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
Poliovirus

(+) Viral RNA

Infection

Cultured cells

Transfection

cDNA synthesis and cloning

Transfection

Transfection

In vitro RNA synthesis

(+) strand RNA transcript

Poliovirus DNA

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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
Adenovirus vectors

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

To prevent interactions with immune system elements (reduce immunogenicity)

Modification of Ad vectors with lipidic microvesicles or polymers

Modification with peptides and natural ligands linked on polymers

FGF2

FGF2

Geneic engineering of the fiber protein to insert peptides or to generate chimeric fibers

Change the tropism of the Ad vector

Targeting specific receptors expressed on different cells

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Adenovirus-associated virus vectors

4.6 kb

mRNAs

5' [ssDNA]

Rep 78
Rep 68
Rep 52
Rep 40

Rep ORF

cap ORF

p5 p19 p40

TR

TR

4.6 kb

3.9 kb

3.6 kb

3.3 kb

2.3 kb

A AUG

A AUG

A AUG

A AUG

VP1

VP2

VP3

AAP
Adenovirus-associated virus vectors

- Long-term gene expression
- Multiple serotypes

Diagram showing the components of a viral vector, including ITR, rep, cap, and the transgene, with additional helper functions such as E1, E2, E4, and VA.
Vector modifications

cap genes from different AAV

Mutagenesis

Shuffling

Peptide insertion

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Retrovirus vectors

Simple genome (ALV) Proviral DNA
Core Enzymes Envelope
gag pol env
LTR 5' gag pol env LTR 3'
MA CA PR RT IN SU TM

Complex genome (HIV-1) Proviral DNA
Core Enzymes Envelope
vif rev RRE
LTR 5' gag pol env LTR 3'
vpr vpu tat nef rev
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

- Capsid plasmid
- Envelope plasmid
- Transgene

- Virus without viral genome
- Retrovirus containing foreign gene
Modified vaccinia virus Ankara (MVA)

- Replication-deficient vector: smallpox vaccine, infectious in avian but not mammalian cells (passaged in chicken cells, assembly block)
- BSL-1
- Intrinsic adjuvant
- Large capacity
Modified vaccinia virus Ankara (MVA)
<table>
<thead>
<tr>
<th>Vector</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrovirus</td>
<td>Moderate capacity (9-10 kb)</td>
<td>Low titers</td>
</tr>
<tr>
<td></td>
<td>High transduction efficiency</td>
<td>Oncogenic potential</td>
</tr>
<tr>
<td></td>
<td>Broad tropism</td>
<td>Low capsid stability</td>
</tr>
<tr>
<td></td>
<td>Stable expression (provirus)</td>
<td>No transduction resting cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Moderate capacity (9-10 kb)</td>
<td>Moderate titers</td>
</tr>
<tr>
<td></td>
<td>High transduction efficiency of resting cells</td>
<td>Oncogenic potential</td>
</tr>
<tr>
<td></td>
<td>Broad tropism</td>
<td>Low capsid stability</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>AAV</td>
<td>High titers</td>
<td>Immunogenic</td>
</tr>
<tr>
<td></td>
<td>Genome persistence in resting cells</td>
<td>Low capacity (&lt;5 kb)</td>
</tr>
<tr>
<td></td>
<td>Non-pathogenic in humans</td>
<td>Low transduction efficiency in cell culture</td>
</tr>
<tr>
<td></td>
<td>Modified targeting by capsid modifications</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus (3rd gen)</td>
<td>High capacity (37 kb)</td>
<td>Immunogenic</td>
</tr>
<tr>
<td></td>
<td>No acute toxicity</td>
<td>Transient effects in cycling cells</td>
</tr>
<tr>
<td></td>
<td>High titers</td>
<td>Low transduction of Car cells</td>
</tr>
<tr>
<td></td>
<td>High transduction efficiency</td>
<td>Liver targeting in vivo</td>
</tr>
<tr>
<td></td>
<td>Broad tropism</td>
<td>Genome persistence in resting cells</td>
</tr>
<tr>
<td></td>
<td>Liver targeting in vivo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genome persistence in resting cells</td>
<td></td>
</tr>
</tbody>
</table>
Indications addressed by gene therapy clinical trials

- Cancer diseases 64.2% (n=1376)
- Monogenic diseases 9.2% (n=196)
- Infectious diseases 8% (n=172)
- Cardiovascular diseases 7.8% (n=168)
- Neurological diseases 1.8% (n=39)
- Ocular diseases 1.5% (n=33)
- Inflammatory diseases 0.7% (n=14)
- Other diseases 1.9% (n=41)
- Gene marking 2.3% (n=50)
- Healthy volunteers 2.6% (n=52)

- Adenovirus 22.5% (n=496)
- Retrovirus 18.8% (n=415)
- Naked/Plasmid DNA 17.5% (n=386)
- Vaccinia virus 7.3% (n=162)
- Adeno-associated virus 5.8% (n=127)
- Lipofection 5.2% (n=115)
- Lentivirus 4.6% (n=101)
- Poxvirus 4.5% (n=100)
- Herpes simplex virus 3.1% (n=68)
- Other vectors 7.4% (n=163)
- Unknown 3.3% (n=73)
**DIRECT DELIVERY**

- **Therapeutic transgene**
  - The therapeutic transgene is packaged into a delivery vehicle such as a virus.
  - ...and injected into the patient.
  - ...and readministered to the patient.

**CELL-BASED DELIVERY**

- **Therapeutic transgene**
  - The therapeutic transgene is introduced into a delivery cell such as a stem cell that is often derived from the patient.
  - The genetically modified cells (e.g., stem cells) are multiplied in the laboratory.

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AIDS Immunoprophylaxis with AAV

Kinetics of early CTL response peak as early viremia falls

Adverse effect of removing CD8+ T cells in SIV-infected macaques, CTL correlate with slower disease in humans

Viral escape, CD4 T cell loss contributes to dysfunction

© Principles of Virology, ASM Press
# Ebolavirus GP vaccine in Ad/MVA

<table>
<thead>
<tr>
<th>Vector</th>
<th>Dose (PU)</th>
<th>Protection$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single shot</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChAd3</td>
<td>$1 \times 10^{11}$</td>
<td>2/4</td>
</tr>
<tr>
<td>ChAd3</td>
<td>$1 \times 10^{10}$</td>
<td>0/4</td>
</tr>
<tr>
<td><strong>Prime-boost</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChAd3/ChAd63</td>
<td>$1 \times 10^{10}/1 \times 10^{10}$</td>
<td>1/3</td>
</tr>
<tr>
<td>ChAd3/ChAd63</td>
<td>$1 \times 10^{10}/1 \times 10^{10}$</td>
<td>1/4</td>
</tr>
<tr>
<td>ChAd3/MVA</td>
<td>$1 \times 10^{10}/1 \times 10^{8}$</td>
<td>4/4</td>
</tr>
</tbody>
</table>

IM challenge  

GSK, NIAID/NIH
IN challenge, 1000 pfu, 28 da
RhCMV-based SIV vaccine in macaques

Group A
(RhCMV/SIV vector-vaccinated)

Plasma viral load (Log copy eq. per mL)

Follow up discontinued

Time post infection (weeks)

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
<table>
<thead>
<tr>
<th>Disease</th>
<th>Defect</th>
<th>Incidence</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe combined immunodeficiency</td>
<td>Adenosine deaminase (25%)</td>
<td>&lt;1 in $10^5$</td>
<td>Retrovirus</td>
</tr>
<tr>
<td></td>
<td>Common cytokine receptor $\gamma$ chain</td>
<td>1 in 50-100,000</td>
<td></td>
</tr>
<tr>
<td>Liproprotein lipase deficiency</td>
<td>Lipoprotein lipase</td>
<td>1-2 in $10^6$</td>
<td>AAV</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Factor IX deficiency</td>
<td>1 in 30,000 males</td>
<td>AAV</td>
</tr>
<tr>
<td>Hemoglobinopathies and thalassemias</td>
<td>Defects in $\alpha$- or $\beta$- globin gene</td>
<td>1 in 600 in specific ethnic groups</td>
<td>Lentivirus</td>
</tr>
<tr>
<td>$\alpha$1-antitrypsin deficiency (emphysema, liver disease)</td>
<td>$\alpha$1-antitrypsin not produced</td>
<td>1 in 3,500</td>
<td>AAV</td>
</tr>
<tr>
<td>Retinal degenerative disease, Leber’s congenital amaurosis</td>
<td>Retinal pigment epithelium-specific 65 kDa protein</td>
<td>Inherited retinopathies (1 in 2000) &lt;10% LCA (1 in 80,000)</td>
<td>AAV</td>
</tr>
<tr>
<td>X-linked adrenoleukodystrophy</td>
<td>ABCD1 transporter</td>
<td>1 in 20-50,000</td>
<td>Lentivirus</td>
</tr>
<tr>
<td>Wiskott-Aldrich syndrome (eczema-thrombocytopenia-immunodeficiency syndrome)</td>
<td>Was protein</td>
<td>1-10 in $10^6$ males</td>
<td>Lentivirus</td>
</tr>
</tbody>
</table>
First human viral gene therapy: 1993

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

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

- Mutations in \textit{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 (2 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

http://www.microbe.tv/viruswatch/cancer-killing-viruses/
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

a  Positive targeting

Healthy cell

Tumour cell

Virus

Promoter

b  Negative targeting

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

- Identified as a contaminant of cell culture medium, from bovine serum or porcine trypsin
- Selective tropism for cancers with neuroendocrine 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, $\gamma$-emitting isotopes given allow visualization of virus replication in tumor
- Administration of $\beta$-emitting isotopes can induce radiation poisoning
Two patients with multiple myeloma given $10^{11}$ particles IV

One of two had complete remission
Herpesvirus - Talimogene laherparepvec

- 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

aka T-VEC
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 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.
Adenovirus - CG0070

- Armed with GM-CSF
- Preferentially replicates in Rb-deficient tumors
- Phase II, III for bladder cancer (intravesical infusion)
Adenovirus - CG0070

E2F promoter

GM-CSF gene

E1A

Activation

Adenovirus - CG0070

Time after infection

Ad2

1E

E1A

E

E2

E2F

iVa2

L4

Antirepression Relief of promoter occlusion

Ad5

Ori

Packaging sequences

E1A

TR

ORI

E1A

E3 promoter

GM-CSF gene

Hdac

Rb

Cdk2

E1A, LT

E7

Cyclin D-

Cdk4/6

Cyclin E-

Cdk2

Ink4

DNA synthesis

Rb

P

P

Cdk2

Cyclin A-

Cyclin E-

Cyclin D-

E2f

P

Anaphase-promoting complex

Increased Cyclin B

Mitosis

E1A

Activation

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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, thyroid, pancreatic, gastric, ovarian, mesothelioma)
Vaccinia virus JX-594

- Virus replicated in tumors in nearly half of patients ($\beta$-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
• Amphotropic murine leukemia retrovirus armed with cytosine deaminase
• Given intratumoral or intravenous with 5-fluorocytosine
• Phase I and II for glioma
- 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!