

Gene Therapy

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April 16th, 2025

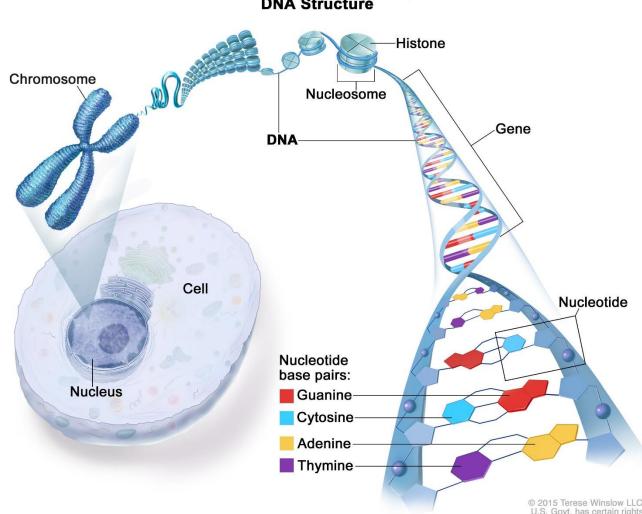




Agenda

- 1. Background Foundational Biology
- 2. Gene Therapy Approaches
- 3. Key Takeaways

Basic Architecture of Genes and DNA

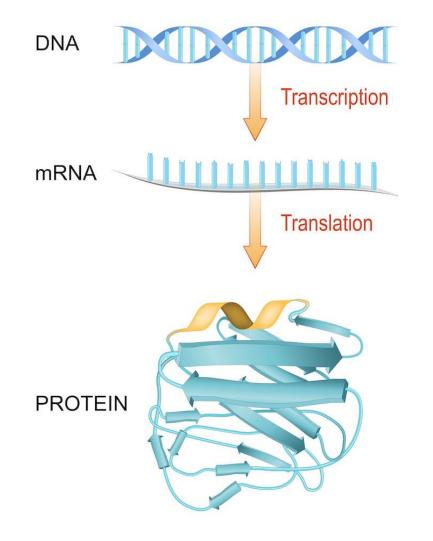


DNA Structure

- Gene A basic genetic unit encoding a function
 - Located in a specific section of DNA on a specific chromosome
 - Encoded by the DNA nucleotide "alphabet" A, G, T & C
 - Genes encoding overt functions typically code for proteins
 - *Alterations* in genes can, but do not always, cause dysfunction
- CTNNB1 gene is located on Chromosome 3 [3p22.1]
- DMN1 gene is located on Chromosome 9 [9q34.11]
- ATM gene is location on Chromosome 11 [11q22.3]



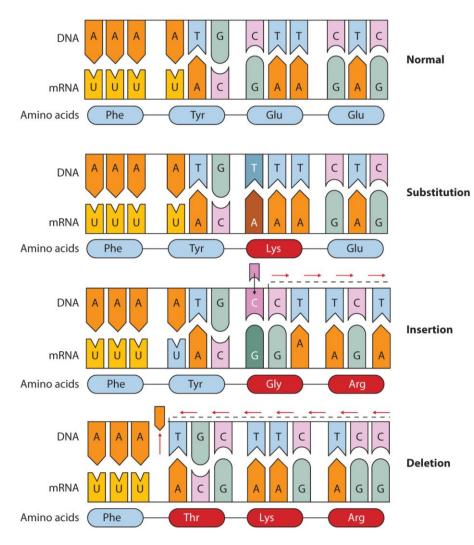
DNA Encodes for Proteins with Specific Functions



DNA encodes ~20,000 genes with discrete cellular • functions utilized by our cells & bodies for our day-to-day existence

- These functions are facilitated by pathways that • utilize the 'DNA -> mRNA -> Protein' program to provide the necessary elements to carry out these day-to-day tasks
 - Examples of protein functions include processing of • metabolites, muscle contraction, DNA replication, movement of other proteins, activation or inhibition of other proteins, and much more...
- Gene therapy seeks to replace or repair these • specific pathways & molecules when typical function is not being provided due to mutation(s)

Common Types of DNA Mutations



THE CAT ATE THE RAT THE KAT ATE THE RAT

Silent Mutation

The meaning of the sentence is the same.

THE CAT ATE THE RAT THE HAT ATE THE RAT

<u>Substitution Mutation</u>

The meaning of the sentence is changed.

THE CAT ATE THE RAT THE <mark>E</mark>CA TAT ETH ERA T Insertion Mutation The sentence no longer makes sense.

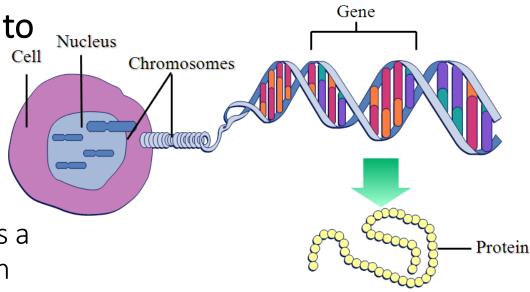
THE CAT ATE THE RAT THE CAA TET HER AT **Deletion Mutation**

The sentence no longer makes sense.



Basic Definition of Gene Therapy

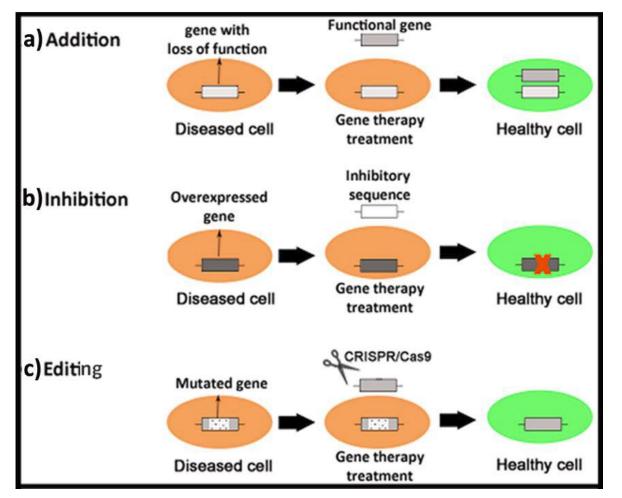
- Treatment or prevention of a [genetic] disease via introduction of genetic material expected to provide a necessary function
- First developed in <u>1972</u> when Theodore Friedmann and Richard Roblin published a paper in *Science* called "Gene therapy for human genetic disease?"
- The first patient to be treated with gene therapy was a four-year-old girl treated at the NIH Clinical Center in <u>1990</u>
 - She had a congenital disease called adenosine deaminase (ADA) deficiency which severely affects immunity and the ability to fight infections
 - Treatment was considered successful (*ex vivo*)



http://igbiologyy.blogspot.com/2014/03/chromosomes-dna-genes-and-alleles.html



Gene Therapy Approaches



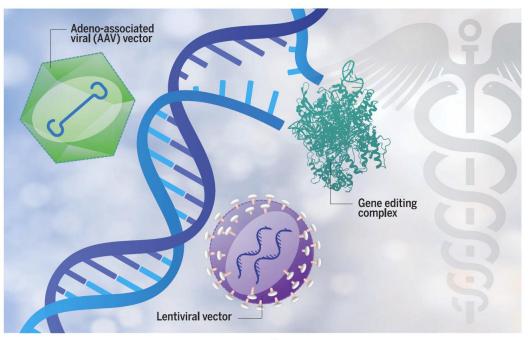
Int. J. Mol. Sci. 2021, 22, 7545. https://doi.org/10.3390/ijms22147545

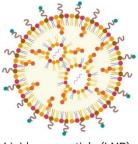
- Gene therapy approaches include the delivery of a package/cargo designed to:
 - <u>Add back</u> a functional or wildtype copy of a mutated or missing gene that is causing disease
 - <u>Inhibit</u>, inactivate, or "knock out," a mutated or overexpressed gene that is functioning improperly
 - <u>Edit</u> a mutated gene back to a functional or wildtype copy of that gene

Note – "wildtype" refers to the natural or common version of a specific protein, DNA, virus, etc. that exists in nature

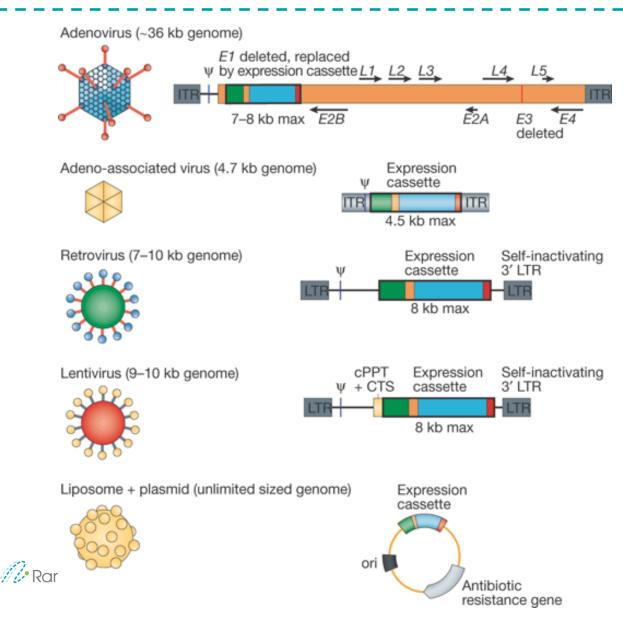
Common Tools of Gene Therapy

- The 3 most basic tools currently utilized for Gene Therapy are:
 - Adeno-associated virus (AAV)
 - Delivery Vehicle and Cargo
 - Lentivirus
 - Delivery Vehicle and Cargo
 - Gene Editing complex
 - Cargo
 - Must be coupled with Delivery Vehicle
 - mRNA
 - Delivery via Lipid Nanoparticles (LNPs)
 - Genetic therapy





Extended Toolbox of Gene Therapy Vectors



Adenovirus

- Antiviral vaccines; Anticancer therapy; Larger transgene size; Potential immune response to vector
- AAV
 - Ideal for targeting non-dividing cells; Ideal for *in vivo* delivery; Ideal for gene replacement; Smaller transgene size

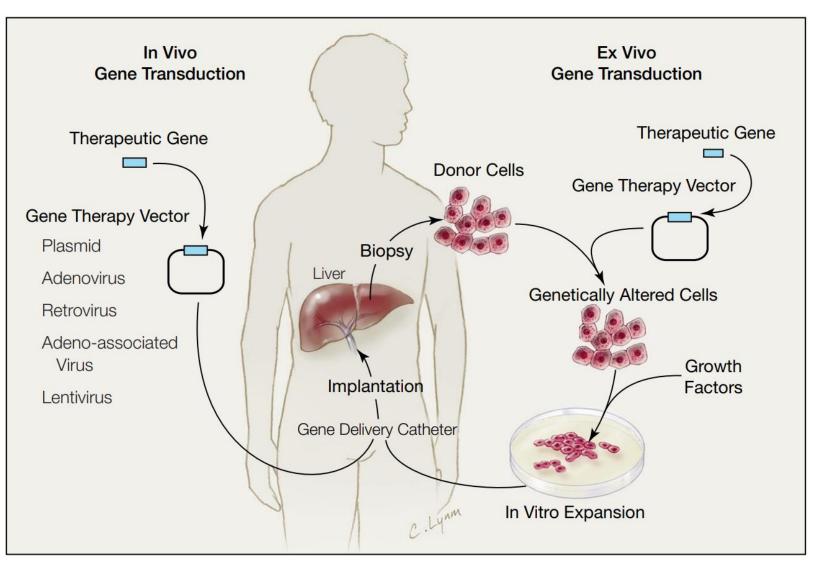
Retrovirus

• Require active cell division for infection (target dividing cells); Lower safety profile than lentivirus due to higher rate of oncogenesis

• Lentivirus

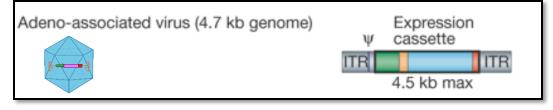
- Ideal for targeting non-dividing cells; Ideal for *ex vivo* delivery; Larger transgene size than rAAV, less than Adenovirus
- Liposome + Plasmid
 - Very large transgene size; Complicated 19 manufacturing and delivery

In Vivo versus *Ex Vivo* Gene Delivery Requirements Influence Vector Choice





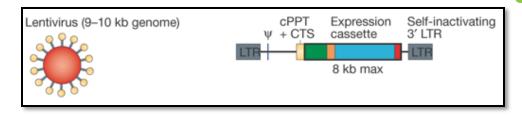
Primary Viral Tools of Gene Therapy – AAV & Lentivirus



- Advantages
 - Ideal for *in vivo* delivery (directly into patient)
 - Ideal for gene replacement
 - Can be used for CRISPR, but small genome size limitations exist
 - Very low integration rates
 - Multiple capsid choices tailor cellular tropism
 - Academic and industry manufacturing experience
- Challenges

A Re

- Small genome size (≤5 kilobases)
- Expensive to manufacture
- Single dose limitation*
- Immune reactions to treatment in small number of patients not well understood

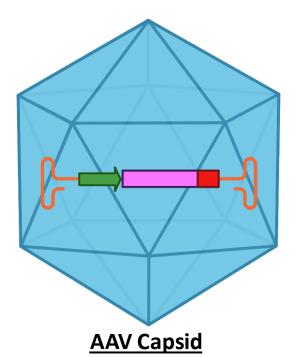


- Advantages
 - Ideal for *ex vivo* delivery (into isolated patient cells, which are then returned to patient)
 - Ideal for targeting nondividing cells
 - Infect wide variety of cell types
 - Larger transgene size than rAAV (Lenti ≈ 9 kilobases), smaller than Adenovirus (~36 kilobases)
- Challenges
 - Lentiviral integration poses higher oncogenic/genotoxic risk than AAV
 - Potential activation of neighboring genes post-integration

What Makes a "Good Candidate" for Addition Gene Therapy?

• Does your transgene fit?

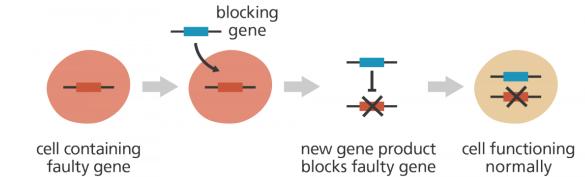
- AAV maximum packaging capacity is ~5 kilobases of DNA
- Lentivirus maximum packaging capacity is ~9 kilobases DNA
- Note This maximum capacity is not for transgene alone, but requires consideration of *required* viral and regulatory elements as well (used to turn gene expression on, package into virus, terminate mRNA)
- Can the delivery vehicle (AAV/Lentivirus) infect and transfer genetic material to the target cell(s) important for correcting the disease?



- Are the target cell(s) amenable to the gene therapy approach/cargo you are using? (addition vs. inhibition)
 - Important to consider gene expression levels associated with a disease phenotype, especially in CNS-related diseases [Goldilocks scenario]

What Makes a "Good Candidate" for Inhibition Gene Therapy?

- Is there a well understood target to inhibit?
 - This target can be direct (e.g., the protein causing the disease) or...
 - This target can be indirect (e.g., a genetic component modifying the protein causing the disease)
 - Examples include expression of an inhibitory protein, an inhibitory sequence or a 'suicide gene' to kill affected cells (cancer or infectious disease)
 - Long term delivery methods (e.g., AAV) mostly used for infectious disease and cancer
- Can the delivery vehicle reach and transfer inhibitory genetic material to the target cell(s) important for correcting the disease?
- Are the target cell(s) amenable to the gene therapy approach/cargo you are using?



Why is Gene Therapy a Compelling Therapeutic Modality?

- *Potential* for halting, treating *or* curing* disease using a one-time** treatment
- Broad experience in global clinical trials
- Increased awareness and acceptance due to recent product approvals and compelling late-stage clinical results
- Regulators and payers becoming more familiar with therapeutic paradigm

* The definition of 'cure' is a complicated, moving target

** Limited by lifetime/dilution of treated cells

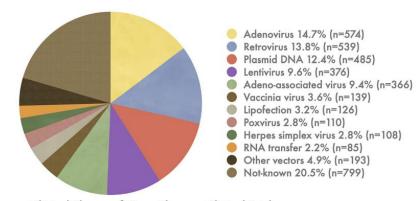


Success Stories of Gene Therapy

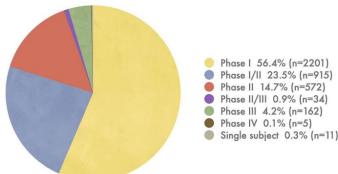
- >3900 GT clinical trials have been initiated. ~1300 GT clinical trials across the 3 most common delivery vehicles
- 24 approved Gene Therapy products; 7 approved Adeno-associated Virus (AAV) products; 7 approved Lentivirus products; 1 approved CRISPR product (for 2 indications);

J Gene Med. 2024:26:e3721

- Luxterna (Spark/Roche) Leber's congenital amaurosis (RPE65 deficiency)
 - Cost = \$850,000, at launch
- Zolgensma (AveXis/Novartis) Spinal Muscular Atrophy (SMN1)
 - Cost = \$2.125M, at launch
- Hemgenix (CSL Behring/UniQure) Hemophilia B (FIX)
 - Cost = \$3.5M, at launch
- Roctavian (BioMarin) = Hemophilia A (FVIII) ۰
 - Cost = \$2.9M, at launch
- Upstaza (PTC Therapeutics) Aromatic I-amino acid decarboxylase deficiency •
 - Cost = \$3.7M, at launch
- Elevidys (Sarepta) Duchenne Muscular Dystrophy (DMD) [≥4 years-old]
 - Cost = \$3.2M, at launch
- Beqvez (Pfizer) Hemophilia B (FIX)
 - Cost = \$3.5M, at launch
- Casgevy (CRISPR Therapeutics/Vertex) Sickle Cell Disease and transfusion-dependent Beta-thallase
 - Cost = \$2.2M, at launch
- Kebilidi (PTC Therapeutics) Aromatic L-amino Acid Decarboxylase Deficiency (AADC)
- *∭*[∕• Rare Bootcamp™
 - Cost = \$3.71M, at launch



Clinical Phases of Gene Therapy Clinical Trials



| 15

Vectors Used for Gene Transfer in Gene Therapy Clinical Trials

https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products

Keys to Recent Successful Gene Therapy Outcomes

- Solid basic and clinical science
- High unmet medical need
- Good cellular/tissue target choice
- Increased platform understanding by Health Authorities

• What limitations exist?

- Commercial CMC
- Continued stringency of Health Authority CMC requirements
- Extension of clinical success to new targets
- Safety concerns with high dose AAV delivery (>1-2x10¹⁴ vg per kg)
 - SMA AAV9
 - XLMTM AAV8
 - DMD-AAV9
- Continued study of immunological reactions to gene therapy



Key Takeaways

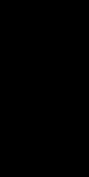
- Gene therapy has the potential for halting, treating or curing* disease using a one-time** treatment
 - There are multiple successful, FDA approved examples via recombinant AAV and Lentiviral vectors
- Success is *critically dependent* upon solid basic and clinical science knowledge, a targetable cellular/tissue target choice, and the ability to manufacture the vector
- Each gene therapy strategy and delivery vehicle has pros and cons
 - Strategy and delivery vehicle must be matched to the biology of the disease and target cells
- Gene therapy manufacturing and reimbursement are *expensive*
 - The field is continually working to improve manufacturability and reduce costs
 - Broader adoption of gene therapy treatments and continued understanding of treatment paradigm by regulators and payers should lead to reduced costs over time





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Appendix



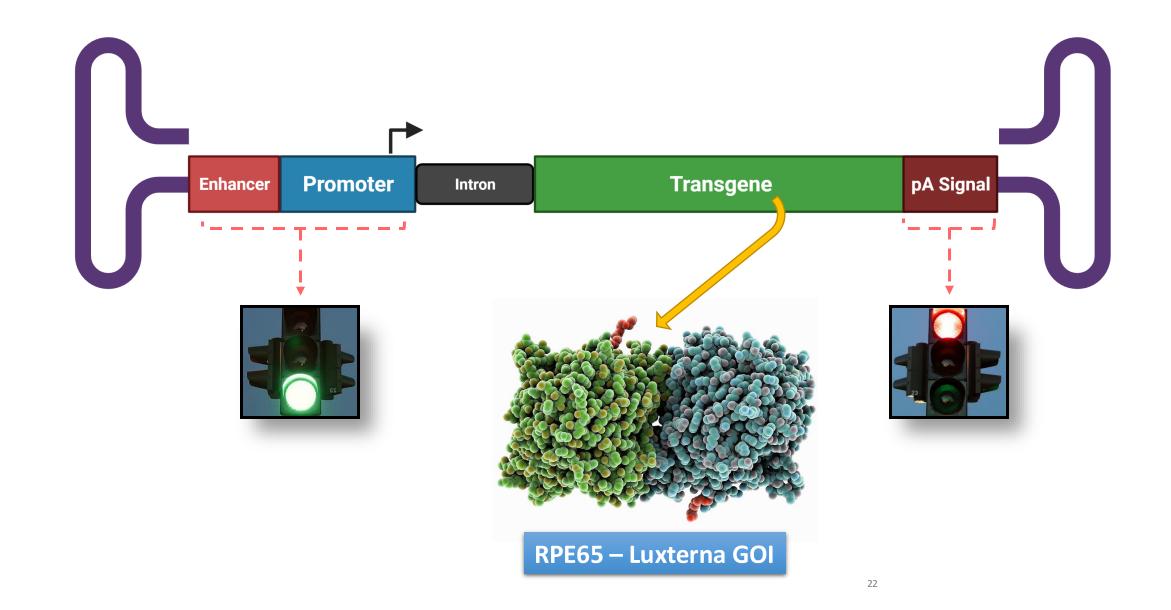


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How to Design and Generate an AAV GT Vector

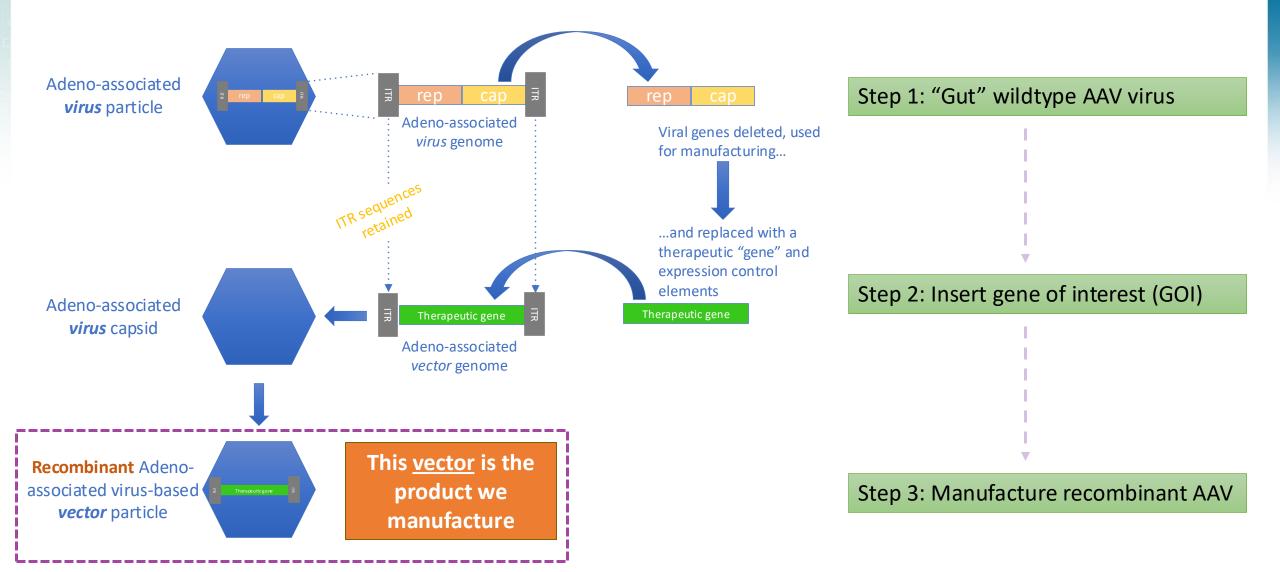
Fundamental Design of a Recombinant AAV





Basic recombinant AAV Molecular Engineering





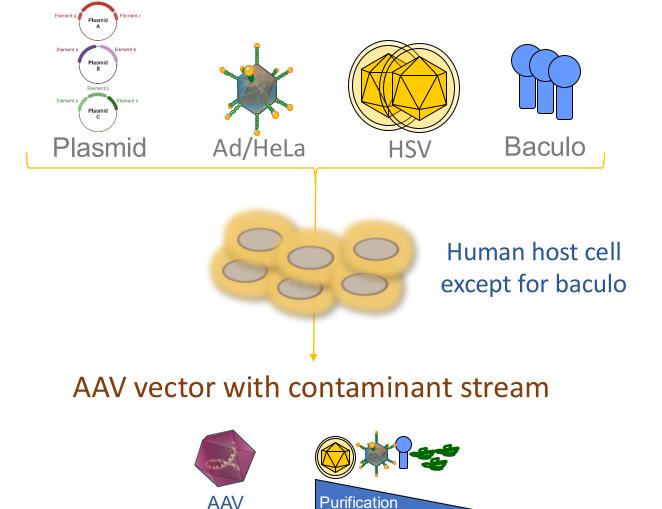
AAV Vector Manufacturing – Overview



AAV rep+cap functions and helper functions must be provided

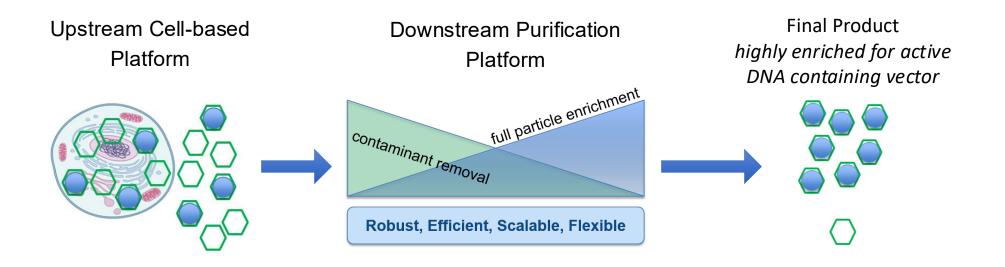
Commercial success requires bioreactor production platform and scale

Scalable operations must be designed to purify the product at acceptable yield and quality, while removing contaminants

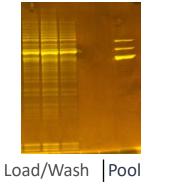


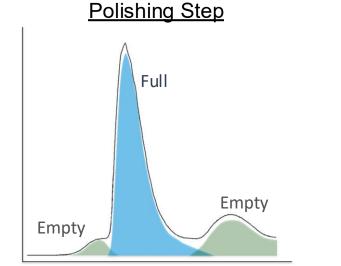
AAV Vector Purification



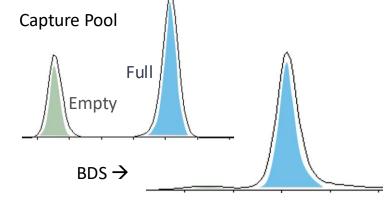








Analytical Ultracentrifuge



Key Takeaways



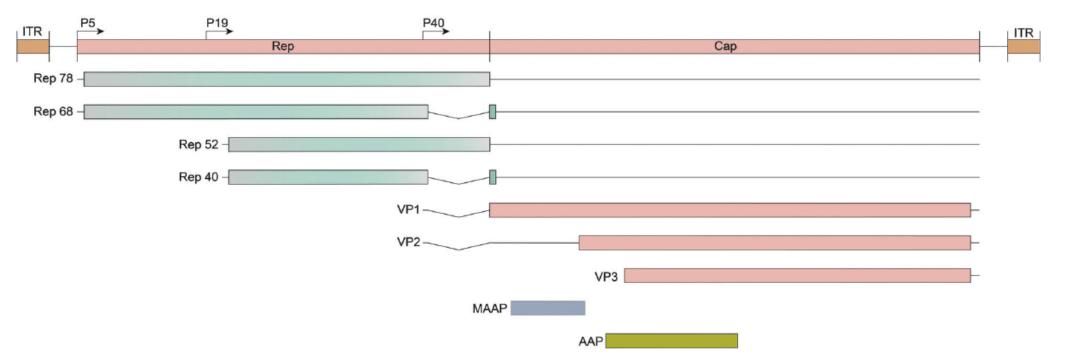
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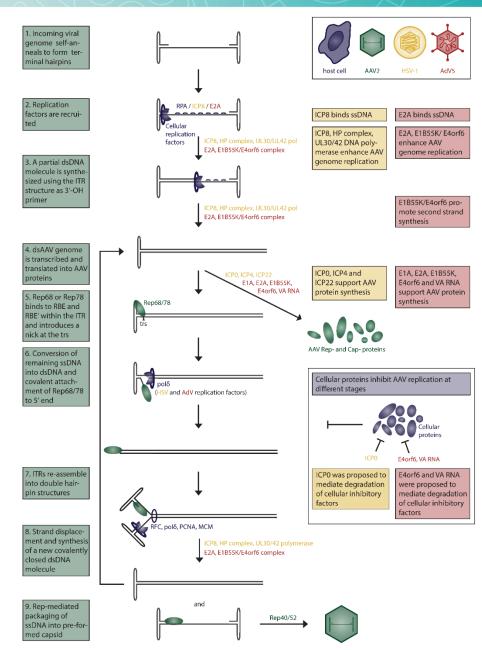
A bit more detailed information on AAV

AAV Protein Expression



- AAV "Large Rep" proteins provide a nicking function required for genome replication [Rep78 and Rep68]
- AAV "Small Rep" proteins are involved in packaging the genome into the preformed capsid [Rep52 and Rep 40]
- AAV Capsid proteins ("Cap") form the structure that the genome is packaged into and is the delivery mechanism of gene therapy [VP1, VP2, VP3]

AAV Replication – Helper Virus

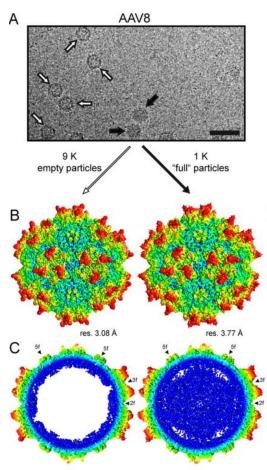


- AAV co-opts both cellular and viral factors to facilitate completion of its life cycle (genome replication and packaging)
- Cellular factors are used to replicate the AAV genome
- Helper virus factors are used to support replication of the AAV genome
- AAV Rep proteins are used to facilitate replication and packaging of the AAV genome
- AAV Cap proteins associate to form the capsid

AAV Capsid Selection

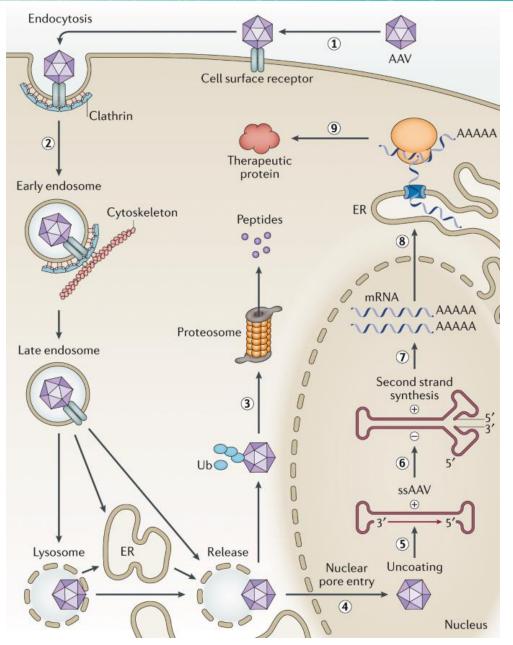


AAV serotype	Origin of isolation	Primary receptor	Co-receptor	Tissue tropism	Condition (ClinicalTrials.gov identifier)	Approved drug
AAV1	Monkey	Sialic acid	AAVR	Muscle, CNS, heart	Muscle diseases (NCT01519349)	None
					Heart failure (NCT01643330)	
					AAT deficiency (NCT01054339, NCT00430768)	
AAV2	Human	Heparin	Integrin, FGFR, HGFR, LamR, AAVR	Liver, CNS, muscle	Eye diseases (NCT00643747)	Luxturna for Leber congenital amaurosis
					Haemophilia (NCT00515710)	
					CNS diseases (NCT00400634)	
					AAT deficiency (NCT00377416)	
AAV3	Human	Heparin	FGFR, HGFR LamR, AAVR	Muscle, stem cells	No trials underway	None
AAV4	Monkey	Sialic acid	Unknown	Eye, CNS	Eye diseases (NCT01496040)	None
AAV5	Human	Sialic acid	PDGFR, AAVR	CNS, lung, eye	Haemophilia (NCT03520712)	None
					Eye diseases (NCT02781480)	
					AIP (NCT02082860)	
AAV6	Human	Heparin, sialic acid	EGFR, AAVR	Muscle, CNS, heart, lung	Haemophilia (NCT03061201)	None
					CNS diseases (NCT02702115)	
AAV7	Monkey	Unknown	Unknown	Muscle, CNS	No trials underway	None
AAV8	Monkey	Unknown	LamR, AAVR	Liver, muscle, pancreas, CNS	Eye diseases (NCT03066258)	None
					Haemophilia (NCT00979238)	
					Muscle diseases (NCT03199469)	
AAV9	Human	Galactose	LamR, AAVR	Every tissue	CNS diseases (NCT02122952)	Zolgensma for spinal muscular atrophy
					Muscle diseases (NCT03362502)	
AAV10	Monkey	Unknown	Unknown	Muscle	No trials underway	None
AAV11	Monkey	Unknown	Unknown	Unknown	No trials underway	None
AAV12	Human	Unknown	Unknown	Nasal	No trials underway	None 30



AAV Transduction





- AAV capsid interacts with the external cellular receptor and is endocytosed
- AAV capsid interacts with internal endosomal receptor
- Endosome matures from early to late stage, pH change induces conformational change in AAV capsid externalizing VP1
- VP1 phospholipase (PLA) activity opens the endosome allowing capsid escape
- Capsid traffics to the nucleus, disassembles and releases the single-stranded DNA
- WT virus expresses viral proteins and replicates
- Recombinant AAV concatamerizes and resides as an episome loosely associated with cellular chromatin (forms circular DNA molecule)



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Transition Slide

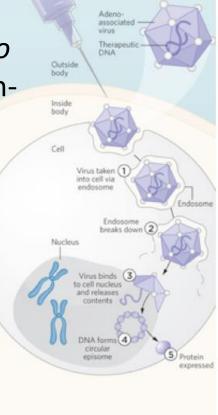


Appendix

GT Overview: Introducing Genetic Material via Viruses



Ideal for in vivo delivery to nondividing cell targets



Non-Integrating (AAV)



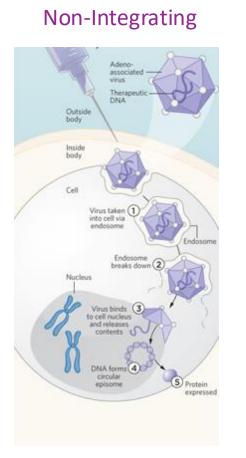
Integrating (Lenti)

Ideal for ex vivo delivery to stem cell targets

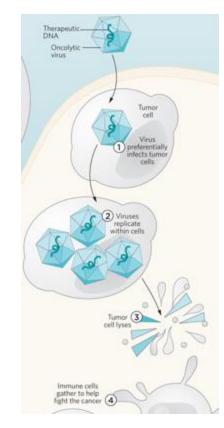
Confidential

Introducing Genetic Material via Viruses

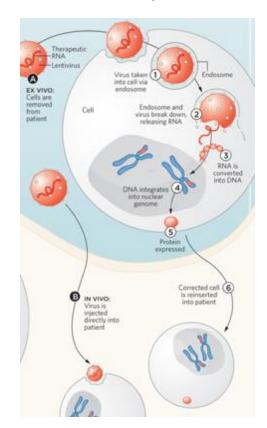




Integrating



Oncolytic





- Capsid the protein shell of a virus; essential component involved in cell binding, internalization, and trafficking within the targeted cell
- Genes the building blocks of inheritance
- Genetic disorder results when genes don't produce the right proteins or don't produce them correctly
- Transgene the gene or genetic material that is being transferred to the cell
- Vector delivery vehicles that encapsulate therapeutic genes for delivery to the cell; include genetically disabled viruses, such as adeno-associated virus



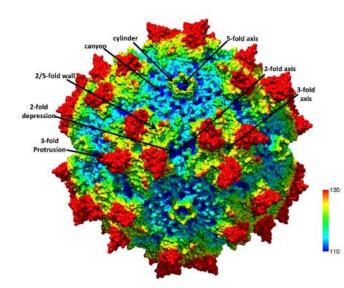
- Non-integrating reducing oncogenic potential
- Multiple capsid types allowing for tailored tropism
- Expertise and experience to manufacture commercial scale product
- Leverages biopharma protein manufacturing experience

AAV: Family Parvoviridae, Genus Dependovirus

Scientific Platform



- AAV discovered in early 1960s
- Wild type AAV is not associated with disease
- Variable seropositivity in human population depending on capsid serotype

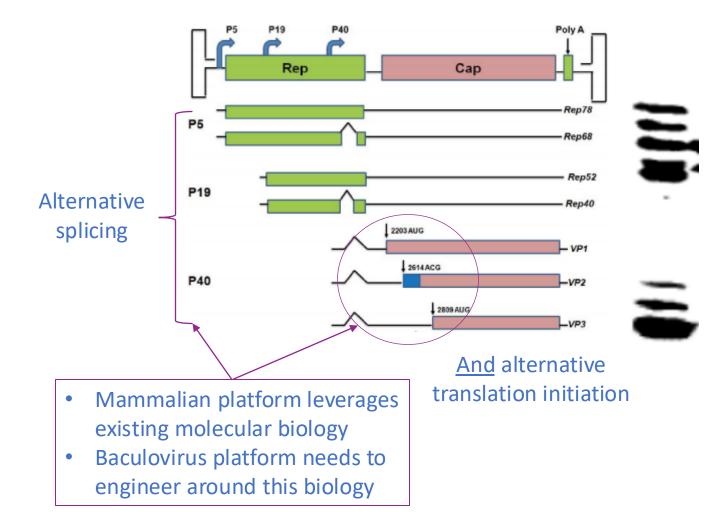


- 20 nm non-enveloped icosahedral capsid
- Virion extremely stable
- Single-stranded genome of 4,680 nt
- Three capsid proteins (VP1,2,3)
- Multiple capsid structural variants available

Dependovirus Genome Structure –

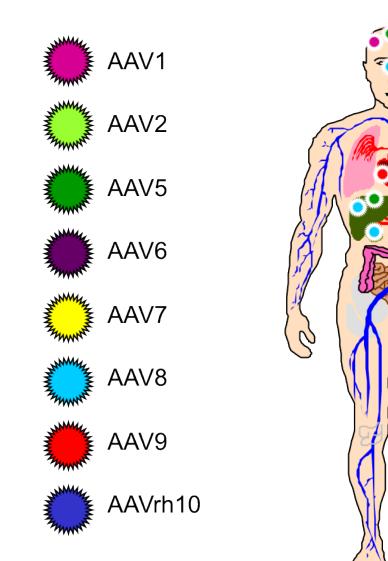


Implications for Manufacturing



Different AAV Capsids Have Different Tropisms



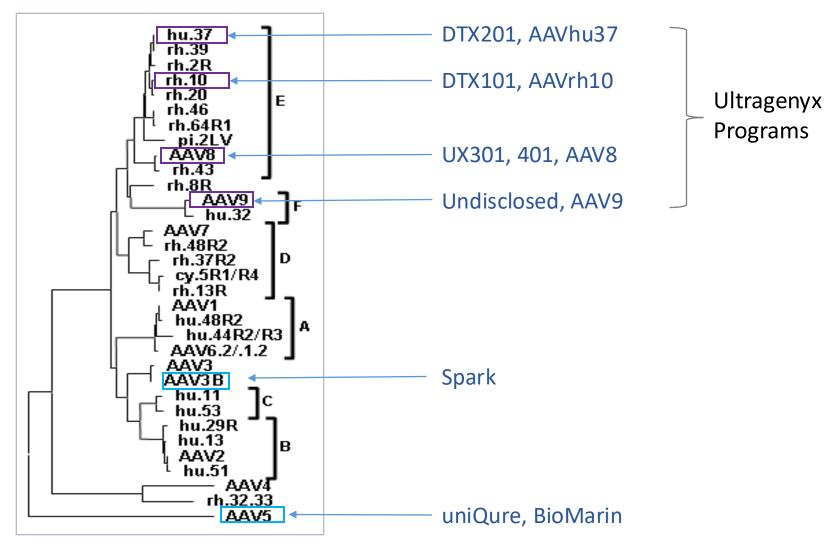


- Tropism is not absolute
- Route of administration can overcome inherent tropism
- Prevalence of anti-capsid antibodies is a major consideration
 - Lack of precision on differences between capsids
 - Extremes are AAV2 with high antibody prevalence and AAV5 with low antibody prevalence

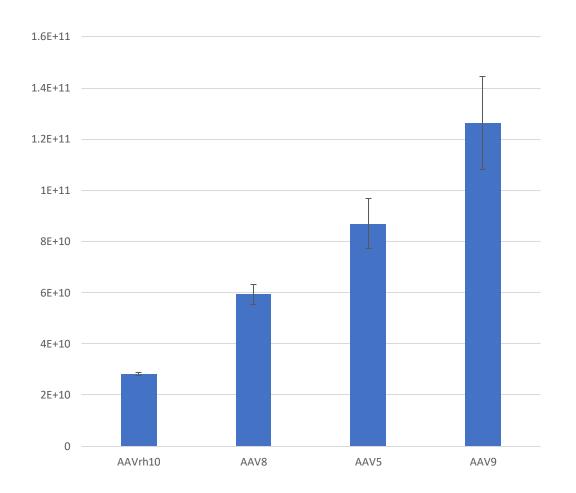
Current Products Based on Clade E Family



Capsids



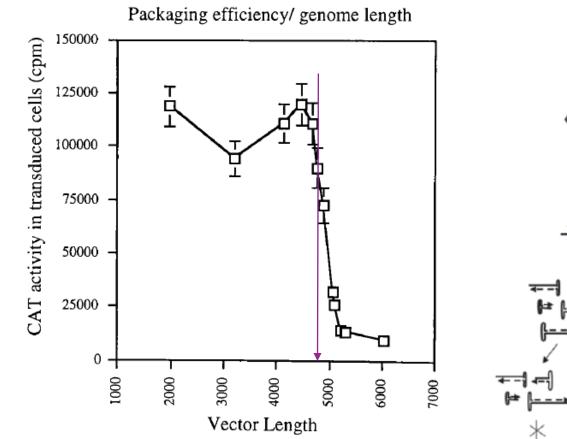


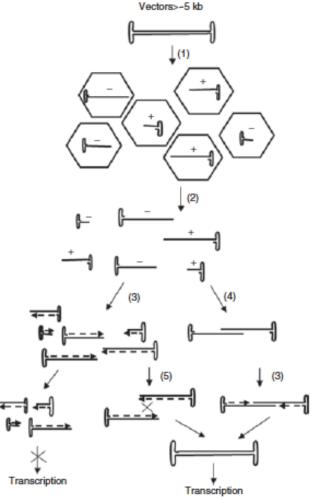


- Packaging of 4 different vector genomes evaluated in 4 different capsids
- Certain downstream unit operations are common in vector purification, others are related but are fine-tuned

AAV Genome Forms – Reduced Packaging Efficiency & Expression Efficiency for Oversized Genomes









A number of viral vector-mediated phase I/II clinical trials have been initiated to treat neurologic disorders

Table 2

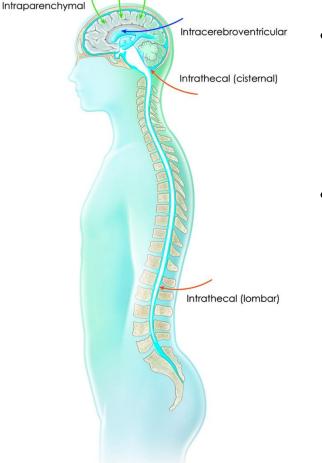
Viral vector-mediated clinical trials for neurological disorders.

Disease	Vector	Transgene	Phase	Trial code	
Ex vivo					
Alzh eimer's disease	Retrovirus	NGF	1	US-0322	
Metachromatic leukodystrophy	Lentivirus	ARSA	I, II	Biffi et al., 2013	
Multiple sclerosis	Retrovirus	MBP	I, II	US-0851	
Wiskott-Aldrich syndrome	Lentivirus	WASP	I, II	Aiuti et al., 2013	
X-linked adrenoleukodystrophy	Lentivirus	ABCD1	I, II	Cartier et al., 2009	
In vivo					
AADC deficiency	AAV	AADC	I, II	NCT01395641	
Alzheimer's disease	AAV	NGF	1, 11	NCT00087789, NCT00876863	
Batten disease	AAV	CLN2	1	NCT00151216	
Batten disease	AAV	CLN2	1, 11	NCT01414985	
Canavan disease	AAV	ASPA	1	Leone et al., 2012	
Giant axonal neuropathy	AAV	GAN	1	NCT02362438	
Glioblastoma	Oncolytic poliovirus	-	1	NCI01491893	
Glioblastoma multiforme (GBM), other gliomas	Oncolytic adenovirus	-	1	NCT00805376, NCT01956734, NCT02197169	
Glioblastoma multiforme, other gliomas	Retrovirus	CD	I, II/III	NCT01470794, NCT02414165	
Glioblastoma, other gliomas	Oncolytic HSV	-	1	NCT02031965	
Glioblastoma, other gliomas	Oncolytic HSV	-	1	NCT00028158, NCT00157703	
Leber's hereditary optic neuropathy	AAV	MT-ND4	1	NCT02161380	
Metachromatic leukodystrophy	AAV	ARSA	I, II	NCT01801709	
MPS IIIA (Sanfilippo Disease Type A)	AAV	SGSH, SUMF1	I, II	NCT01474343, NCT02053064	
Parkinson's disease	AAV	GAD	1, 11	NCT00195143, NCT00643890	
Parkinson's disease	AAV	NTRN	1, 11	NCT00252850, NCT00400634	
Parkinson's disease	Lentivirus	TH, AADC, CH1	1, 11	NCT00627588, NCT01856439	
Parkinson's disease	AAV	GDNF	1	NCT01621581	
Parkinson's disease	AAV	AADC	1, 11	NCT02418598	
Parkinson's disease	AAV	AADC	1	NCT00229736	
Pompe disease	AAV	GAA	1, 11	NCT00976352	
Pompe disease	AAV	GAA	I	NCT02240407	
Spinal muscular atrophy type 1	AAV	SMN	I	NCT02122952	

Potential delivery sites for CNS AAV gene



therapy



Global delivery

• IV: easiest delivery, requires high vector doses, and may not target sufficient cells in regions of interest due to low penetration of BBB

CSF based delivery

 ICV, IT (cisternal or lumbar): potentially challenging delivery method, may not reach deep brain structures, but will target a higher % of neurons compared to IV at a similar dose

Intraparenchymal delivery

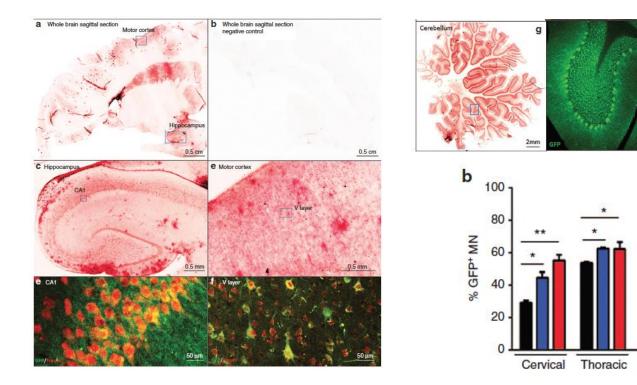
Potentially target a large percentage of neurons but only in a select area

45



Meyer et al., 2015. Molecular Therapy (Brian Kaspar's lab)

- scAAV9-CBA-GFP was delivered to n=5, 1 yr old NHPs (cynos) via sacral-IT (1x10¹³ vg/kg) used Trendelenburg position (head tilted down by 15-30 degrees for 10 min following infusion)
- Animals were sacrificed after 2 weeks
- GFP was noted in all regions of the brain, with particularly strong signal in the hippocampus, motor cortex and cerebellum



Note: others have reported similar results but with less robust brain delivery

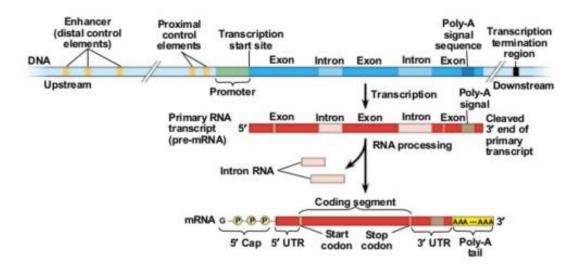
Lumbar

No tilting 5 min

10 min

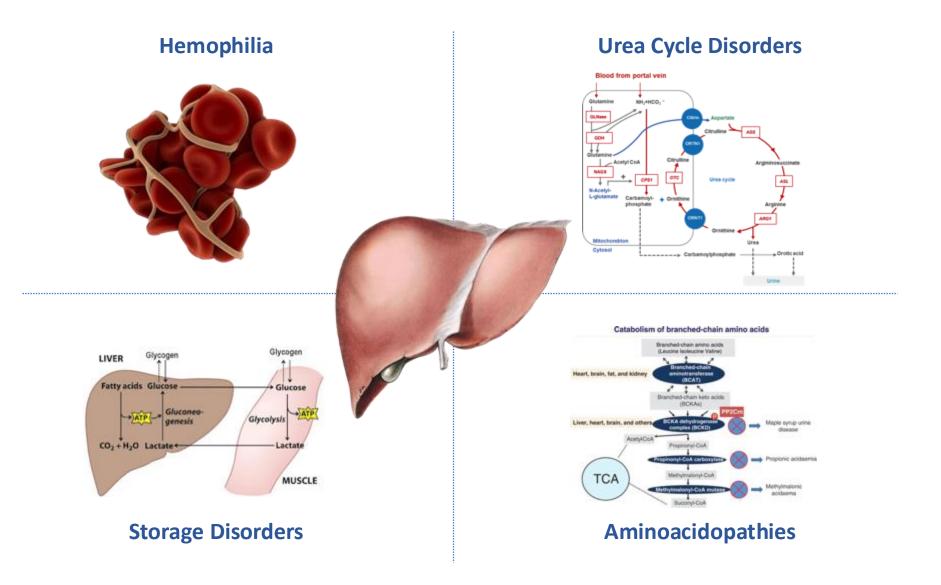


- Most therapeutic gene coding sequences will use their minimal cDNA format
- Overstuffing capsid has negative yield and quality consequences
- Additional elements, such as introns & 5' and 3' UTR sequences, can sometimes be included
- Greatest technical challenge is engineering small enhancer and promoter combinations to achieve tissue specific gene expression



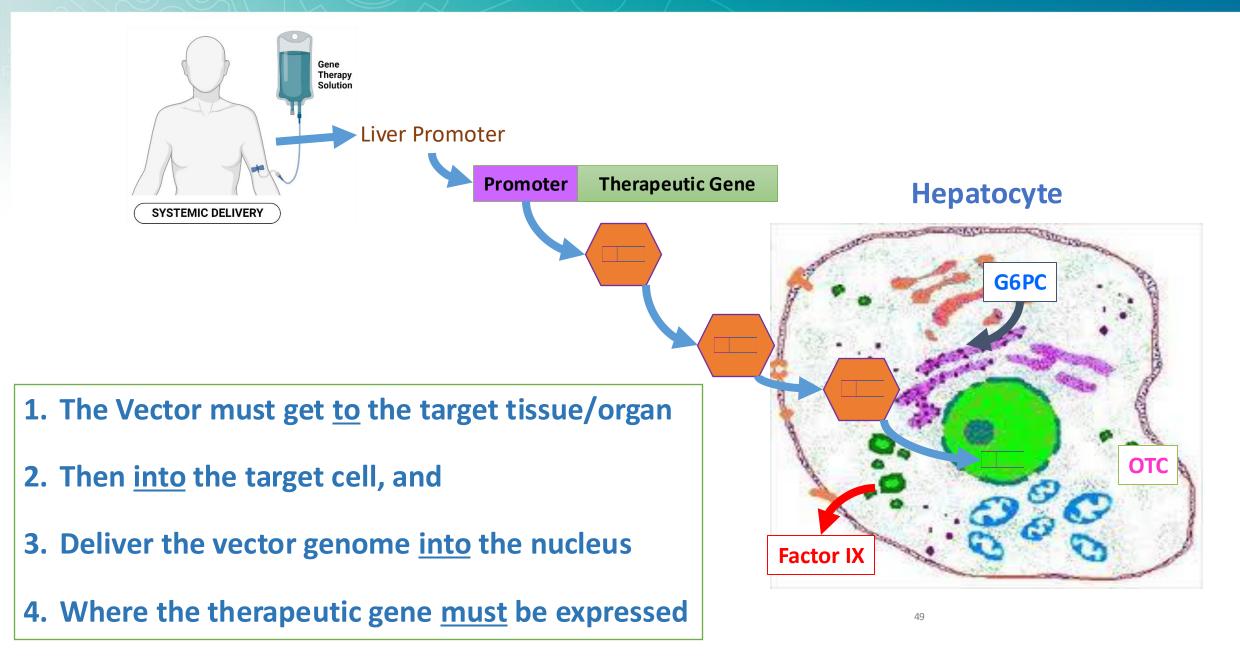
Targeting the Liver – A Master Hub for Rare Diseases



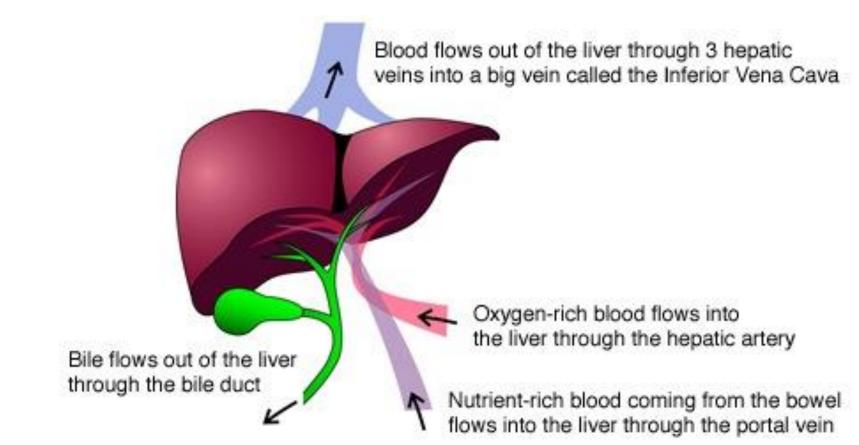


Gene Therapy Delivery is a Complicated, Multi-Step Process

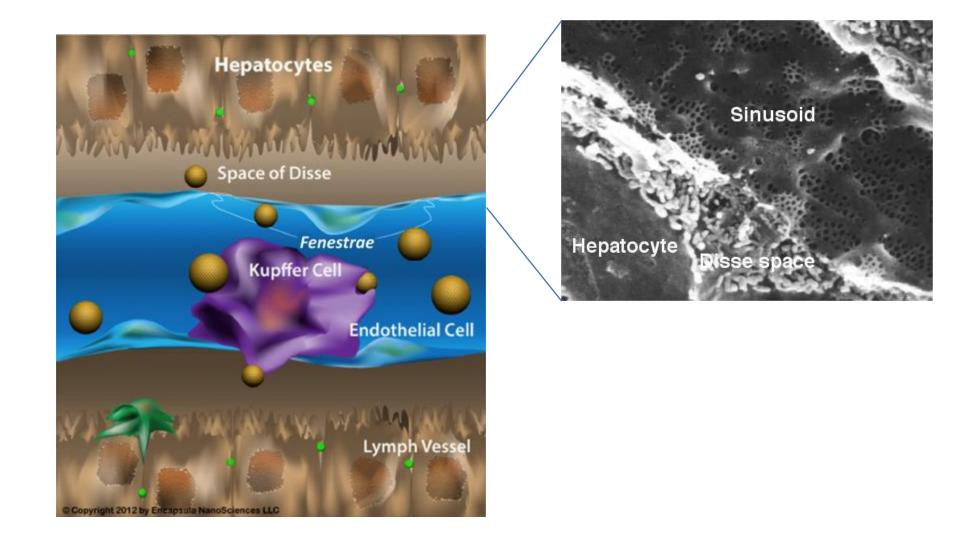




Blood Flows Through the Liver at 1.5L per min in Bloot AMP



Liver Sinusoidal Endothelium Is Fenestrated



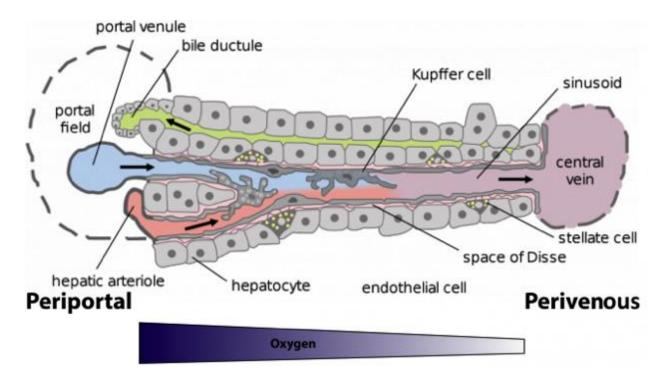
Hepatocytes Are Divided Into Functional



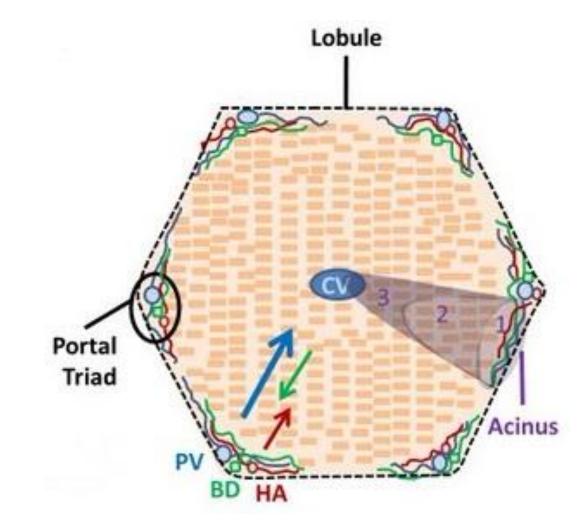
Zones

High O2 processes

Low O2 processes

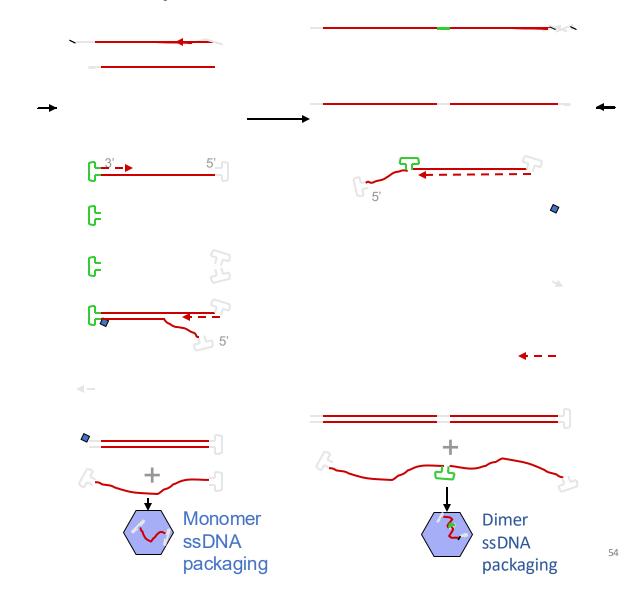


Functional Zones of the Liver (the big picture) I PARE BOOTCAMP



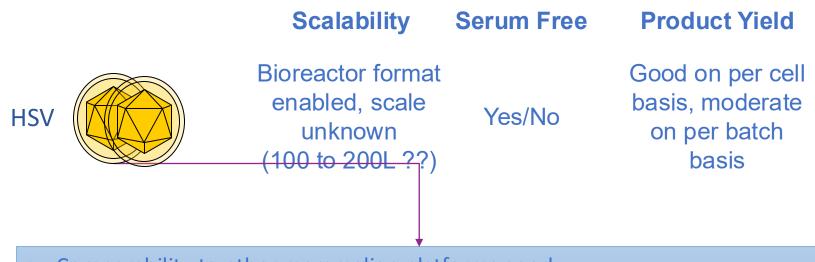
AAV Genome Forms – Single Strand versus Self Complementary

3 , 5





AAV Vector Manufacturing – HSV Advantages

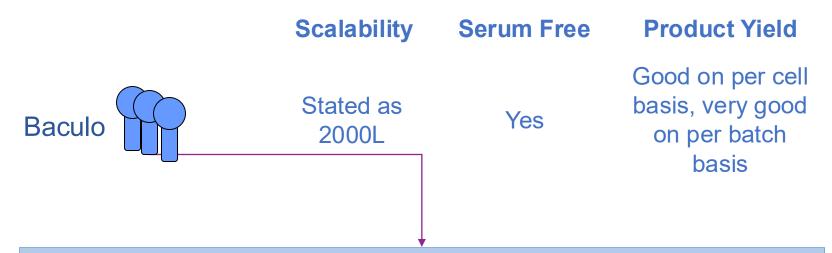


- Comparability to other mammalian platforms good
- Key features/bugs Good product quality, easy removal of rHSV/Limited by stability of rHSV, requires 2 rHSV vectors as complex GMP raw material supply train
- Alternate bioreactor format would be required to implement at Woburn; different format for vector engineering

AAV Vector Manufacturing – Baculo



Advantages and Limitations

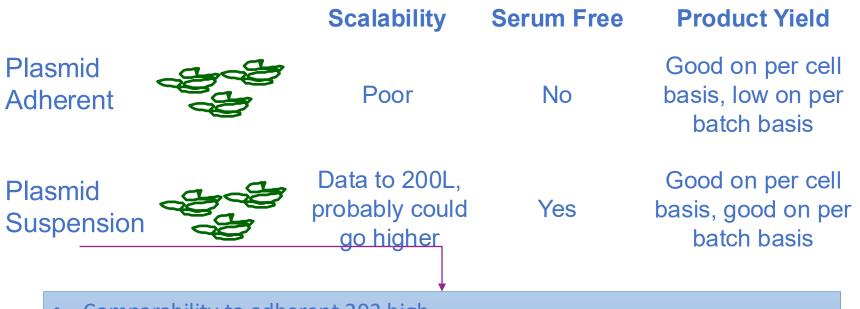


- Comparability to other mammalian platforms a question
- Key features/bugs high yield & serum free, high cell density/requires 2 or 3 rBac viruses as supply chain, non-optimal stability of rBac (genetic & storage), complex molecular engineering required to support fidelity of AAV life cycle
- EMA Glybera Assessment highlights several gaps in CMC
- Alternate bioreactor format would be required to implement at Woburn; different format for vector engineering

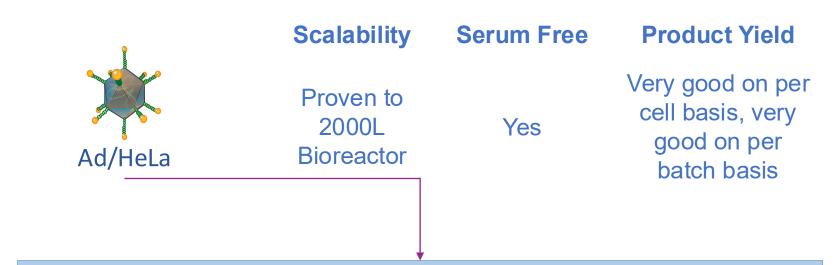
AAV Vector Manufacturing – HEK293



Advantages and Limitations



- Comparability to adherent 293 high
- Key features/bugs HEK293 platform used for majority of AAV gene therapy trials/plasmid represents high GMP raw material burden
- Readily implemented at Woburn; leverage our core AAV science, support basic PD, de-risk CMO tech transfer

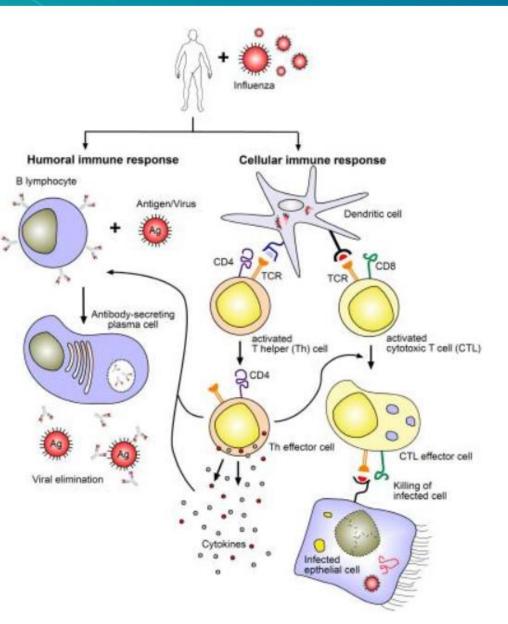


- Comparability to other mammalian platforms good
- Key features/bugs only clonal system enabling screen for desired features;
 E/F, rcAAV, other requires high titer Ad helper GMP raw material supply train
- Readily implemented at Woburn; leverage from our core AAV science, support basic PD, de-risk CMO tech transfer

The Immune System – A Primer

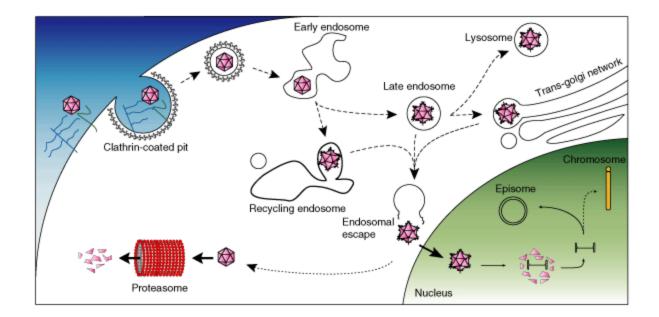


- Two pathways humoral and cellular
- Key signaling includes T-cell, B-cells and NK cells
- AAV vectors retain some viral coding that may be recognized as foreign
 - Capsid
 - Transgene
 - Genome



Potential Goals of immune intervention in AAV GT

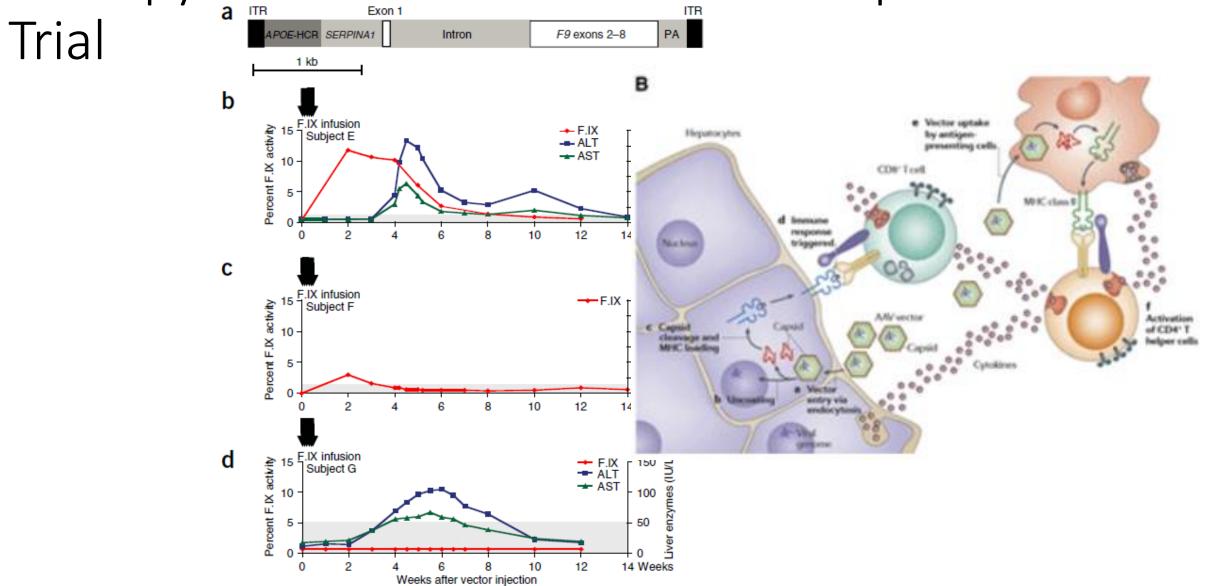
- T cell responses to capsid antigens?
- T cell responses to therapeutic protein?
- B cell responses to capsid antigens?
- B cell responses to therapeutic protein?





First Observation of a Reduction in AAV Gene 🔃 🖄 RATE DE DOUTCAMP

Therapy – First Liver Delivered Hemophilia



First Introduction of Steroids to Address Reduction in Efficacy – Nathwani 2011

- Steroids broadly suppress the immune system
- Nathwani's use of steroid was likely serendipitous
- Outcome was favorable and patients have sustained activity beyond 6 years

