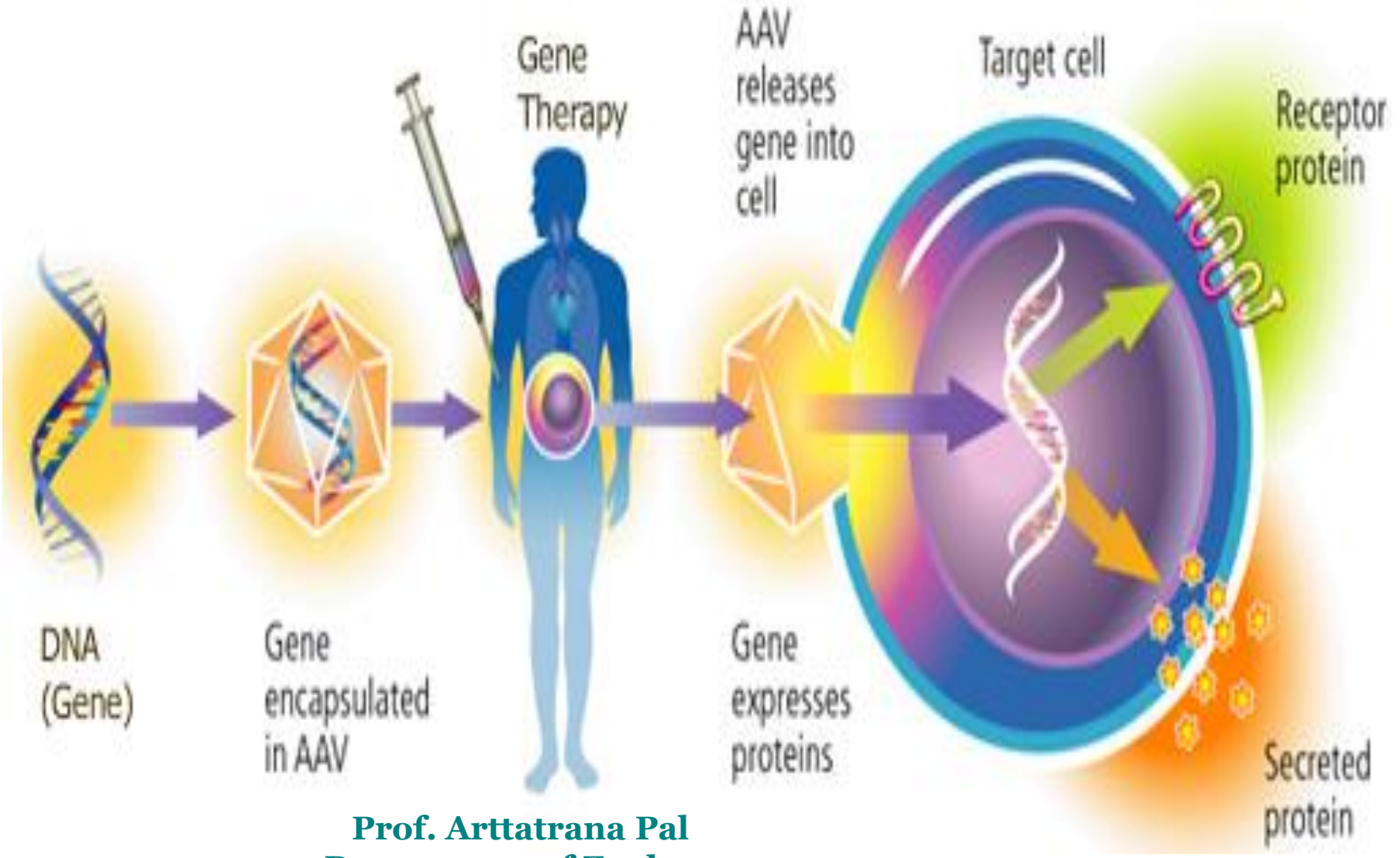
Gene Therapy- I



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What is gene therapy? Why is it used?

- Gene therapy is the application of genetic principles in the treatment of human disease

- Gene therapy = Introduction of genetic material into normal cells in order to:
  - counteract the effect of a disease gene or
  - introduce a new function

- GT is used to correct a deficient phenotype so that sufficient amounts of a normal gene product are synthesized → to improve a genetic disorder

Can be applied as therapy for cancers, inherited disorders, infectious diseases, immune system disorders, etc.

History of gene therapy

1930's	"genetic engineering" - plant/animal breeding
60's	first ideas of using genes therapeutically
50's-70's	gene transfer developed
70's-80's	recombinant DNA technology
1990	first GT in humans
2001	596 GT clinical trials (3464 patients)

Types of gene therapy

1. Monogenic gene therapy
    - Provides genes to encode for the production of a specific protein
        - Cystic fibrosis, Muscular dystrophy, Sickle cell disease, Haemophilia, etc.
2. Suicide gene therapy
    - Provide 'suicide' genes to target cancer cells for destruction
        - Cancer
3. Antisense gene therapy
    - Provides a single stranded gene in an 'antisense' (backward) orientation to block the production of harmful proteins
        - AIDS/HIV

Different Delivery Systems

In vivo* versus *ex vivo

1. ***ex vivo*- cells removed, genetically modified, transplanted back into a patient**
   - **delivery of genes takes place in the body**

2. ***in vivo*- direct transfer of genetic material into patient**
   - **delivery takes place out of the body, and then cells are placed back into the body**

Getting genes into cells

- ***In vivo* versus *ex vivo***
  - ***In vivo* = intravenous or intramuscular or non-invasive (sniffable)**
  - ***Ex vivo* = hepatocytes, skin fibroblasts, haematopoietic cells (bioreactors)**

- **Gene delivery approaches**
  - **Physical methods**
  - **Non-viral vectors**
  - **Viral vectors**

In vivo techniques

***In vivo* techniques usually utilize viral vectors**

- **Virus = carrier of desired gene**
- **Virus is usually "crippled" to disable its ability to cause disease**
- **Viral methods have proved to be the most efficient to date**
- **Many viral vectors can stable integrate the desired gene into the target cell's genome**

Problem: Replication defective viruses adversely affect the virus' normal ability to spread genes in the body

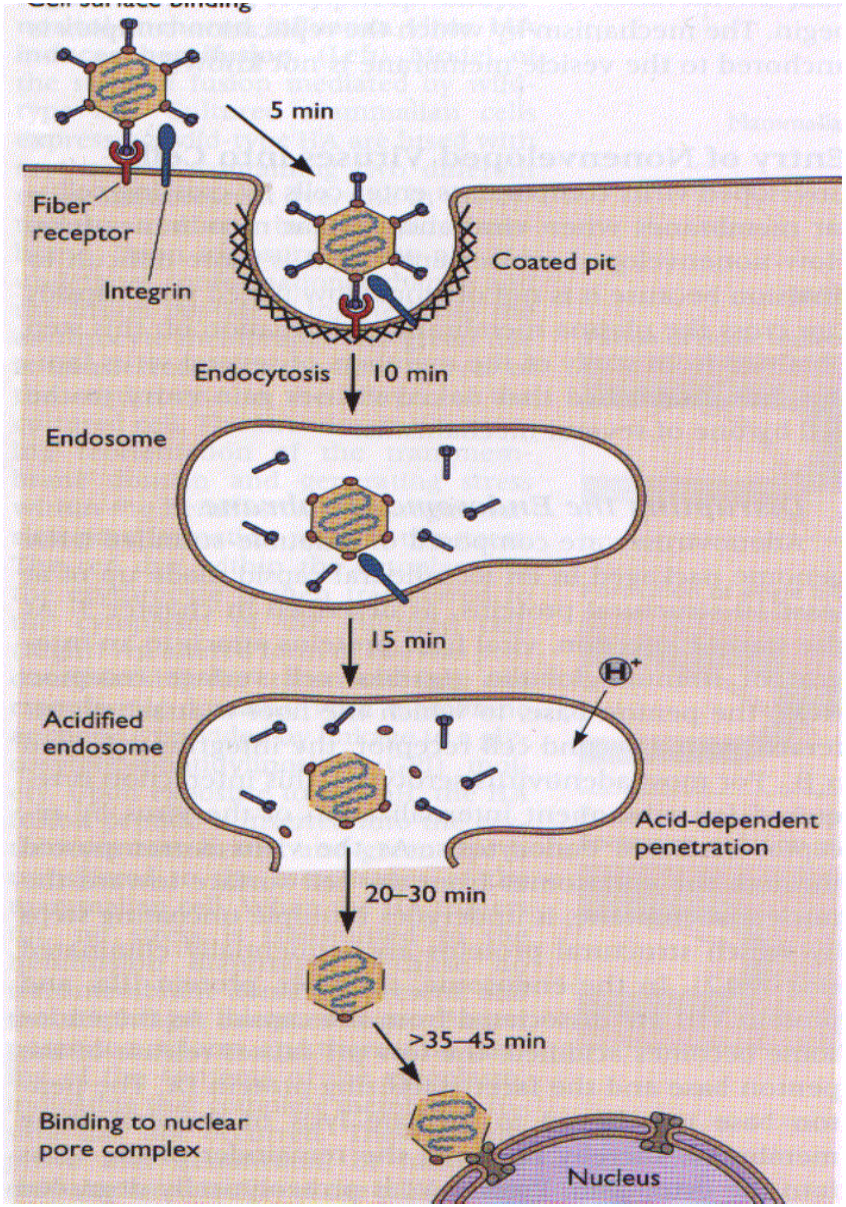
- **Reliant on diffusion and spread**
- **Hampered by small intercellular spaces for transport**
- **Restricted by viral-binding ligands on cell surface → therefore cannot advance far**

Viral vectors

"Viruses are highly evolved natural vectors for the transfer of foreign genetic information into cells" [Kay et al 2001]

But to improve safety,
they need to be replication defective

Viral vectors



Compared to naked DNA, virus particles provide a relatively efficient means of transporting DNA into cells, for expression in the nucleus as recombinant genes *(example = adenovirus)*.

Flint *et al.* Principles of Virology, ASM Press, 2000]

Ideal Vector for Gene Transfer

- High concentration of virus allowing many cells to be infected or transduced
- Convenience and reproducibility of production
- Ability to transduce dividing and non-dividing cells
- Ability to integrate into a site-specific location in the host chromosome, or to be successfully maintained as stable episome
- A transcriptional unit that can respond to manipulation of its regulatory elements
- Ability to target the desired type of cell
- No components that elicit an immune response

Introduction of Genes Into Animals

METHODS	MAJOR LIMITATIONS
Calcium Phosphate	
DEAE (Diethylaminoethyl) Dextran	Low Efficiency
Cationic Lipids, Liposomes	
Diethylaminoethyl cellulose (DEAE)	
Direct DNA Injections	Low Efficiency
Electroporation	Transient expression

Introduction of Genes Into Animals

VIRAL VECTORS: MAJOR LIMITATIONS

Papova (SV40, Polyoma)	Size; Host range
Papilloma (BPV)	Size; Integration, Transformation
Adeno associated (AAV)	Size; production
Adeno	Size; antigenicity, episomal DNA, toxic
Herpes/Vaccinia	Pathogenic, cytotoxic, lytic
Retroviruses	Inability to infect post-mitotic cells
Lentiviruses	Safety, integration

Genetic Defects that are Candidates for Gene Therapy

Disease	Defect	Incidence	Target Cells
Severe combined immunodeficiency (SCID)	Adenosine deaminase (ADA) in 25% of SCID patients	Rare	Bone-marrow cells or T lymphocytes
Hemophilia A	Factor VII deficiency	1:10,000 males	Liver, muscle, fibroblasts or bone marrow cells
Hemophilia B	Factor IX deficiency	1:30,000 males	
Familial hypercholesterolemia	Deficiency of low-density lipoprotein (LDL) raeceptor	1:1 million	Liver
Cystic fibrosis	Faulty transport of salt in lung epithelium	1:3000 Caucasians	Airways in the lungs
Hemoglobinopathies thalassemias	(Structural) defects in the α or β globin gene	1:600 in certain ethnic groups	
Gaucher's disease	Defect in the enzyme glucocerebrosidase	1:450 in Ashkenazi Jews	Bone marrow cells, macrophages
α_1 antitrypsin deficiency inherited emphysema	Lack of α_1 antitrypsin	1:3500	Lung or liver cells
Duchenne muscular distrophy	Lack of dystrophin	1:3000 males	Muscle cells

Vectors Based on RNA Viruses

Features	Retroviral	Lentiviral	Alphaviral
Maximum Insert size	7-7.5 kb	7-7.5 kb	5 kb
Concentrations viral particles/ml	$>10^8$	$>10^8$	$>10^9$
Route of gene delivery	Ex vivo	Ex/In vivo	In vivo
Integration	Yes	Yes	No
Duration of expression in vivo	Shorter than theorized	Long	Short
Stability	Good	Not tested	Good
Ease of Preparation scale up	Pilot scale up up to 20-50 liters	Not known	Not known
Immunological problems	Few	Few	Unknown
Pre-existing host immunity	Unlikely	Unlikely, except in AIDS patients	No
Safety problems	Insertional mutagenesis?	Insertional mutagenesis?	Few

Vectors Based on DNA and on DNA Viruses

Features	Adenoviruses	Adeno-associated viruses	Herpesviruses	Vaccinia virus	Naked DNA /Lipid DNA
Maximum Insert size	7.5 kb	4.5kb	~30kb	>25 kb	Unlimited size
Concentrations viral particles/ml	$>10^{10}$	$>10^{12}$	$>10^{8}$	10^{7}-10^{9}	No limitation
Route of gene delivery	Ex/In vivo	Ex/In vivo	Ex vivo	Ex/In vivo	Ex/In vivo
Integration	No	Yes/No	No	No	very poor
Duration of expression in vivo	Short	Long	Short/ Long in CNS?	Short	Short
Stability	Good	Good	Unknown	Good	Very good
Ease of Preparation scale up	Easy to scale up	Difficult to purify, difficult to scale up	Not yet tried	Vaccine production facilities exist	Easy to scale up
Immunological problems	Extensive	Not known	Not known	Extensive	None
Pre-existing host immunity	Yes	Yes	Yes	Diminishing as unvaccinated population grows	No
Safety	Inflammatory response, toxicity	Inflammatory response, toxicity	Neurovirulence? Insertional mutagenesis	Disseminated vaccinia in immunocompromised hosts	None?

Adenoviral vectors

Advantages

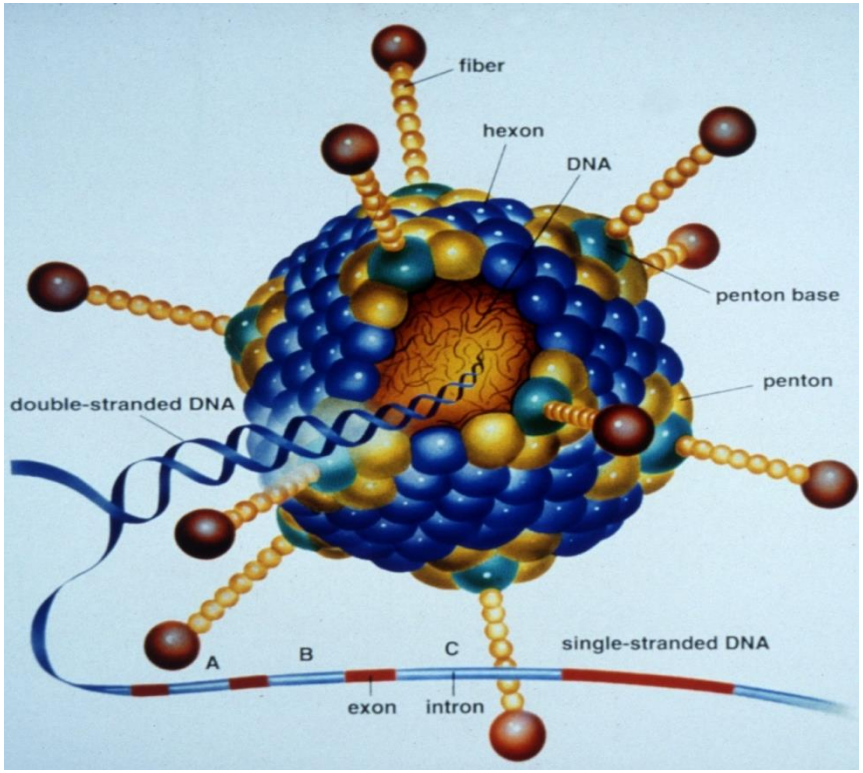
- Higher titer
- Efficient transduction of nondividing cells
- In vitro and in vivo

Disadvantages

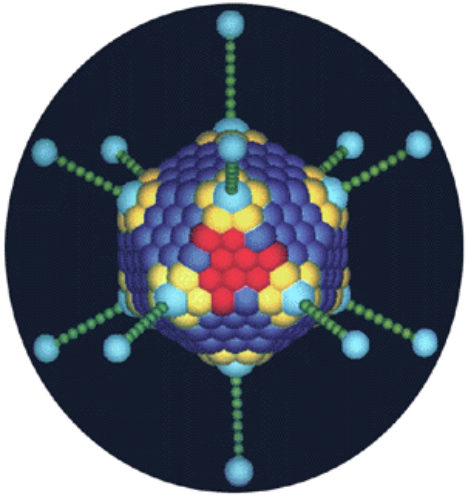
- Toxicity
- Immunological response
- Prior exposure

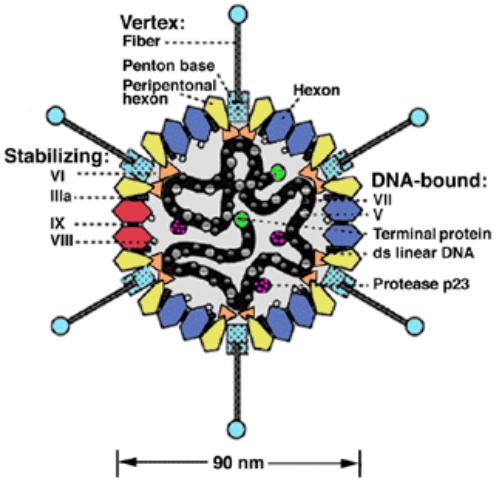
Structure

- **Size: 70-90nm**
- **Non-enveloped icosahedral virus**
- **Capsid comprised of 3 surface coat proteins**
  - **Fibers**
  - **Pentons**
  - **Hexons**

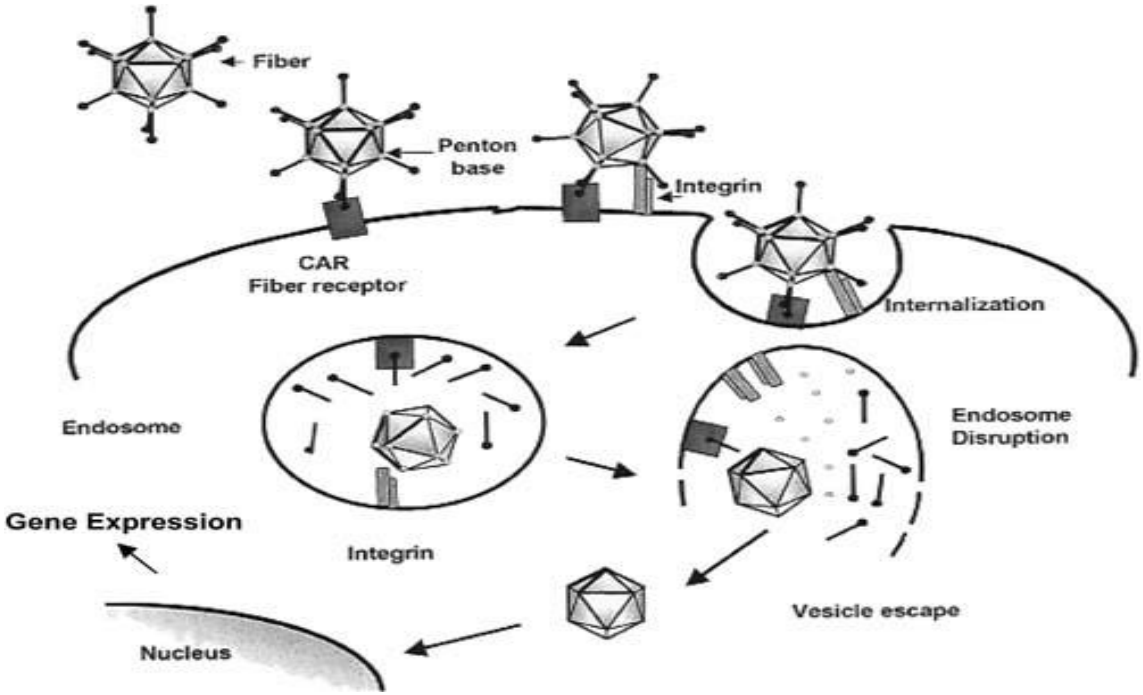


- **Contains linear double stranded DNA**
- **Does not integrate into the host genome**
- **Replicates as an episomal element in the nucleus**





Replication Cycle



1) **Absorption and Penetration**
   - Bind to cell surface receptor
   - Enters cell by endocytosis

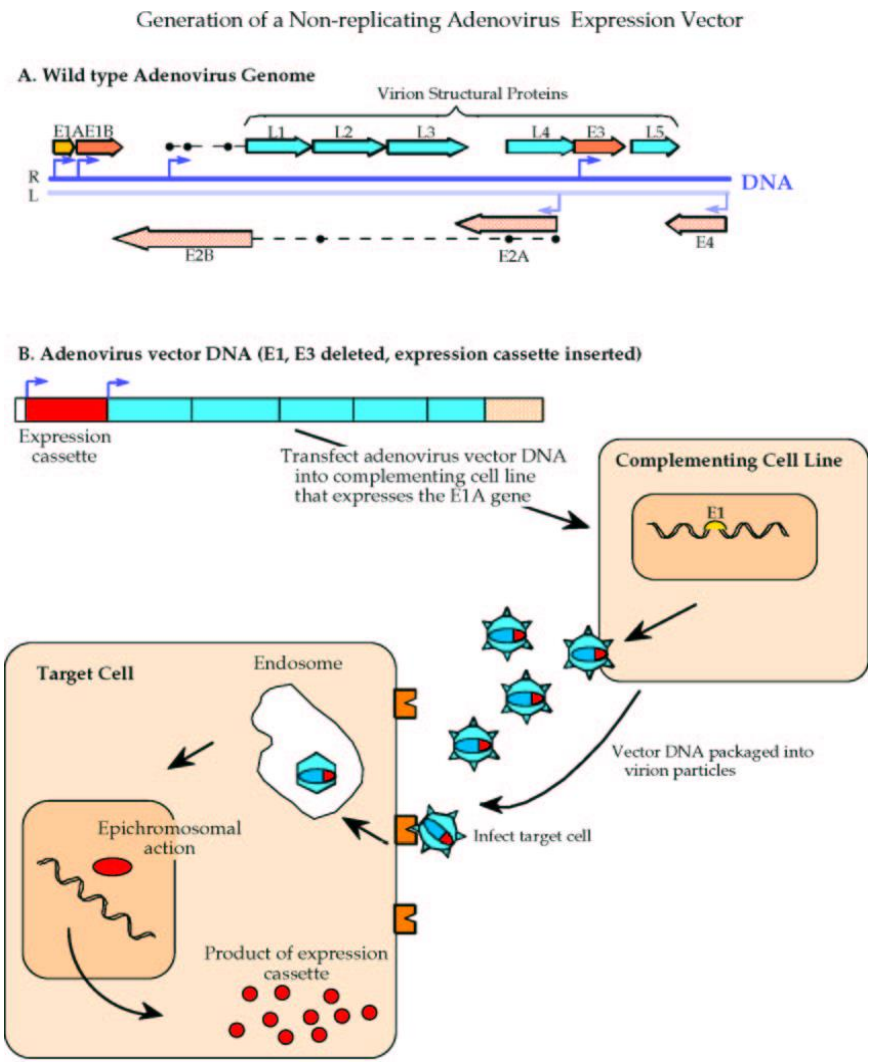
2) **Transcription**
   - Early transcription- Codes for non-structural, regulatory proteins
   - Late transcription- Codes for replication substrates and machinery

3)Assembly

4) Exit- Cell lysis

Adenoviral vectors

- Double-stranded DNA viruses, usually cause benign respiratory disease; serotypes 2 and 5 are used as vectors
- Can infect dividing and non-dividing cells, can be produced at high titers
- Replication-deficient adenovirus vectors can be generated by replacing the E1 or E3 gene, which is essential for replication



The recombinant vectors are then replicated in cells that express the products of the E1 or E3 gene and can be generated in very high concentrations

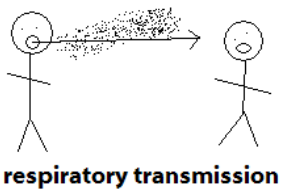
- Cells infected with recombinant adenovirus can express the therapeutic gene, but because essential genes for replication are deleted, the vector can't replicate

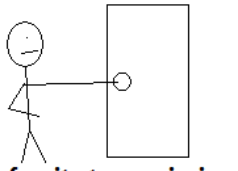
Normal Viral Transmission

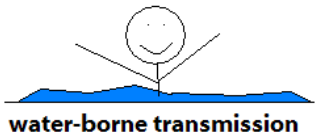
Airborne and Waterborne

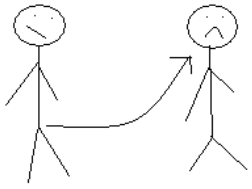
Transmission Routes: Direct and Indirect Contact

- Hand-eye Contact
- Fecal/Oral Contact
- Venereal Contact
- Respiratory Droplet Transmittance
  - *Incubation period of 2-9 days



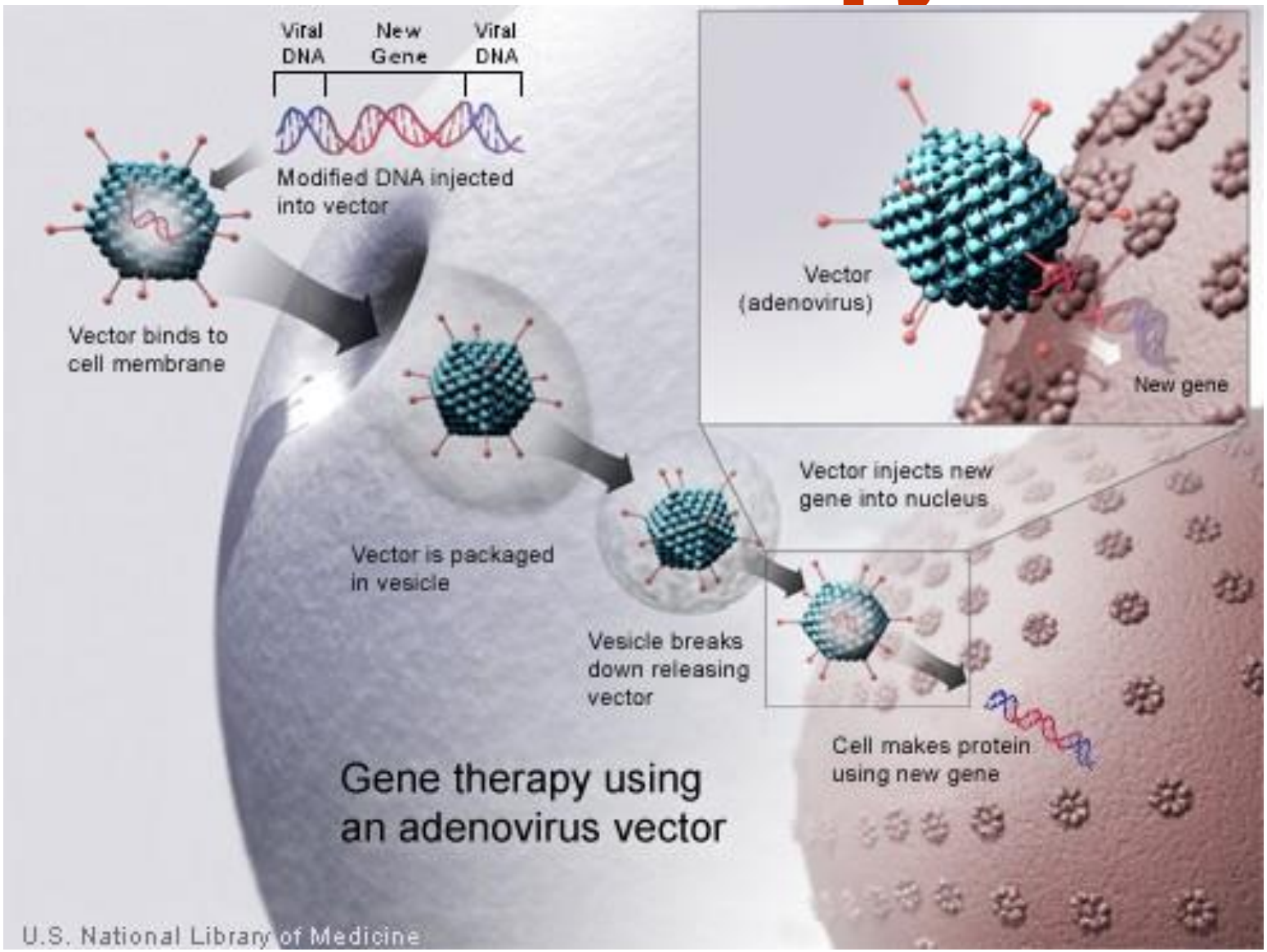






- www.stanford.edu/group/virus/adeno/2005/

Adenovirus use in Gene Therapy



Why adenoviruses are good vectors for gene therapy in cancer patients

- Gene therapy works by manipulating viruses to contain "good genes" in which they can transport to the cells to code for needed protein/hormone/enzyme/etc
- They do not incorporate their genes into the host genome
- The Adenovirus is ubiquitous- it has been isolated from a large number of different species with over 100 known serotypes
- Can rapidly infect a large range of human cells
- Low pathogenicity in humans
- Can hold large segments of DNA
- Genome does not undergo rearrangement at high rates
- DNA is easy to manipulate with current recombinant DNA techniques

How this virus is used in cancer therapy

1) Mutagen compensation
   - **Replacement or inactivation of oncogenes**
2) Molecular chemotherapy (Suicide)
   - **Also known as suicide of target tumor cells**
3) Genetic immunopotentiation
   - **Modifies tumor or immune cells to amplify immunological recognition of neoplastic cells**
4) Genetic modulation of resistance/sensitivity
   - **Modify sensitivity or resistance of cells to chemotherapy (Chemoprotection)**
5) Antiangiogenic gene therapy (tumor suppressor)
   - **Targets the development of new vessels in tumor tissue, inhibiting its growth**

Cancer Trials with

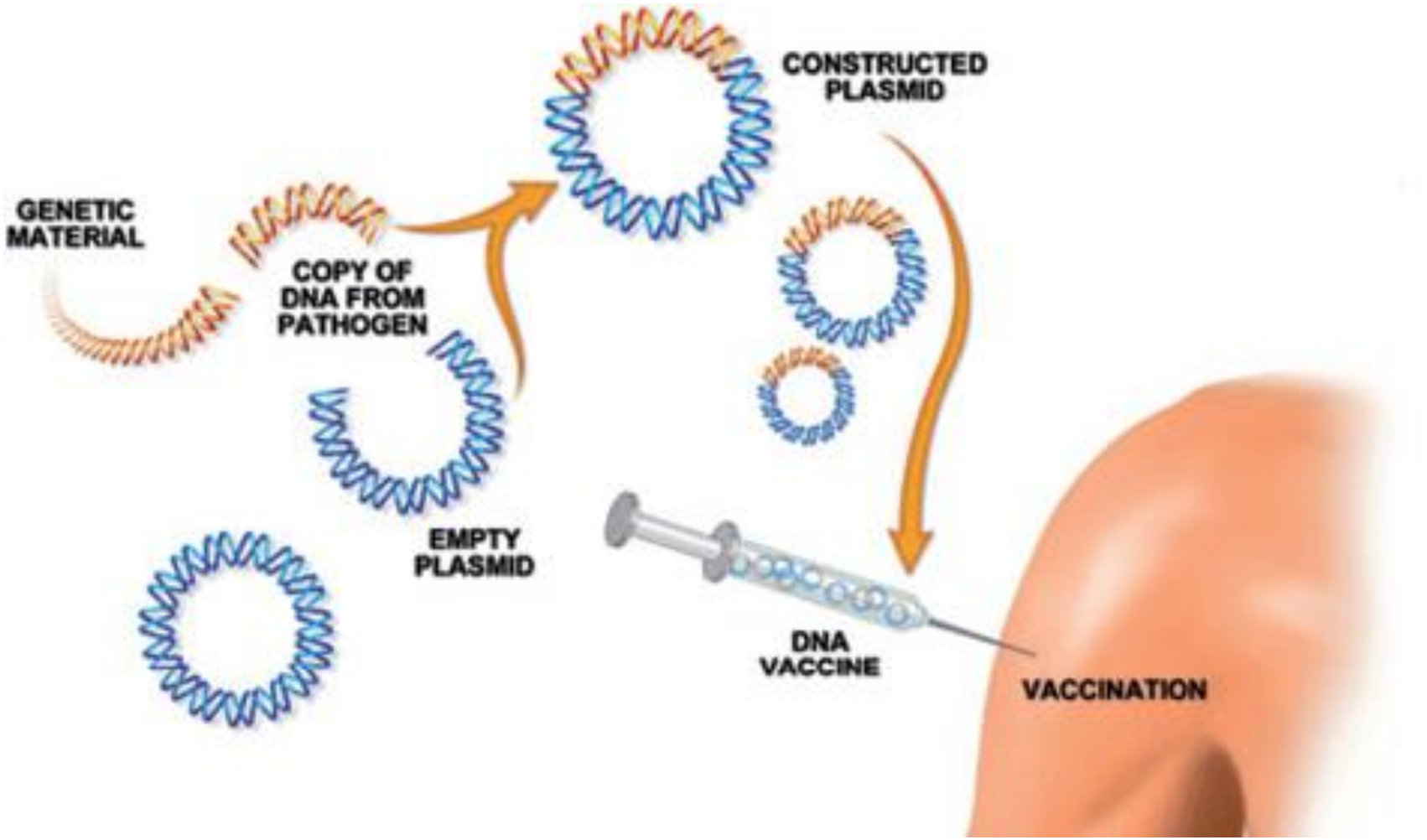
Table 2. Ongoing phase II clinical trials of gene therapy with adenoviral vectors for treatment of cancer

Indication	Gene delivered	Action	Combination	Route of administration	*n* of pts. to date	Investigators
Head and neck cancer	E1b del	Cytolysis	Chemo	Intratumoral	30	*Link*
Prostate cancer	*p53*	Gene transfer		Intratumoral	n/c	*Logothetis*
Head and neck cancer	*p53*	Gene transfer	Chemo	Intratumoral	78	*Breau*
NSCLC	*p53*	Gene transfer	Chemo	Intratumoral	n/c	*Dobbs*
Head and neck cancer	*p53*	Gene transfer		Intratumoral	39	*Dreicer*
NSCLC	*p53*	Gene transfer	XRT	Intratumoral	6	*Swisher*
Melanoma	MART/1 + gp100	Vaccination	IL-2	Subcutaneous	36	*Haluska*
Hepatic metastases from colon cancer	*p53*	Gene transfer	Chemo	Intrahepatic	n/c	*Venook*
Prostate cancer	HSV-tk	Suicide with Valacyclovir	XRT	Intratumoral	50	*Butler*
Prostate cancer	PSA replicative virus	Cytolytic PSA guided	XRT	Intratumoral and intravenous	n/c	*Terris*
NSCLC	GM-CSF	Cytokine vaccination		Intradermal	22	*Smith II*
Renal cell carcinoma	Mod B-7.1	Immuno-stimulation	IL-2	Subcutaneous	n/c	*Anonia*
Chronic lymphocytic leukemia	*CD 154*	Immuno-stimulation		Intravenous	n/c	*Wierda*

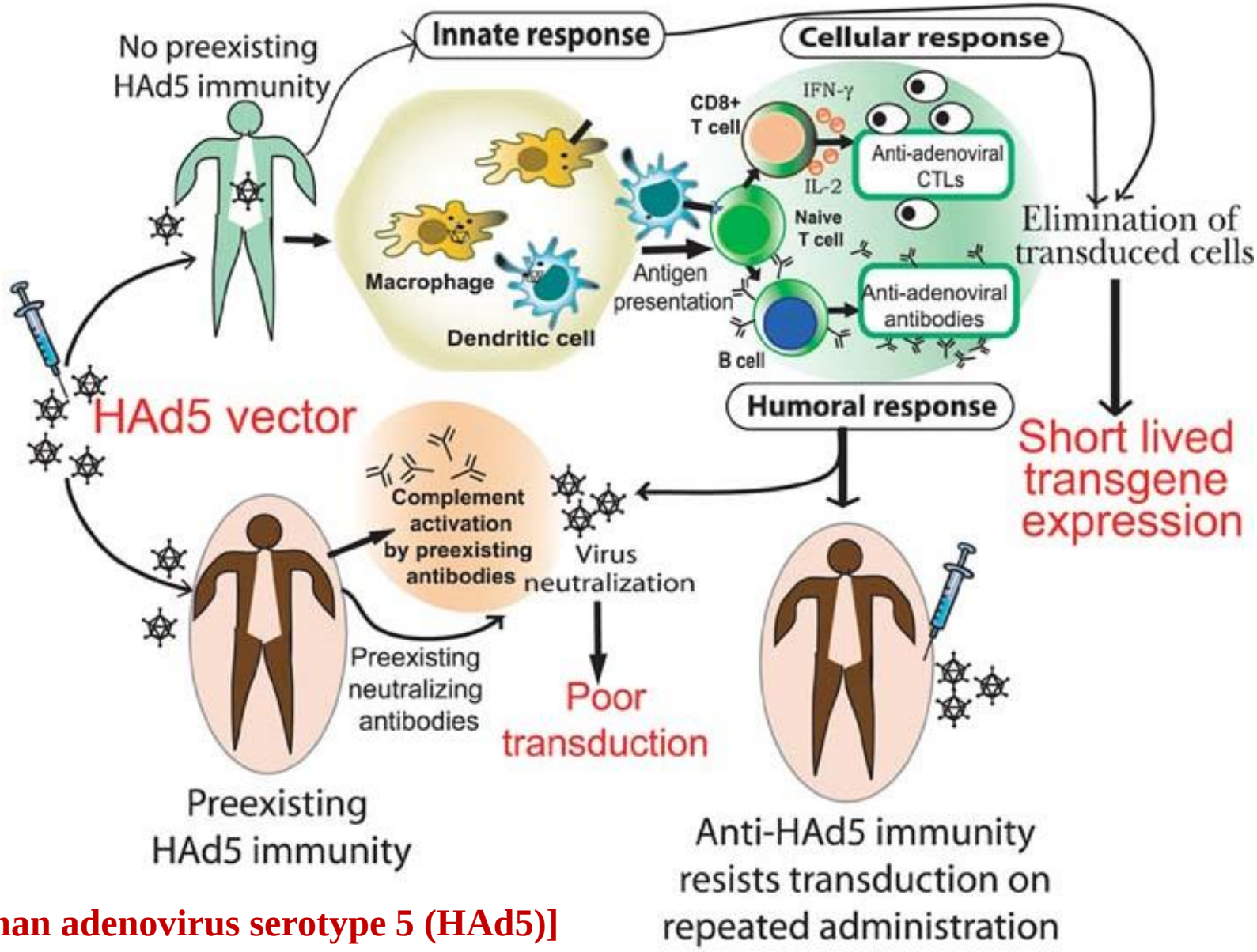
Abbreviations: Chemo = chemotherapy; del = deleted; HSV-tk = herpes simplex virus thymidine kinase; IL-2 = interleukin-2; n/c = not communicated; NSCLC = non-small cell lung cancer; PSA = prostate-specific antigen; XRT = radiotherapy

Source: *Journal of Gene Medicine* website (http://www.wiley.co.uk/wileychi/genmed)

Adenovirus use as a vector in vaccines



Using a Human Adenovirus Vector in Vaccination



[Human adenovirus serotype 5 (HAd5)]

Adenovirus vaccine use in humans

Human Adenovirus Serotypes

Over 100 Known Serotypes

Most Common:

- **HA-vd4**
- **HA-vd 7**

Diagnosis

Respiratory Tract Infection

- **Common cold symptoms**
- **Sore Throat**
- **Severe cough**
- **Swollen lymph nodes**
- **Headache**
- **Non-productive “croupy” cough**

Diagnosis

Intestinal Tract Infection

- **Abrupt onset of water diarrhea**
- **Fever**
- **Abdominal Tenderness**
- **Vomiting**

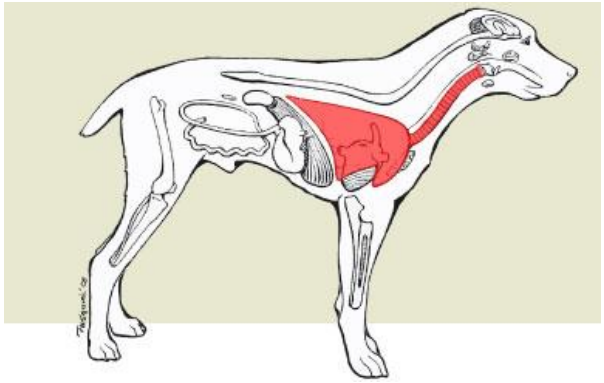
NB: Both cases have very similar symptoms to common cold and influenza

- **Respiratory secretion culture**
- **Stool culture**
- **Chest x-ray**
- **Blood work**

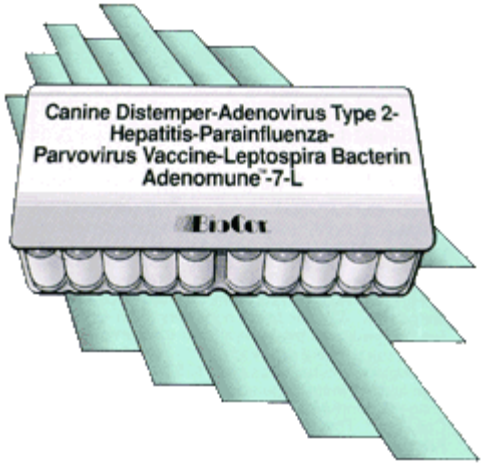
Adenovirus vaccine use in tracheobronchitis “kennel cough”

- **CA-v1:** causes Infectious Canine Hepatitis

- **CA-v2:** causes Infectious Tracheobronchitis



-Type 2-Measl Canine Distemper-Adenovirus es-Parainfluenza Vaccine is a Modified Live Vaccine



-Vaccination against CAdv-2 is done through a 5-way or 7-way vaccine in which other infectious viral strains are vaccinated against.

-Administered in 2 forms: Intranasal & SubQ

-Freeze-Dried and stored at 2-7°C

Future work using adenovirus as a vector

- The majority of clinical trials done using the adenovirus in gene therapy have been phase 1
  - **Phase 1 trials are used to determine safety, feasibility, and toxicity of the process**

- The Phase 1 trials have set the stage for future work with the next generation of Adeno vectors that will show less stimulation of the host immune system and can be selectively targeted to specific tissues

- Live recombinant adenovirus vaccine are being developed
  - **Provides hope for practitioners in using a more economical vaccine that provides a longer lasting immunity**
  - **Ongoing research is aimed at determining effectiveness of recombinant vaccines in animals**

Adenoviral vectors- Limitations

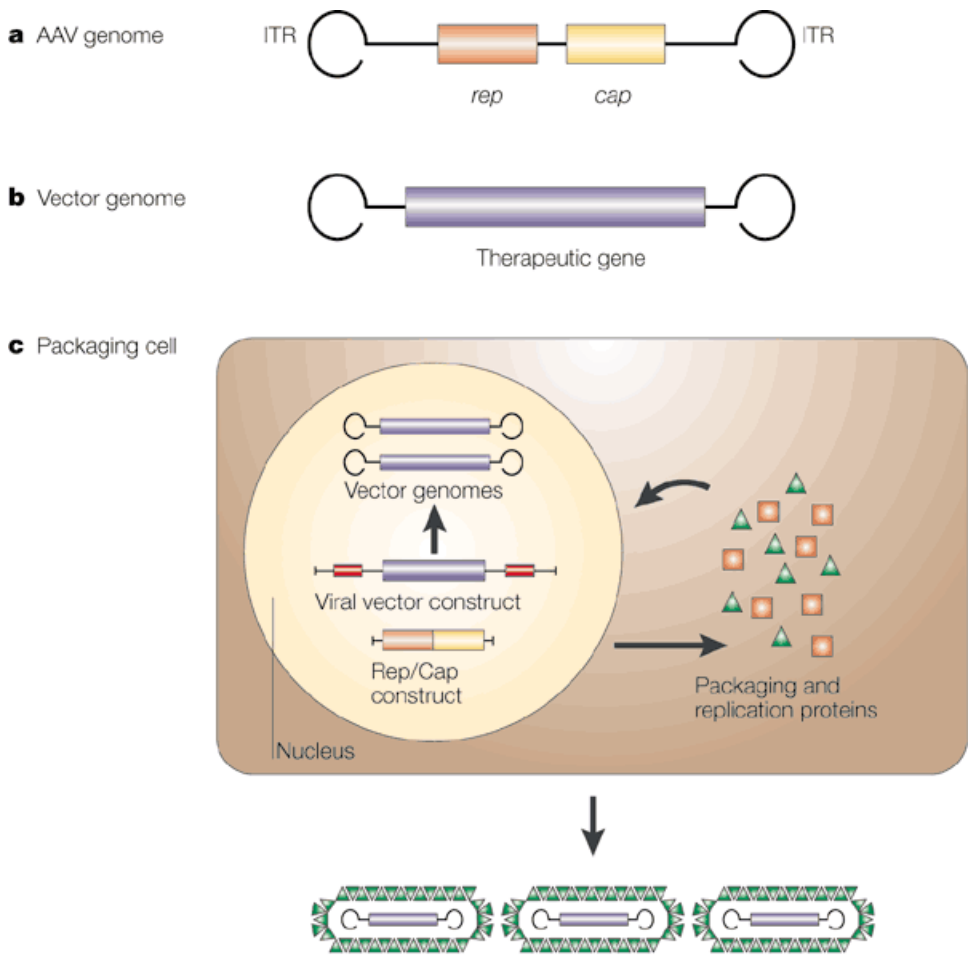
- Adenoviral vectors can infect cells in vivo, causing them to express high levels of the transgene. However, expression lasts for only a short time (5-10 days post-infection)

- Immune response is the reason behind the short-term expression

- Immune reaction is potent, eliciting both the cell-killing "cellular" response and the antibody producing "humoral" response

- Humoral response results in generation of antibodies to adenoviral proteins and prevents any subsequent infection if a second injection of the recombinant adenovirus is given

Adeno-associated viral vectors



- **AAV is a simple, non-pathogenic, single stranded DNA virus dependent on the helper virus (usually adenovirus) to replicate and 4.6 kilobases**

- **It has two genes (cap and rep), sandwiched between inverted terminal repeats (ITRs) that define the beginning and the end of the virus and contain the packaging sequence**

- **The cap gene encodes viral capsid proteins and the rep gene product is involved in viral replication and integration**

- **It can infect a variety of cell types and in the presence of the rep gene product, the viral DNA can integrate preferentially into human chromosome 19**

Adeno-associated viral vectors

- To produce an AAV vector, the rep and cap genes are replaced with a transgene

- The total length of the insert cannot exceed 4.7 kb, the length of the wild type genome

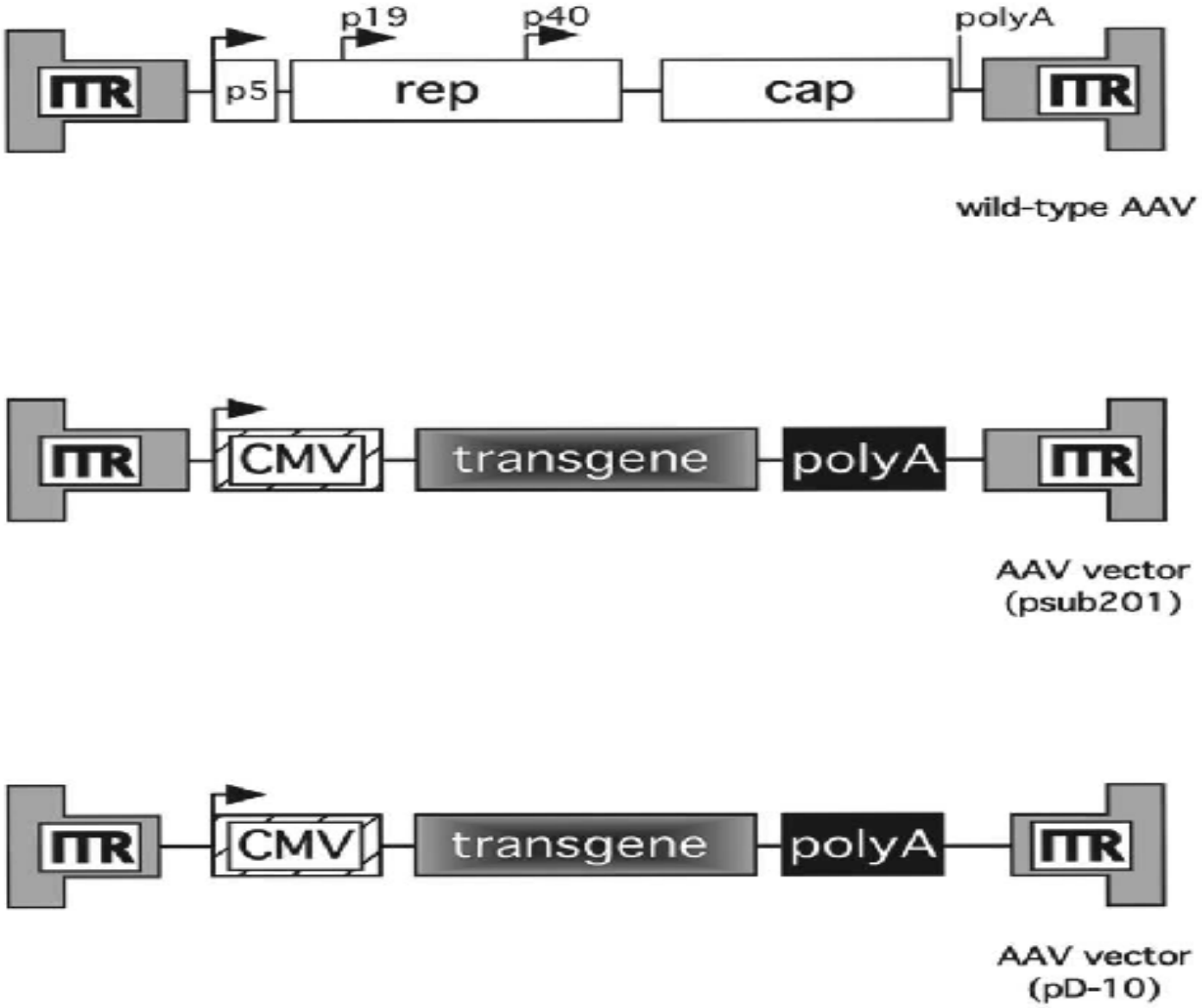
- Production of the recombinant vector requires that rep and cap are provided in trans along with the helper virus gene products

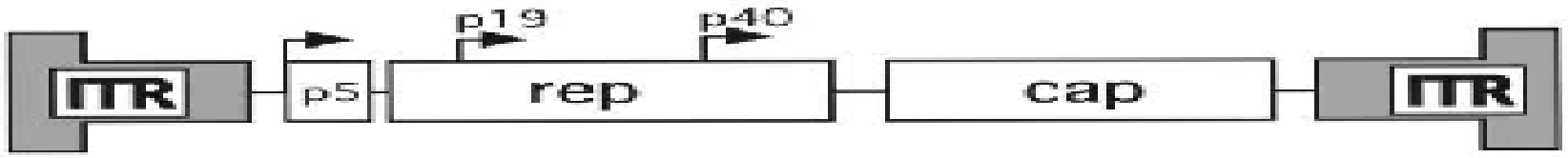
- The current method is to cotransfect two plasmids, one for the vector and another for rep and cap into cells infected with adenovirus

- This method is cumbersome, low yielding and prone to contamination with adenovirus and wild type AAV

- Interest in AAV vectors is due to their integration into the host genome allowing prolonged gene expression

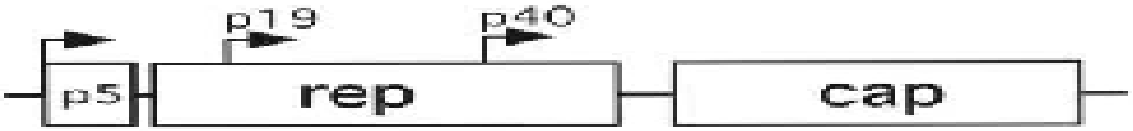
Generation of adeno-associated virus vector



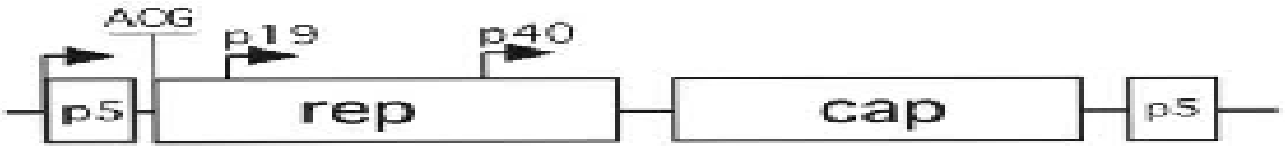


wild-type AAV

AAV packaging plasmids

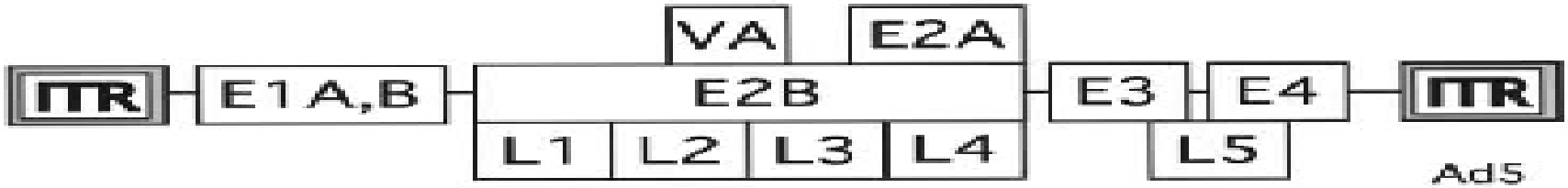


pAAV/Ad

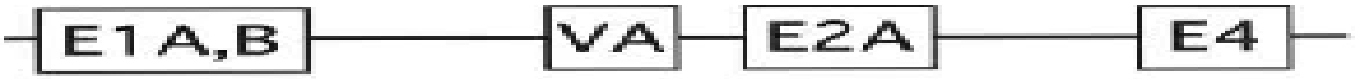


AAV-packaging

AAV helper plasmids



Ad5



Ad-helper

Characteristics of AAV vector

Advantages

- Integration and persistent expression
- No insertional mutagenesis
- Infecting dividing and nondividing cells
- Stable expression
- Safe

Disadvantages

- Size limitation, 4.9 kb (Small genome limits size of foreign DNA)
- Low titer of virus, low level of gene expression
- Labor intensive production
- Status of genome not fully elucidated

Please Follow
Gene Therapy II