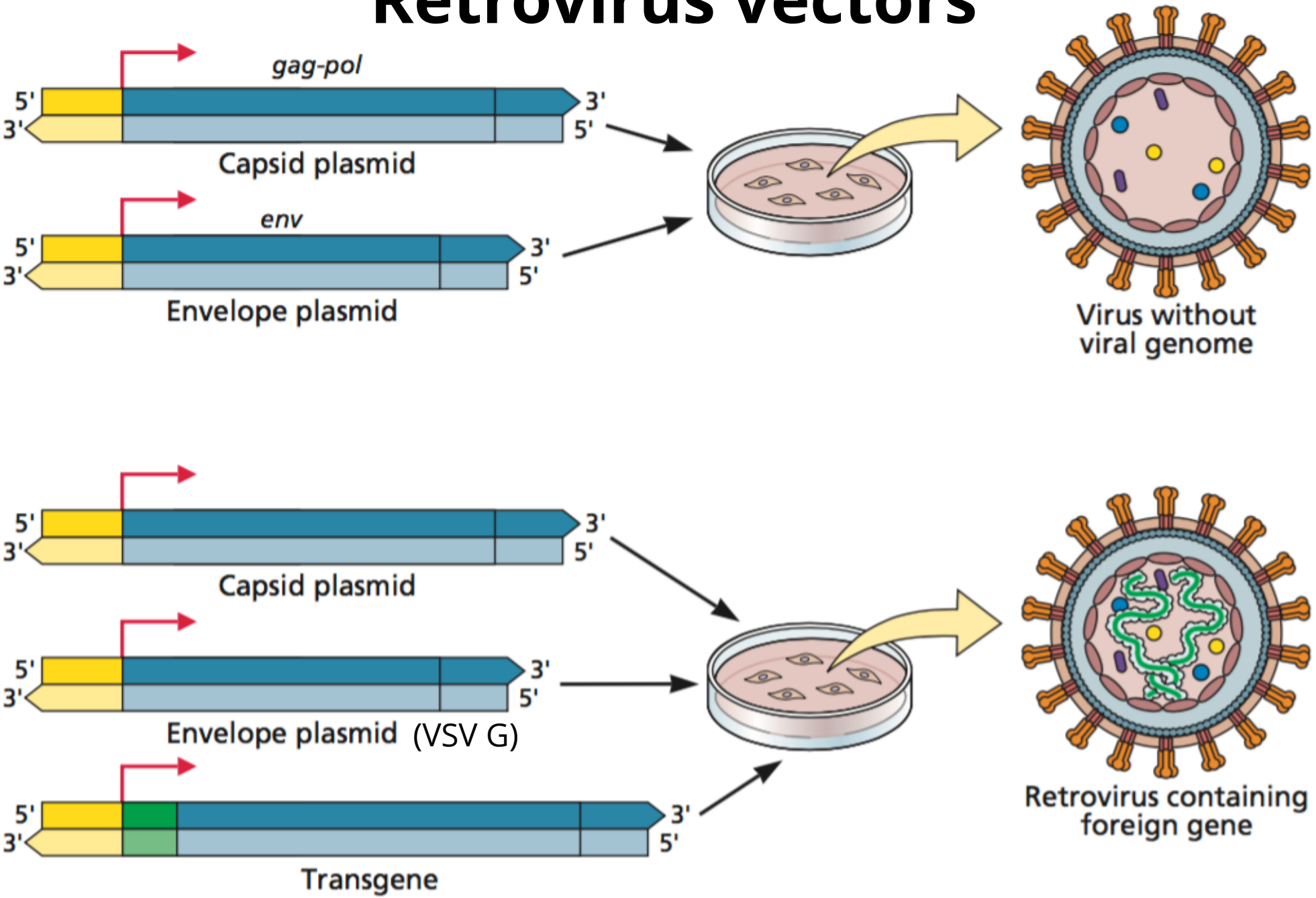


- Based on lentiviruses (HIV-1) or other retroviruses
- HIV can infect non-dividing cells
- Long-term expression (provirus)
- Up to ~8 kb transgene inserts

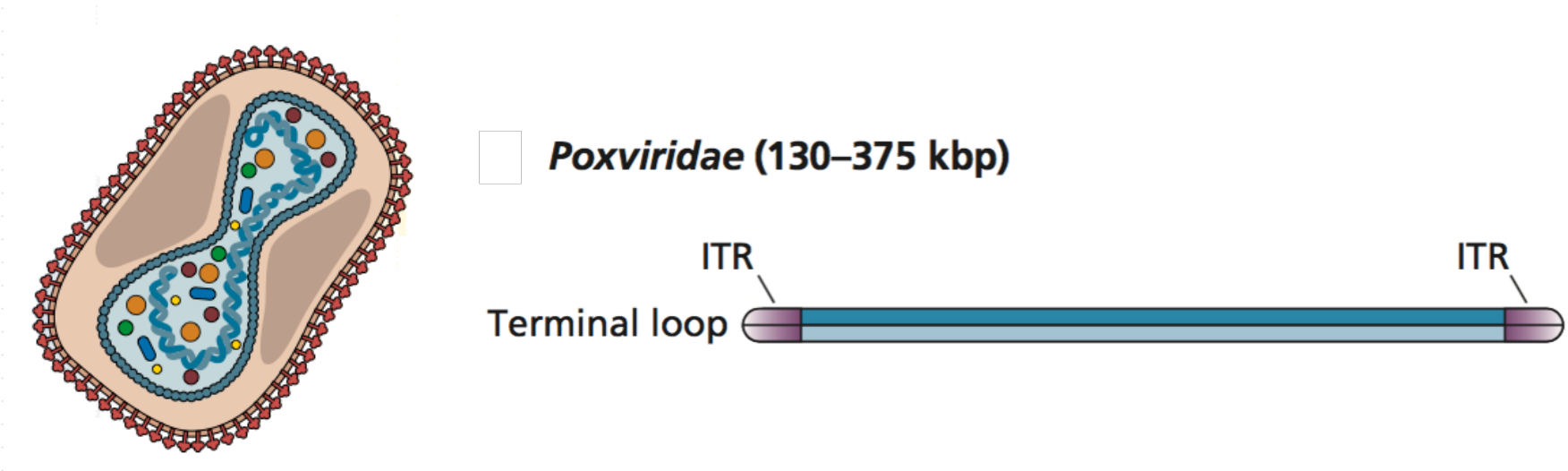


- Possibility for insertional mutagenesis (3'LTR inactivated or integration-deficient)
- Pseudotyping with VSV G

Retrovirus vectors

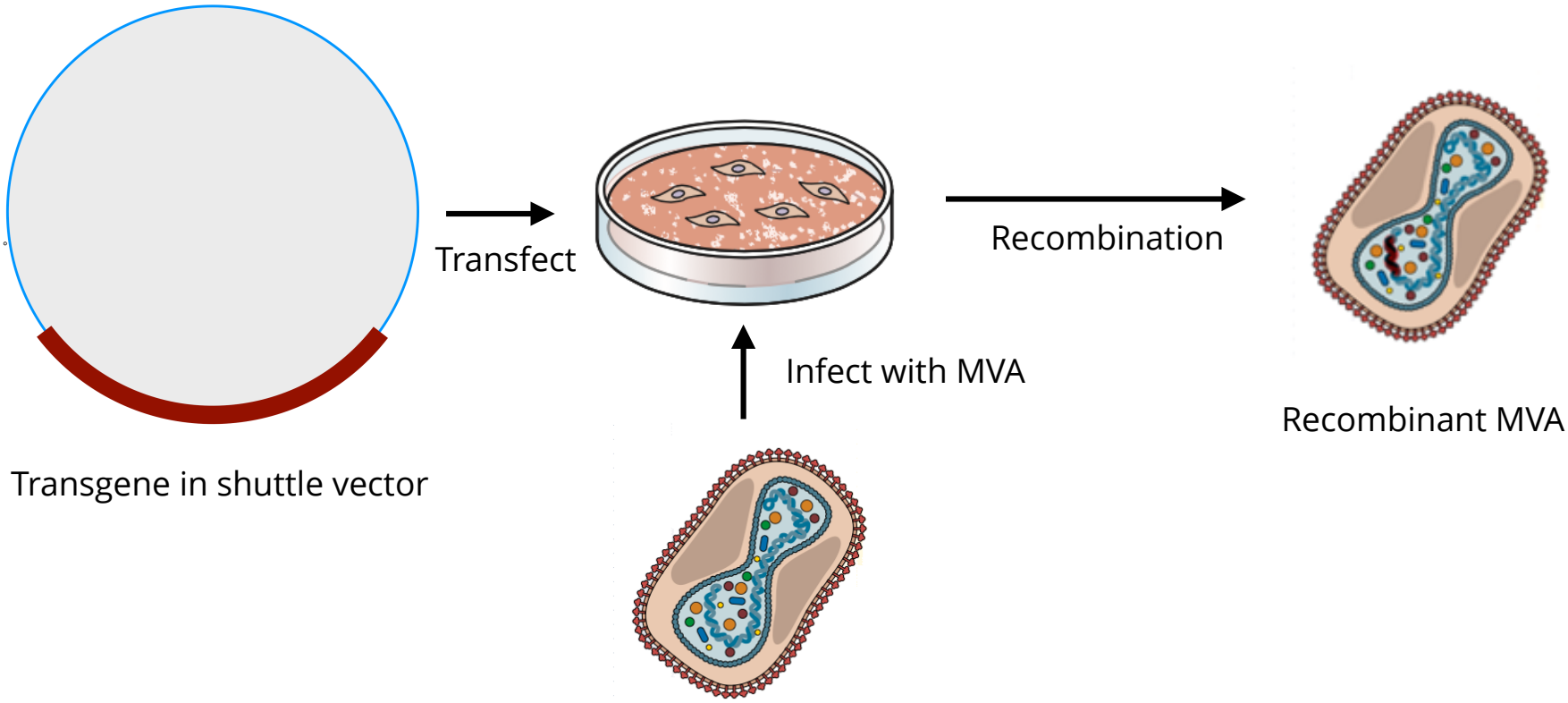


Modified vaccinia virus Ankara (MVA)



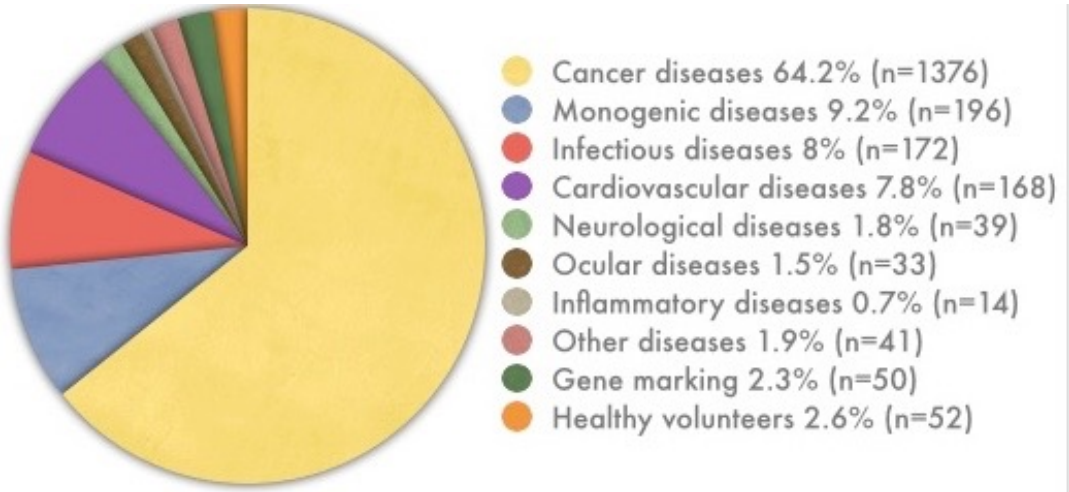
- Replication-deficient vector: smallpox vaccine, infectious in avian but not mammalian cells (assembly block)
- BSL-1
- Intrinsic adjuvant
- Large capacity

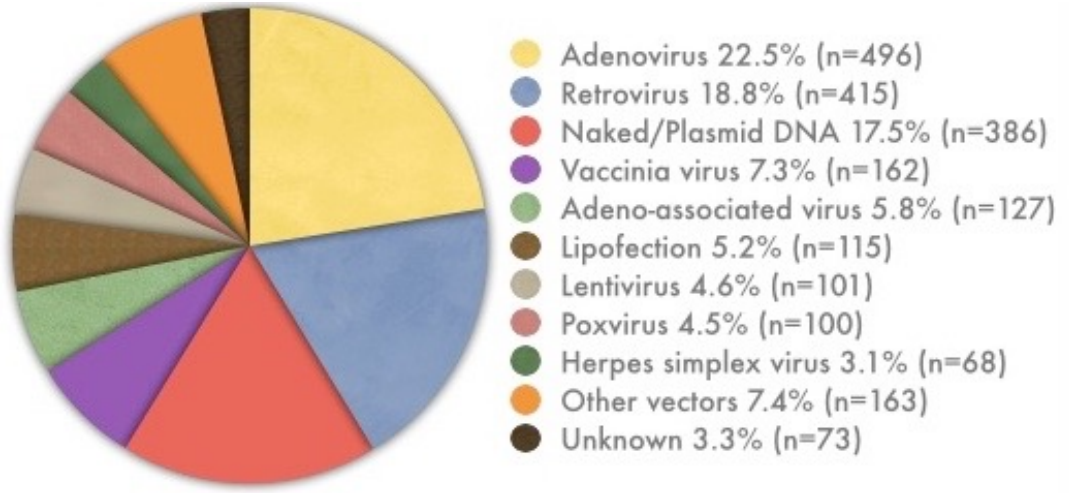
Modified vaccinia virus Ankara (MVA)



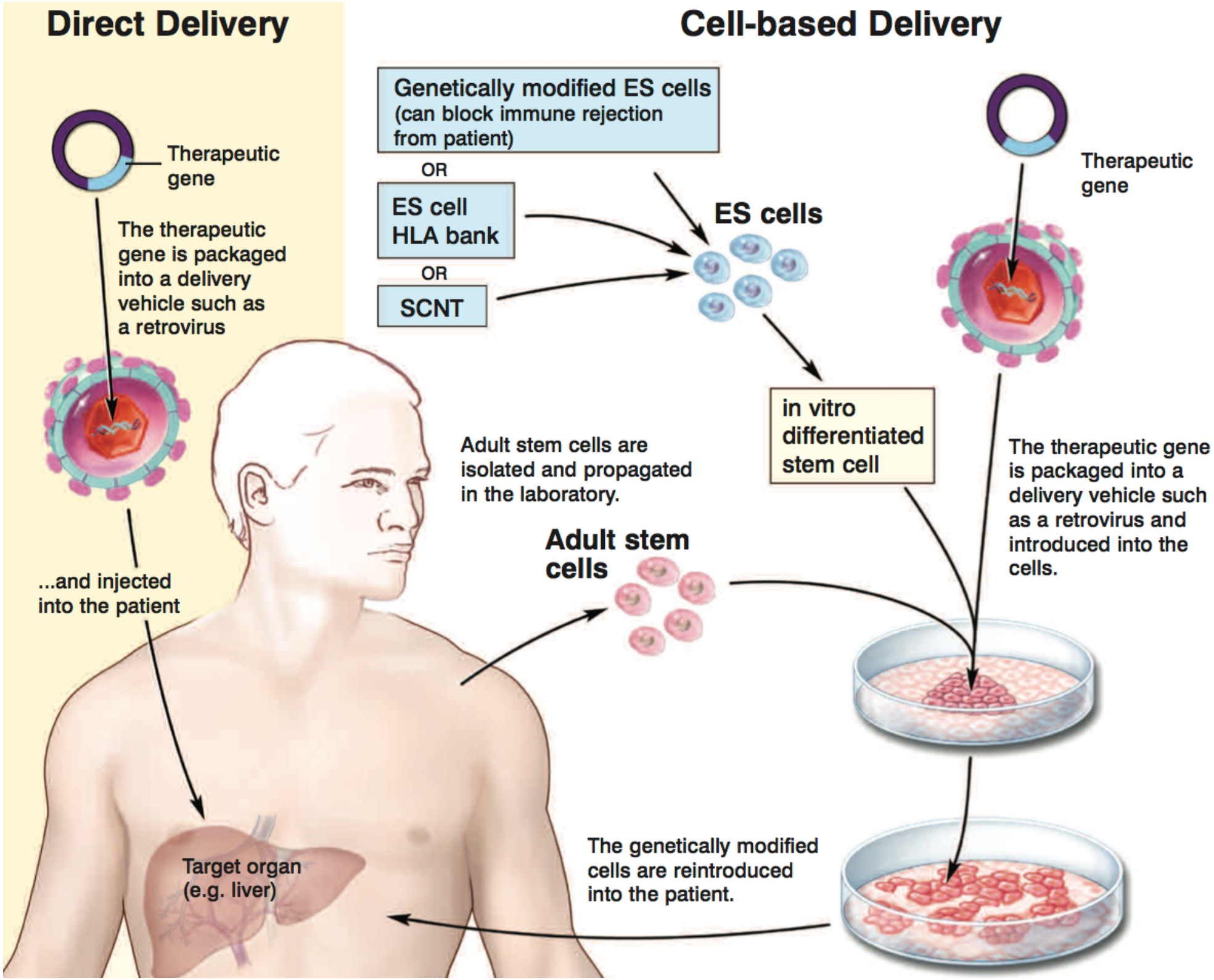
Vector	Advantages	Disadvantages
Retrovirus	Moderate capacity (9-10 kb) High transduction efficiency Broad tropism Stable expression (provirus)	Low titers Oncogenic potential Low capsid stability No transduction resting cells
Lentivirus	Moderate capacity (9-10 kb) High transduction efficiency of resting cells Broad tropism Stable expression (provirus)	Moderate titers Oncogenic potential Low capsid stability
AAV	High titers Genome persistence in resting cells Non-pathogenic in humans Modified targeting by capsid modifications	Immunogenic Low capacity (<5 kb) Low transduction efficiency in cell culture
Adenovirus (3rd gen)	High capacity (37 kb) No acute toxicity High titers High transduction efficiency Broad tropism Liver targeting in vivo Genome persistence in resting cells	Immunogenic Transient effects in cycling cells Low transduction of Car^- cells Liver targeting in vivo

Indications addressed by gene therapy clinical trials

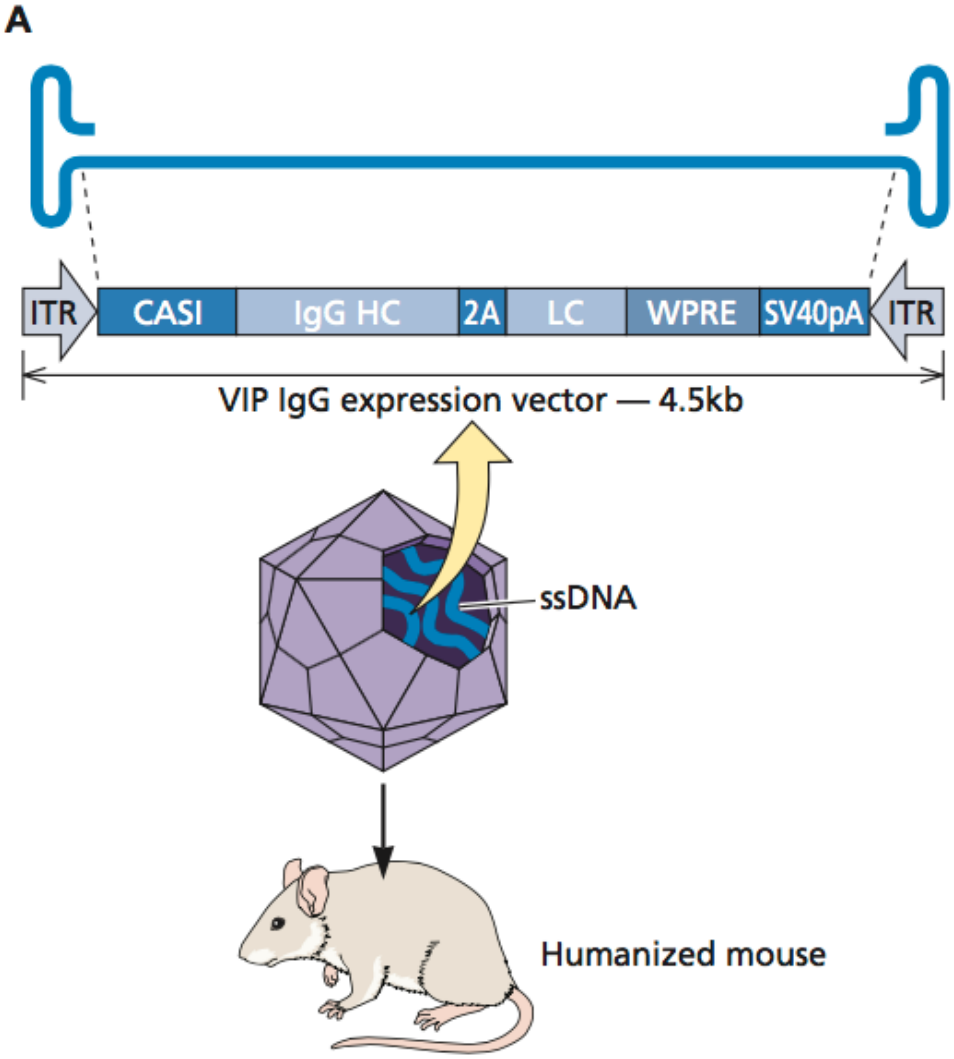


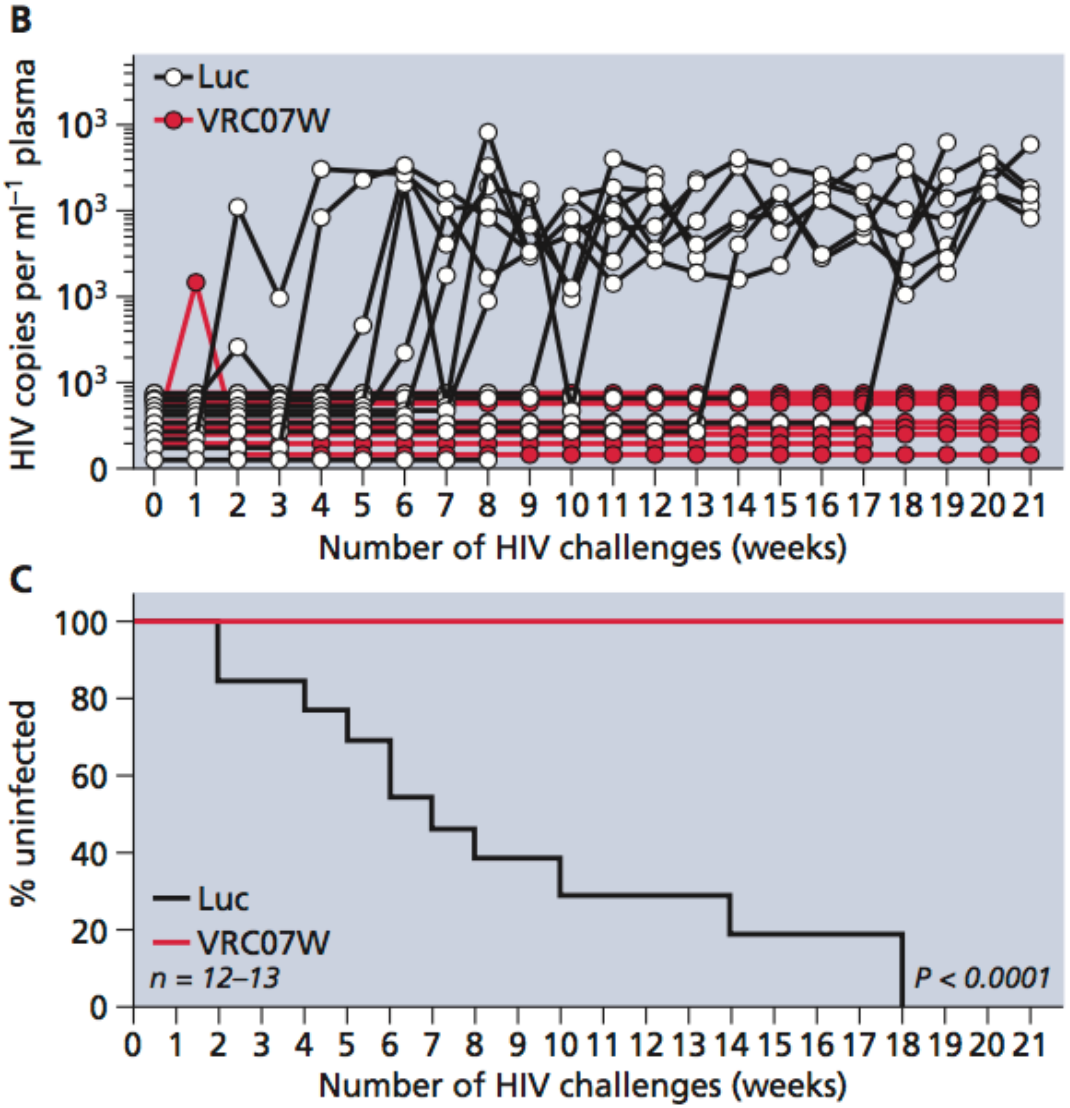


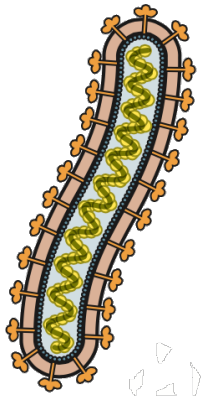
http://www.wiley.com/legacy/wileychi/genmed/clinical/


Direct Delivery
Cell-based Delivery
Genetically modified ES cells
(can block immune rejection
from patient)
OR
ES cell
HLA bank
OR
SCNT
ES cells
Therapeutic
gene
Therapeutic
gene
The therapeutic
gene is packaged
into a delivery
vehicle such as
a retrovirus
in vitro
differentiated
stem cell
Adult stem cells are
isolated and propagated
in the laboratory.
The therapeutic gene
is packaged into a
delivery vehicle such
as a retrovirus and
introduced into the
cells.
Adult stem
cells
...and injected
into the patient
Target organ
(e.g. liver)
The genetically modified
cells are reintroduced
into the patient.

AIDS Immunoprophylaxis with AAV







Ebolavirus GP vaccine in Ad/MVA

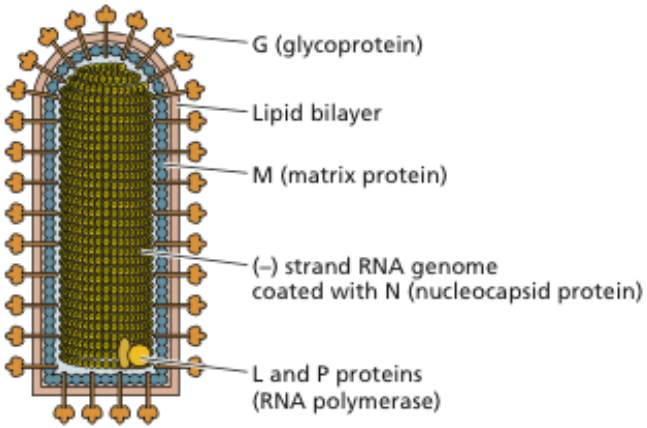
Vector	Dose (PU)		Protection[a]
Single shot			
ChAd3	1×10^{11}	2/4	50%
ChAd3	1×10^{10}	0/4	0%
Prime-boost			
ChAd3/ChAd3	$1 \times 10^{10}/1 \times 10^{10}$	1/3	33%
ChAd3/ChAd63	$1 \times 10^{10}/1 \times 10^{10}$	1/4	25%
ChAd3/MVA	$1 \times 10^{10}/1 \times 10^{8}$	4/4	100%

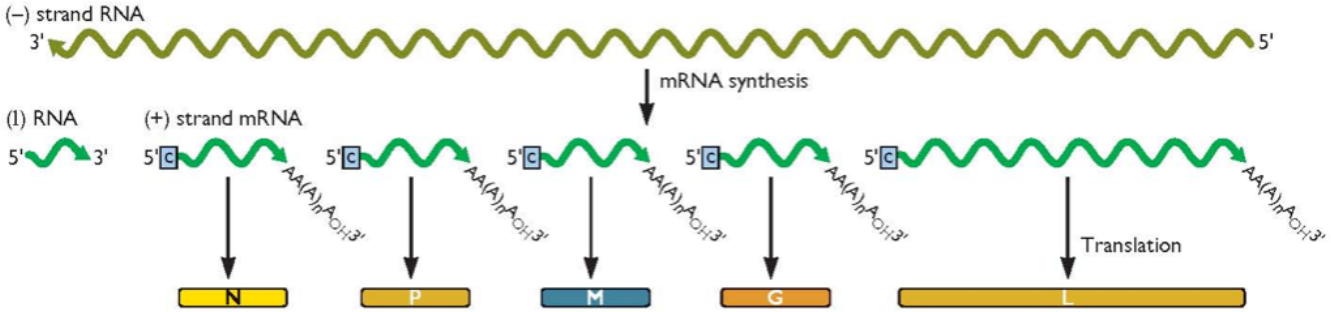
IM challenge

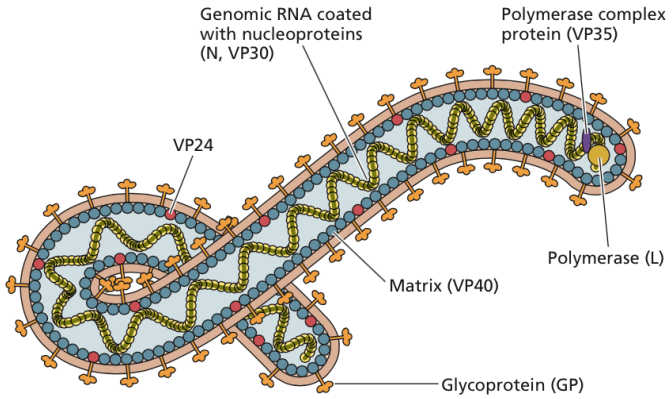
GSK, NIAID/NIH

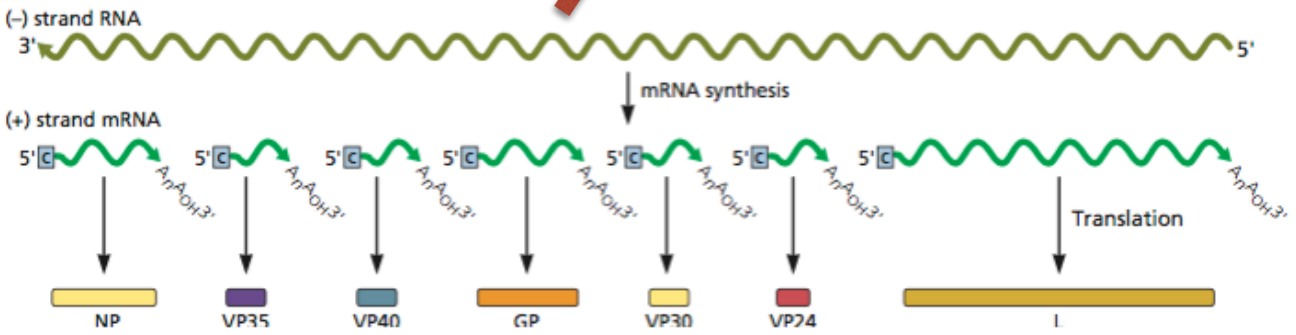
doi:10.1038/nm.3702

Vesicular stomatitis virus - Ebolavirus



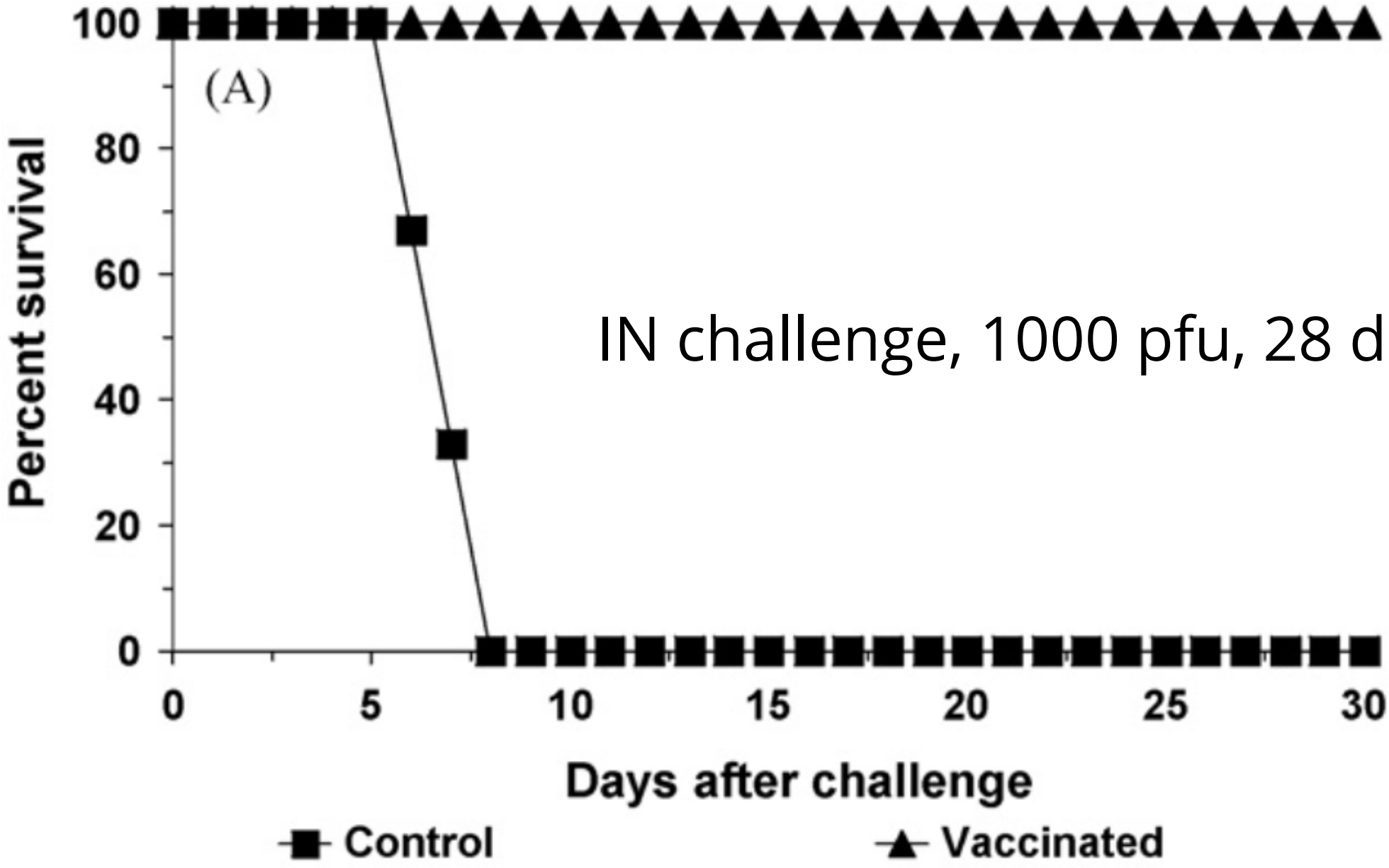






VSV-EBOV

Principles of Virology, ASM Press

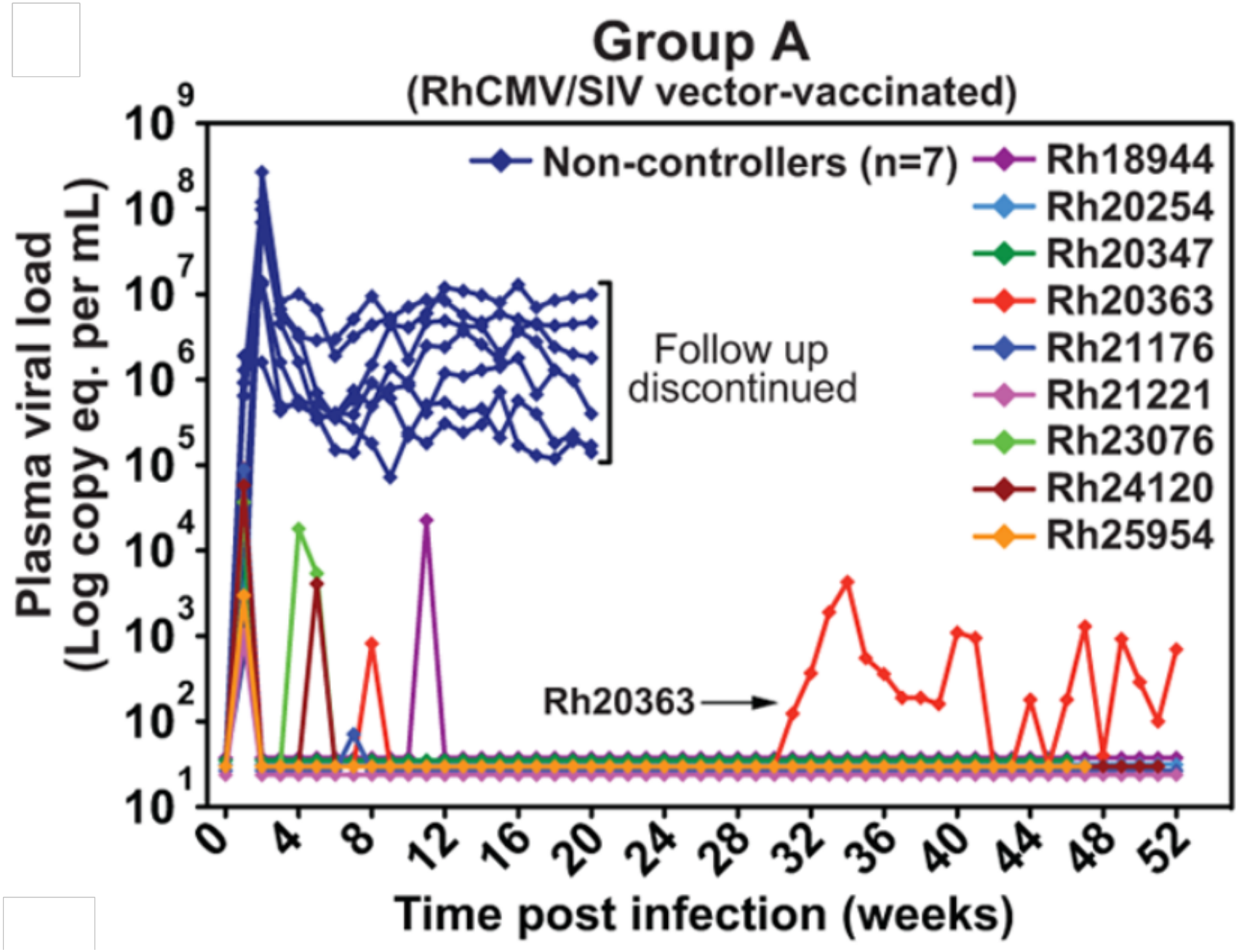


IN challenge, 1000 pfu, 28 da

Public Health Agency, Canada/Newlink

Vaccine 26 (2008) 6894–6900

RhCMV-based SIV vaccine in macaques



Nature. 2013 October 3; 502(7469)

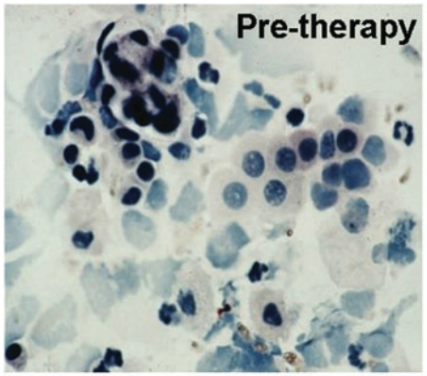
Monogenic diseases

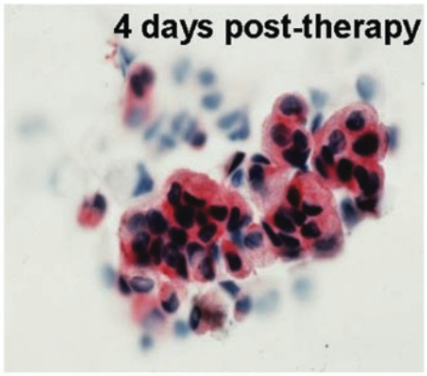
- Caused by mutation in one gene
- >6,000, 1 out of 200 live births
- Amenable to viral gene therapy
- >1,800 clinical trials

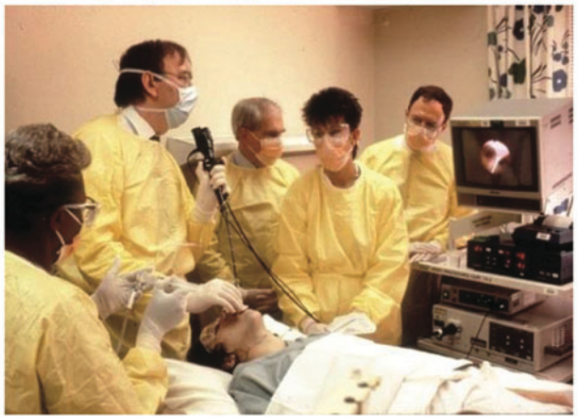
Disease	Defect	Incidence	Vector
Severe combined immunodeficiency	Adenosine deaminase (25%) Common cytokine receptor ɣ chain	<1 in 10^5 1 in 50-100,000	Retrovirus
Liproprotein lipase deficiency	Lipoprotein lipase	1-2 in 10^6	AAV
Hemophilia B	Factor IX defiency	1 in 30,000 males	AAV
Hemoglobinopathies and thalassemias	Defects in α- or β- globin gene	1 in 600 in specific ethnic groups	Lentivirus
α1-antitrypsin deficiency (emphysema, liver disease)	α1-antitrypsin not produced	1 in 3,500	AAV
Retinal degenerative disease, Leber's congenital amaurosis	Retinal pigment epithelium-specific 65 kDa protein	Inherited retinopathies 1 in 2000 <10% LCA (1 in 80,000)	AAV
X-linked adrenoleukodystrophy	ABCD1 transporter	1 in 20-50,000	Lentivirus
Wiskott-Aldrich syndrome (eczema-thrombocytopenia-immunodeficiency syndrome)	Was protein	1-10 in 10^6 males	Lentivirus

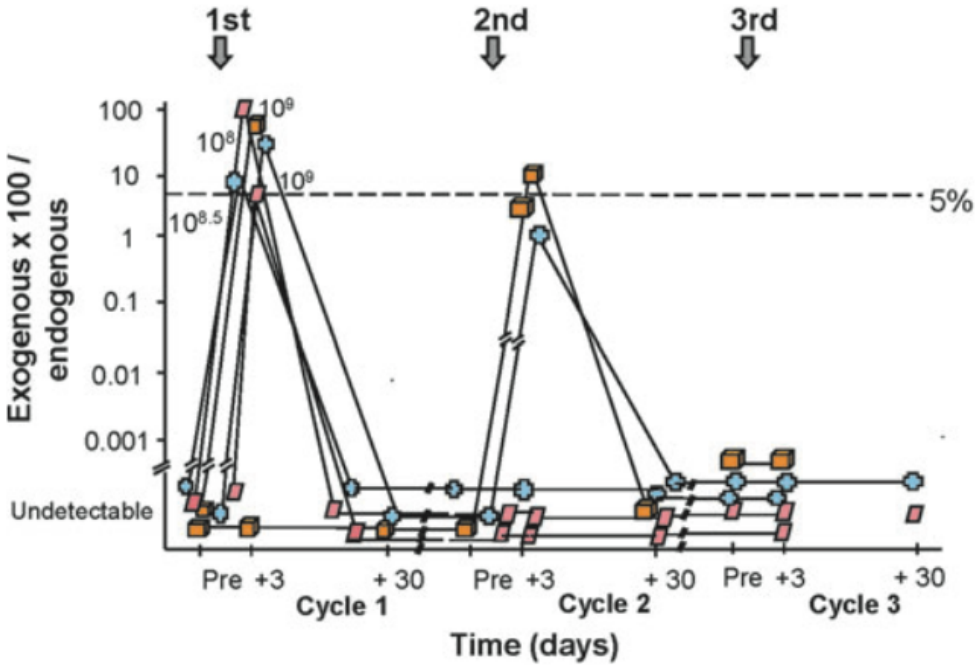
First human viral gene therapy: 1993

- 23 year old male with cystic fibrosis, homozygous for ΔF508 mutation in *CFTR* gene
- 2 x 10^8 pfu $E1^-E3^-$ Ad with CFTR DNA administered to airway epithelium









10.1089/hum.2013.2527

Setback: Jesse Gelsinger

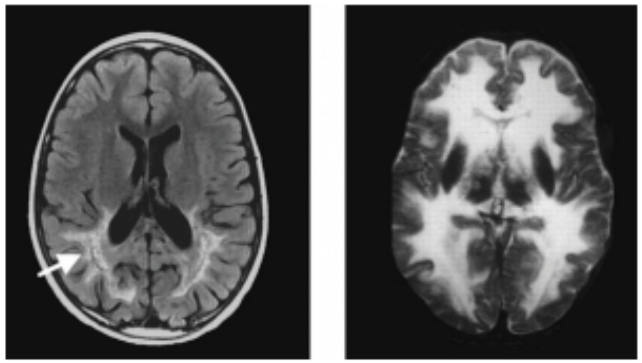
- First person to die in a gene therapy clinical trial
- Ornithine transcarbamylase deficiency
- Given Ad vector with normal *OTC* gene at UPenn
- Died 4 days later: massive immune response, multiple organ failure
- Several rules of conduct broken

X-linked severe combined immune deficiency

- Two trials, London and Paris, giving infants retrovirus with normal *IL2RG* gene (IL-2 receptor ɣ)
- CD34+ bone marrow hematopoietic precursor cells transduced with retrovirus vector, transplanted back into patients
- 4/9* infants in Paris developed T cell leukemia 3-6 years after treatment, 1 in London
- Vector integrated next to oncogene
- 27 trials with retroviral vectors halted

*treatment had worked

X-linked adrenoleukodystrophy

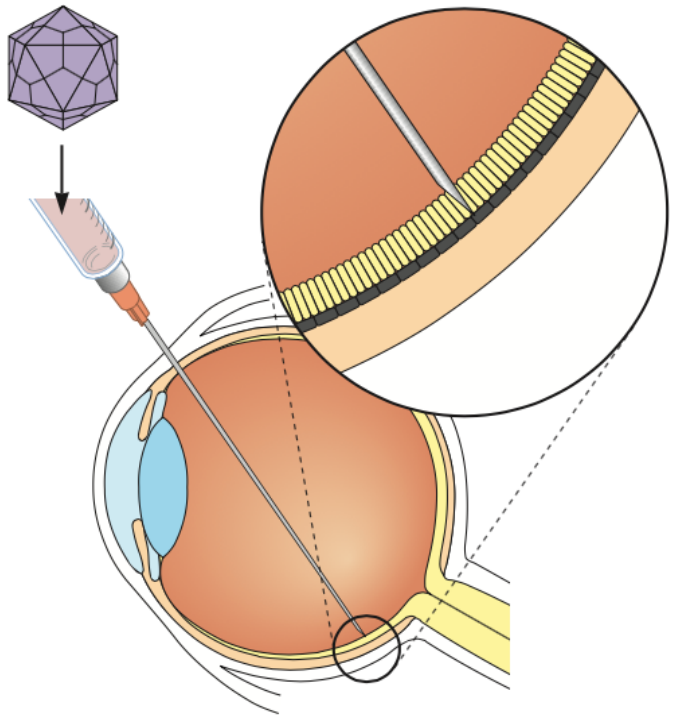


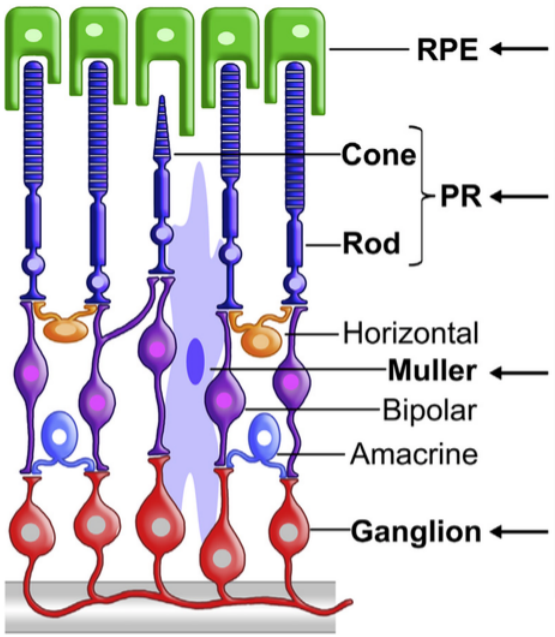
- Defect in ABCD1 transporter
- Patients' marrow derived hematopoietic stem cells infected with lentiviral vector with normal ABCD1 transporter gene
- Re-infused into patients
- Neurologic status stabilized or improved

http://www.virology.ws/2009/11/25/clinical-benefit-of-lentiviral-gene-therapy-in-two-patients-with-a-rare-neurologic-disease/

Inherited retinopathies

- Common untreatable blinding conditions
- Monogenic, mutations in retinal photoreceptors and retinal pigment epithelium
- Many vectors tested in animal models, AAV





AAV	LV	Ad
2/1[1,2] 2/4[2,3] 2/6[4] *2/7m8[5] **2/Tyr mutant[6,7]	HIV-1-VSVG[1,17,18,19] HIV-1-Mokola[1,18] HIV-1-RRV[21] HIV-1-GP64[22] FIV-VSVG[23] SIV-VSVG[25,26,27,43] EIAV-VSVG[30,31,32] BIV-VSVG[28,29]	5[33,34,35,42] 2[22,38] 5/F35++[22] 5ΔRGD[37,41] 6[22] Ch30[22] Ch63[22] 5/F17[37] 5/F35[39]
2/5[1,8,9,10] 2/7[8,10] 2/8[8,10,11,12,13,14] 2/9[8] *2/7m8[5] **2/Tyr mutant[6,7]	HIV-1-VSVG[1,19,20] HIV-1-GP64[22] FIV-VSVG[24] EIAV-VSVG[31,32]	5[22,33,37,42] 5/F35[39] 5ΔRGD[37,41] *5/F37[40]
*2/2[1,10,15] *2/6[15] **2/8[8,10,14] **2/9[8,10] *2/ShH10[16] *2/7m8[5] **2/Tyr mutant[6,7]	HIV-1-VSVG[21] FIV-VSVG[23,24] EIAV-VSVG[31]	**5[22,33,36] 5/F37[41] 5/F17[37] 5/F35[39]
**2/2[1,10,15] *2/6[15] **2/8[10,14] *2/7m8[5] **2/Tyr mutant[6,7]	FIV-VSVG[23] EIAV-VSVG[31]	*5[40] 5ΔRGD[37]

Progress in Retinal and Eye Research 43 (2014) 108e128

Leber congenital amaurosis

- Mutations in *RPE65* gene, encodes protein required for photoreceptor function
- Dog model: single subretinal injection of AAV vector with canine RPE65 gene restores visual function
- Phase I/II trials, safe and leads to sustained (1.5 y) visual improvement

Some viral gene therapy successes

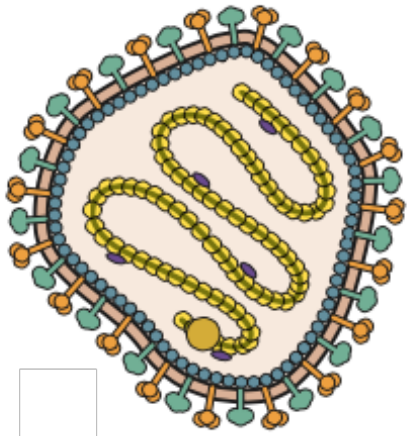
- Severe combined immunodeficiency
- Adenosine deaminase
- Leber congenital amaurosis
- Hemophilia
- beta-Thalassemia
- Lipoprotein lipase (fat metabolism disorder)

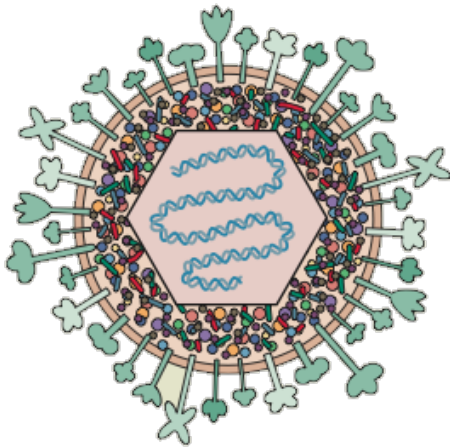
Viral oncotherapy

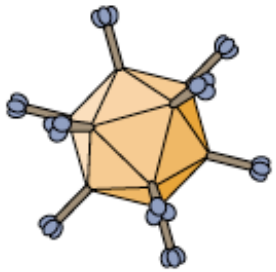
- Destroying tumors with viruses
- Some animal viruses selectively replicate in human tumors (myxoma, Seneca Valley virus)
- Modified viruses to target and kill tumors, often with immune enhancement

Tumor targeting

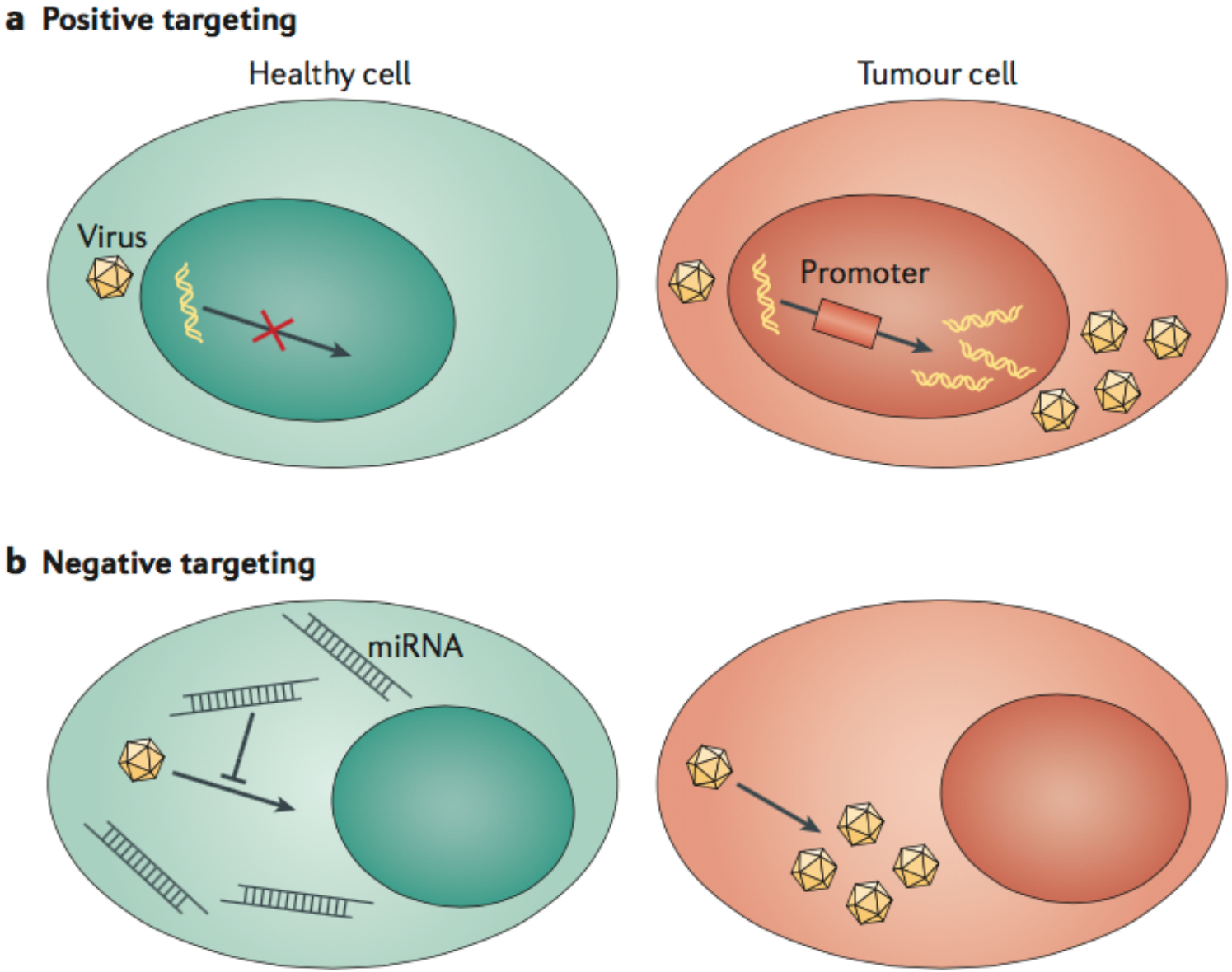
- Receptor targeting
  - Alter measles virus HA to target tumor markers
  - HSV glycoprotein D engineered to contain IL-13, or single chain antibodies against human epithelial growth factor receptor 2, on gliomas and breast tumors
  - Adenovirus: insertion of domains that recognize tumor Ag into fiber
  - Hexon-interlacing protein
  - Adaptors that bind fiber and retarget

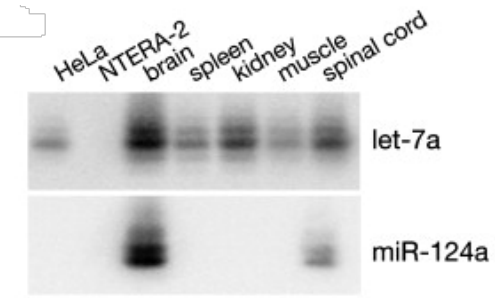






Post-entry targeting

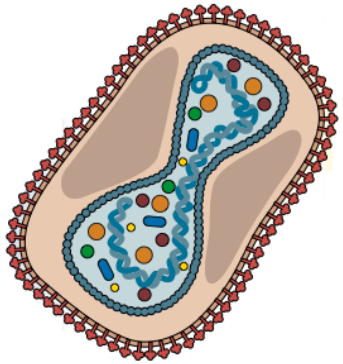




doi:10.1038/nrmicro3140

Arming viral vectors

- Enhance therapeutic efficacy of oncolytic virus: hard to infect 100% of cells
- Strategies that kill tumor cells surrounding those infected - bystander killing
- Prodrug convertases
- Ion transport protein
- Immunostimulatory factors

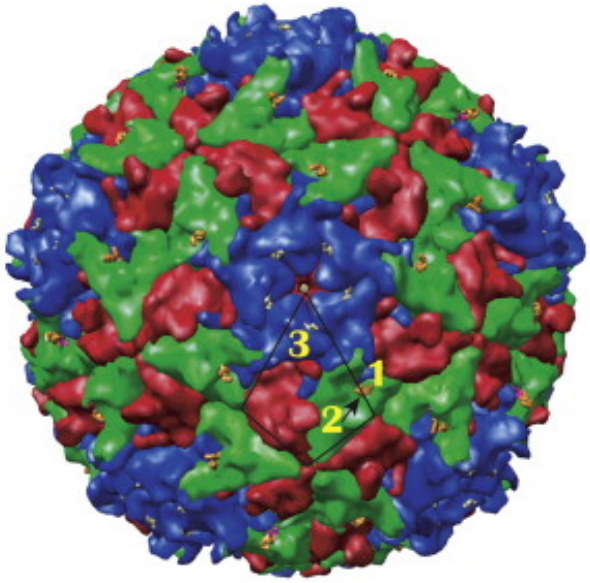


Myxoma virus

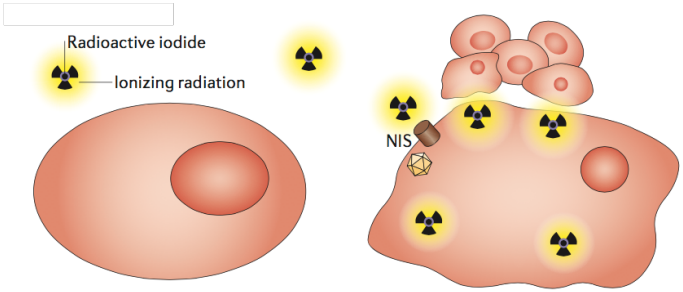
- Same virus introduced into Australia to kill European rabbits
- Does not replicate in any non-rabbit host
- Infects many types of human cancer cells
  - Failure of cells to induce anti-viral response
  - Activation of cell pathways related to transformation

Cancer	Animal model	Tumor establishment	MYXV Administration	Outcome
Acute myeloid leukemia	NSG	Human AML cells in bone marrow xenograft	*Ex vivo*	90% of mice free of human AML cells in BM
Multiple myeloma	NSG	Human MM cells in bone marrow xenograft	*Ex vivo*	100% of mice free of human MM cells in BM
Pancreatic cancer	NOD/SCID	Human pancreatic cancer cells in IP cavity	IP	Reduced tumor burden and prolonged survival
Pancreatic cancer	C57BL/6	Murine pancreatic cancer cells in IP cavity	IP	100% survival combined with gemcitabine
Glioma	CD-1 nude	Human gliomas in mouse brain	Intratumoral	92% of mice cleared of tumors and cured

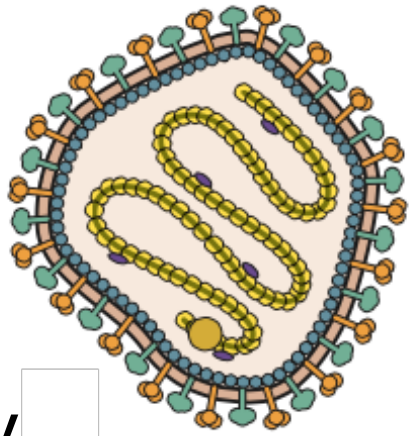
Seneca Valley virus - Picornavirus



- Identified as a contaminant of cell culture medium, form bovine serum or porcine trypsin
- Selective tropism for cancers with neuroendrocrine features: small cell lung cancer, retinoblastoma, neuroblastoma, medulloblastoma, effective in mouse models
- Phase I: safe, II ongoing (iv inoculation)

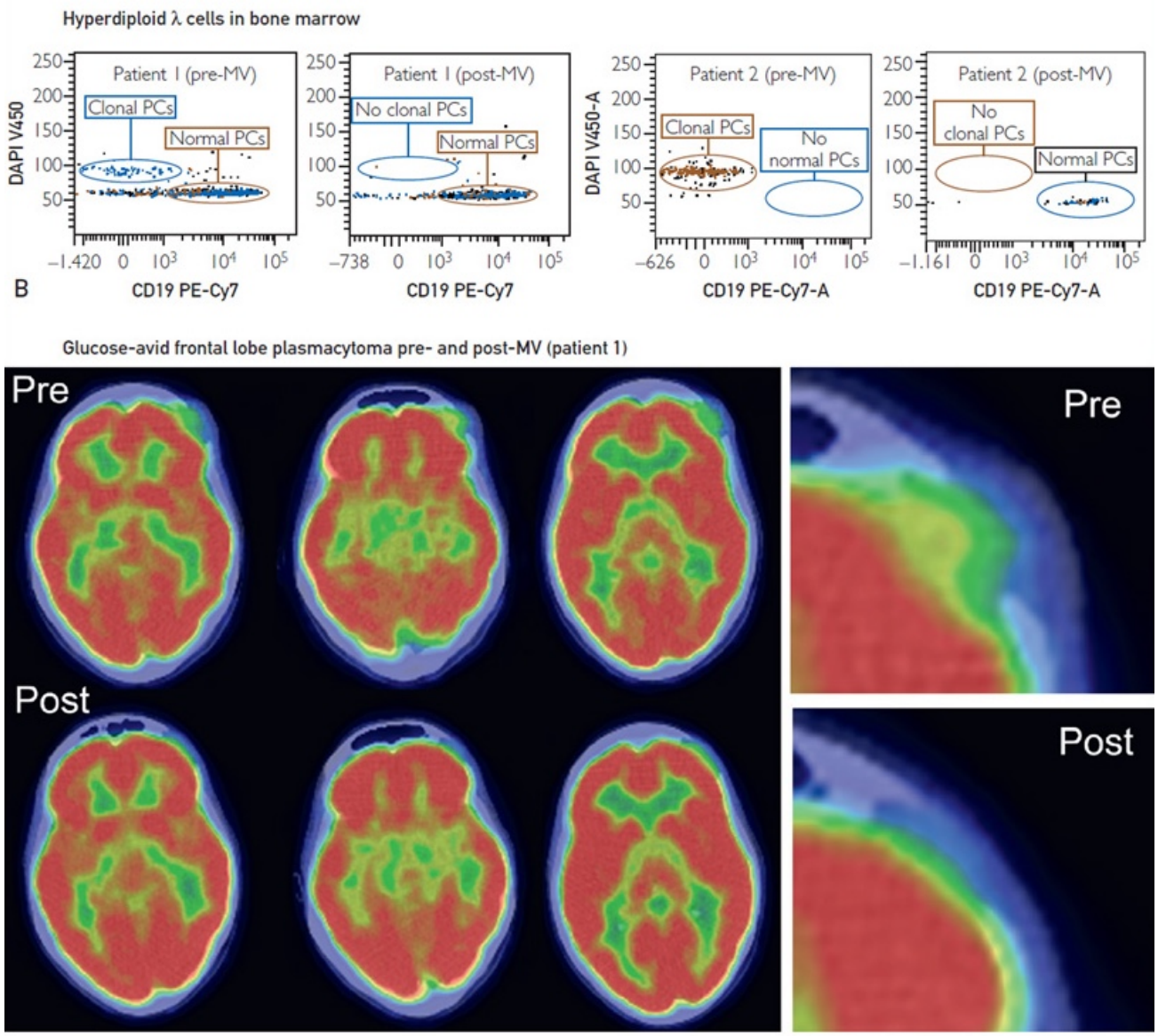


Measles virus



- Attenuated vaccine strain, preferentially replicates in tumors (cannot antagonize STAT1 and MDA5)
- Includes gene for human sodium-iodide symporter (NIS)
- During virotherapy, ɣ-emitting isotopes given allow visualization of virus replication in tumor
- Administration of β-emitting isotopes can induce radiation poisoning

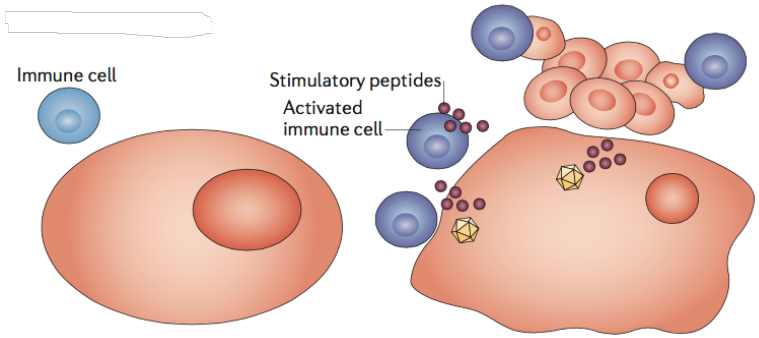
http://www.ncbi.nlm.nih.gov/pubmed/24292552



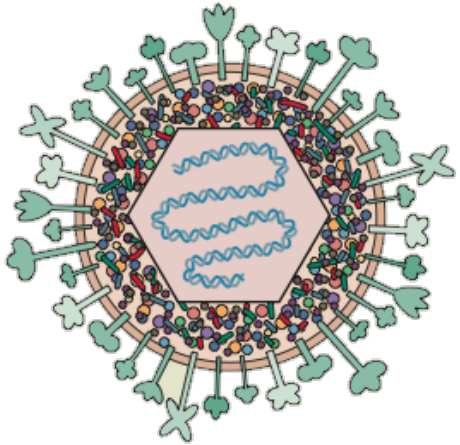
- Two patients with multiple myeloma given 10^{11} particles IV
- One of two had complete remission

http://www.ncbi.nlm.nih.gov/pubmed/24835528

Herpesvirus - Talimogene laherparepvec



aka T-VEC



- Includes gene for GM-CSF: stimulate production of granulocytes and macrophages which stimulate adaptive immunity against tumor antigens
- Deletion of ICP34.5, US11 causes tumor-specific replication
- ICP47 deleted, no inhibition of antigen presentation
- Phase III completed for melanoma, intratumoral: 16% response vs 2% for GM-CSF alone

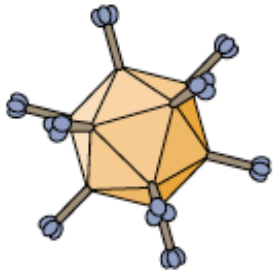
FDA Panel Gives Thumbs-Up To Amgen's Virus-Based Melanoma Drug

Two days after the U.S. Food & Drug Administration signaled it might quash Amgen AMGN +1.29% Inc.'s attempt to usher in a whole new class of virus-based cancer drugs, an advisory panel for the agency voted "yes" on the question of whether the company's experimental melanoma treatment, talimogene laherparepvec (T-VEC), has a favorable enough risk-benefit profile for approval. T-VEC is made from a modified herpes bug and would likely be the first among a number of virus-based cancer treatments in the pharma pipeline to be approved. Of the 23 members on the panel, all but one voted in favor of approval. The FDA doesn't have to follow the direction of its advisory panels but it usually does.

T-VEC, which is injected directly into melanoma tumors, is a version of herpes simplex virus that has been genetically modified so it only replicates in cancer cells, destroying tumors while sparing healthy tissues. It also includes a gene that encodes a type of cytokine, or protein, called granulocyte-macrophage colony-stimulating factor (GM-CSF), which recruits immune-boosting cells to the tumor. The hope is that the combination of the virus with GM-CSF will not only speed up the drug's cancer-killing effect, but also stimulate the immune system to continue killing melanoma cells—even those that have traveled away from the treated tumor.

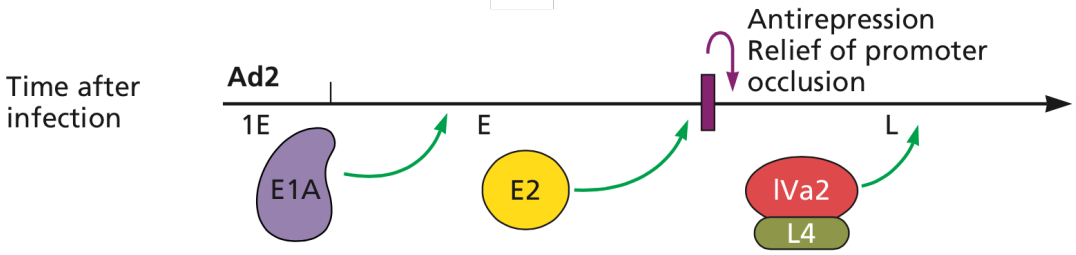
http://www.forbes.com/sites/arleneweintraub/2015/04/29/fda-panel-gives-thumbs-up-to-amgens-virus-based-melanoma-drug/

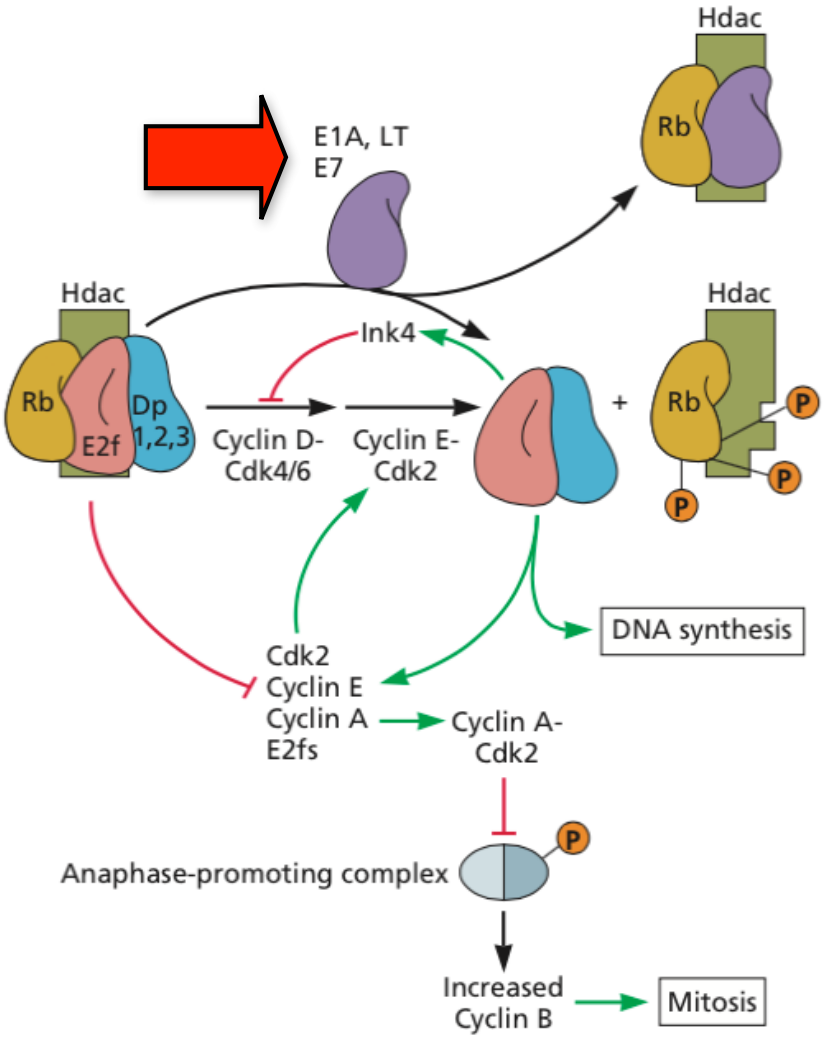
Adenovirus - CG0070

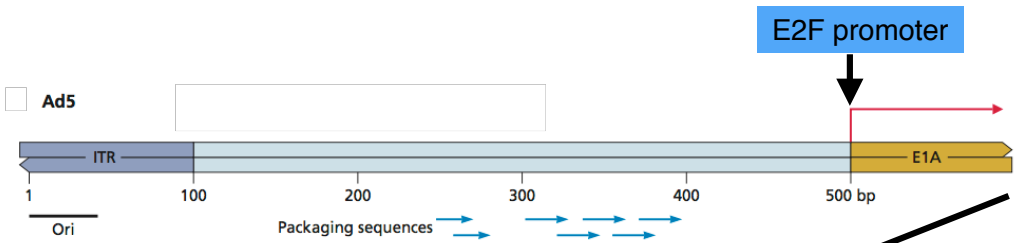


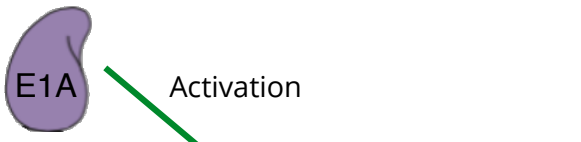
- Armed with GM-CSF
- Preferentially replicates in Rb-deficient tumors
- Phase II, III for bladder cancer (intravesical infusion)

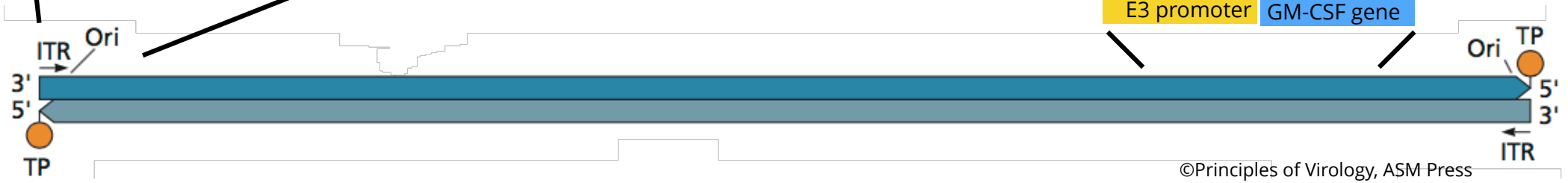
Adenovirus - CG0070





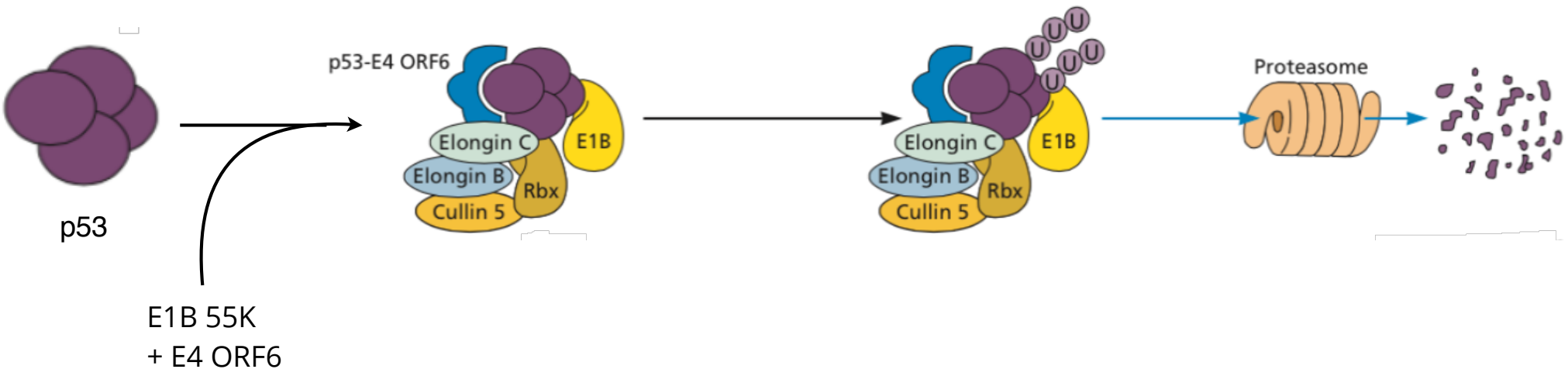




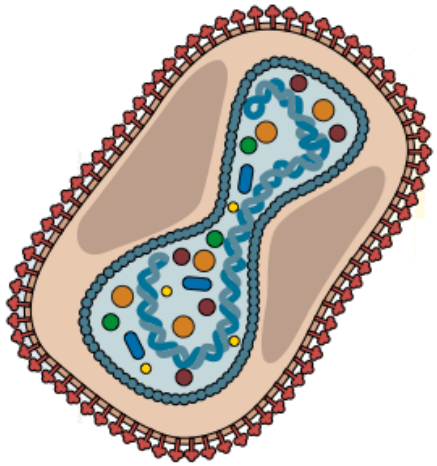


Adenovirus Oncorine

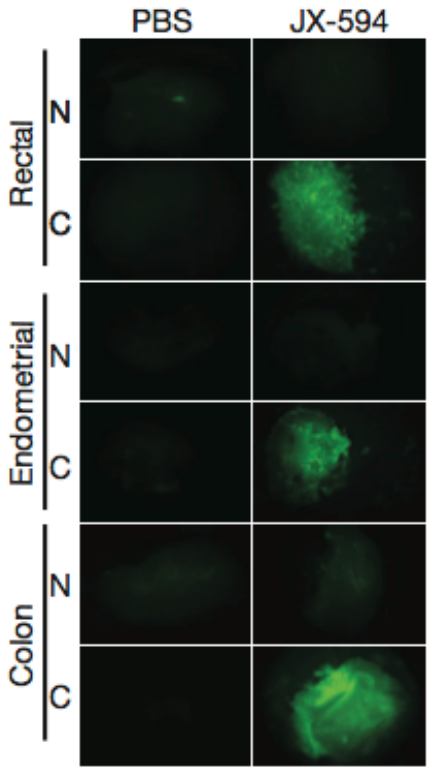
- Licensed in China for treatment of head and neck tumors
- Viral E1B-55K gene is deleted: needed to degrade p53
- Only replicates in p53 deficient tumors



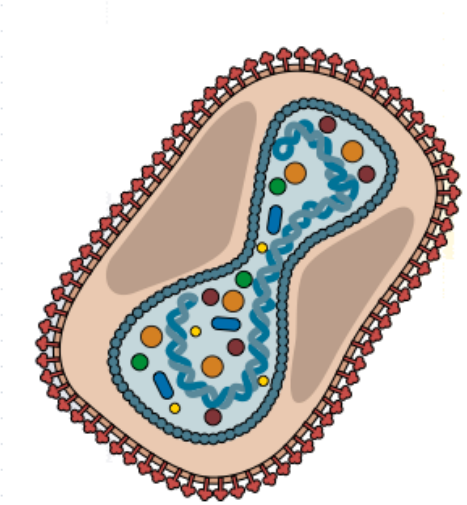
Vaccinia virus JX-594



- Armed with GM-CSF
- Thymidine kinase gene deleted: elevated in tumors
- Tested for the ability to reach metastatic tumors after intravenous delivery (viremia)
- 23 patients with advanced, treatment-refractory solid tumors (lung, colorectal, melanoma, throid, pancreatic, gastric, ovarian, mesothelioma)



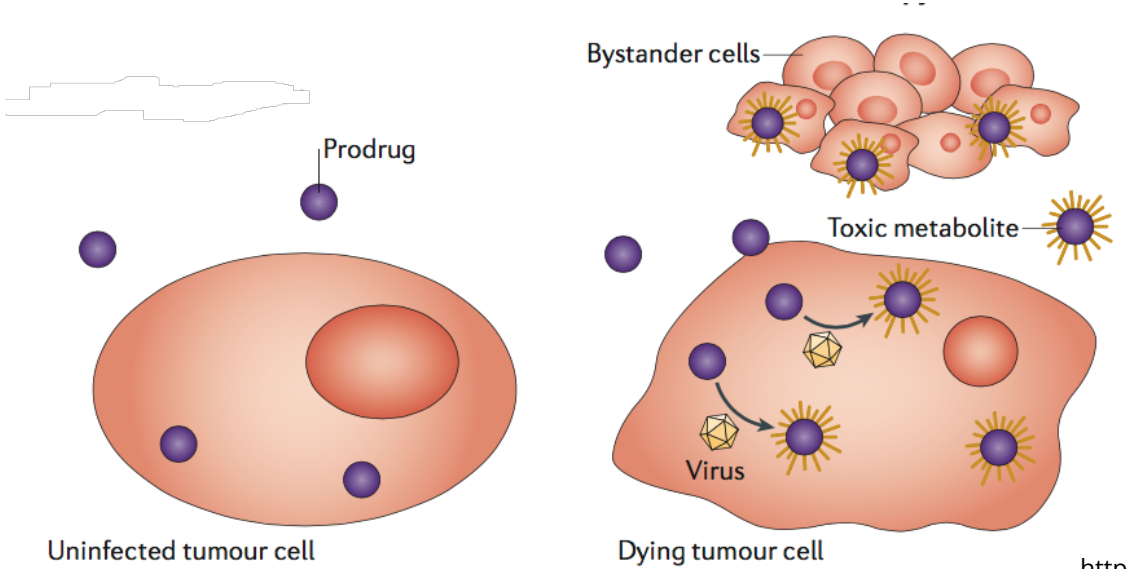
Vaccinia virus JX-594



- Virus replicated in tumors in nearly half of patients (β-gal)
- Anti-tumor activity demonstrated in half of patients
- Proof of concept

Arming with prodrug convertases

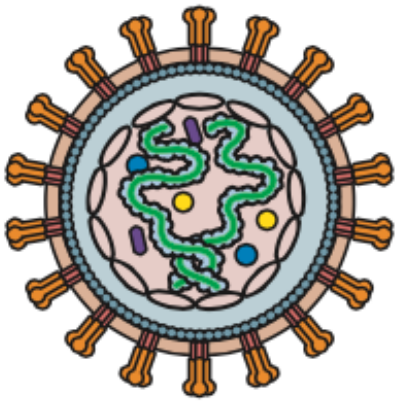
- Thymidine kinase converts ganciclovir to ganciclovir triphosphate
- Cytosine deaminase converts 5-fluorocytosine to 5-fluorouracil
- These nucleoside analogues stop DNA replication of tumor cells



http://www.ncbi.nlm.nih.gov/pubmed/24292552

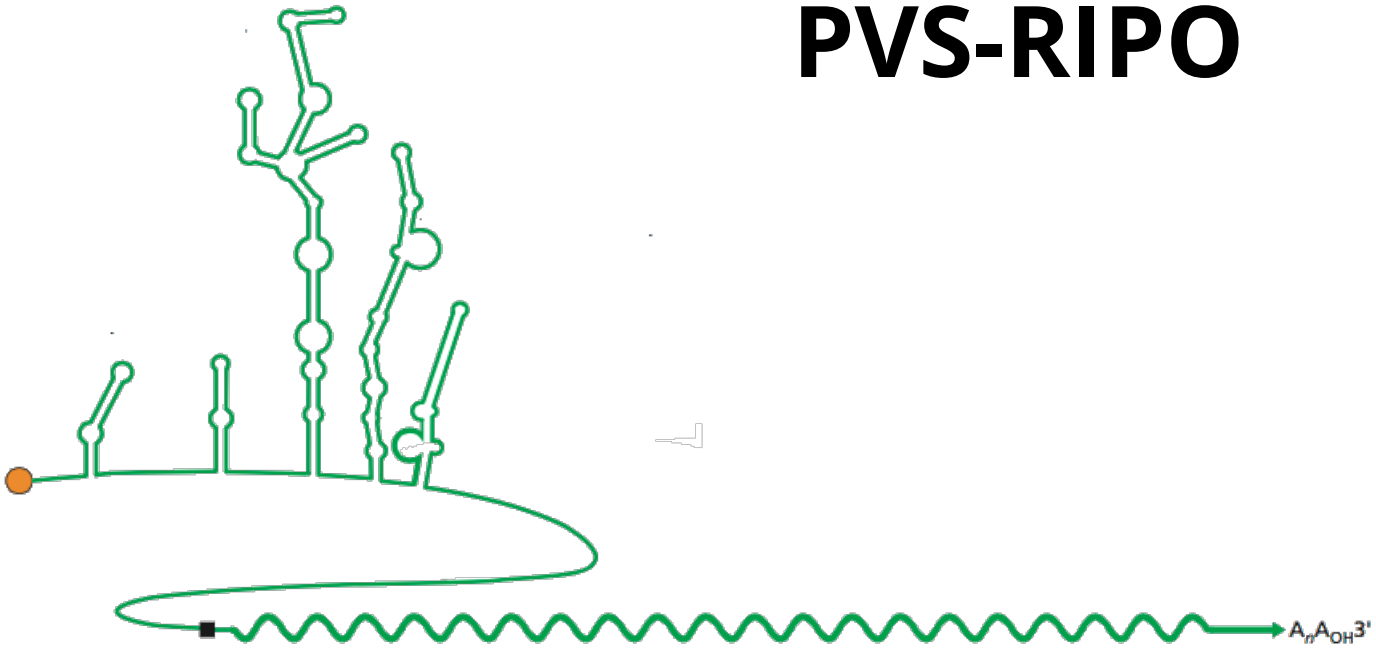
Toca 511

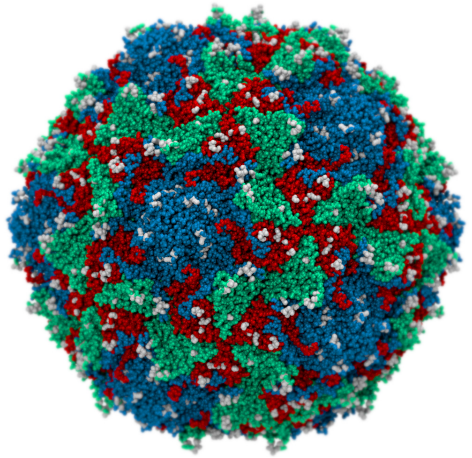
Tocagen
Touching Your Life



- Amphotropic murine leukemia retrovirus armed with cytosine deaminase
- Given intratumoral or intravenous with 5-fluorocytosine
- Phase I and II for glioma

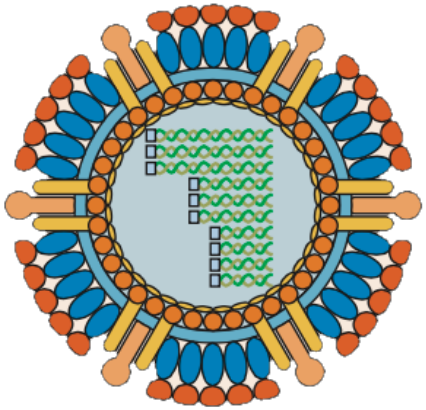
PVS-RIPO





- Poliovirus Sabin with IRES from rhinovirus 2: attenuating
- Tumor cells up-regulate poliovirus receptor
- Intratumoral, Phase I for glioma
- As seen on 60 minutes

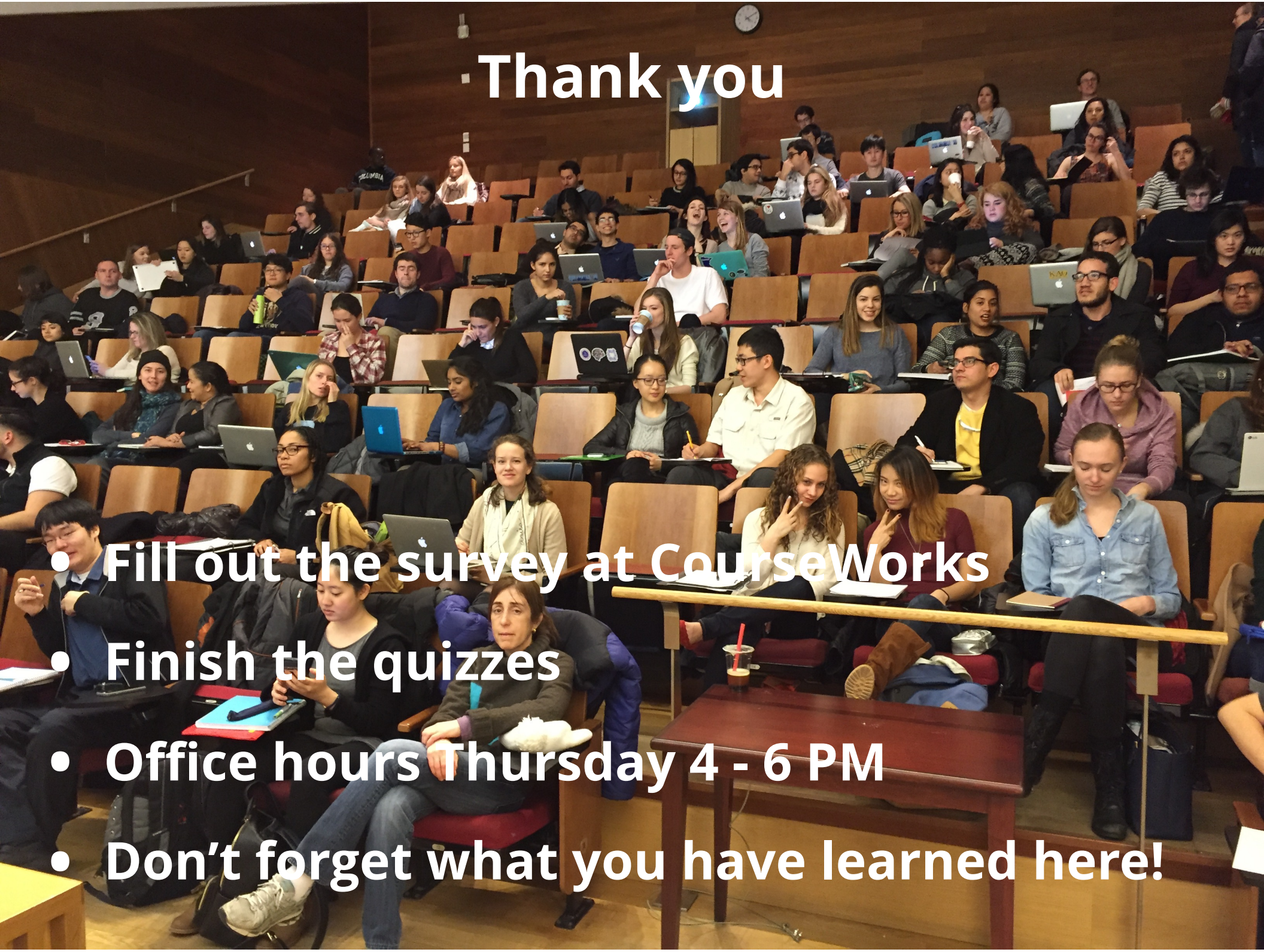
Reolysin



- Reovirus, unmodified, not pathogenic for humans
- Found to kill cells with activated Ras pathway
- Phase III for head and neck tumors, many other studies

The importance of basic research

- Viral gene therapy is possible because of fundamental advances in virology, recombinant DNA, and clinical science
- There must be a balance between translational research and basic research



Thank you

- Fill out the survey at CourseWorks
- Finish the quizzes
- Office hours Thursday 4 - 6 PM
- Don’t forget what you have learned here!