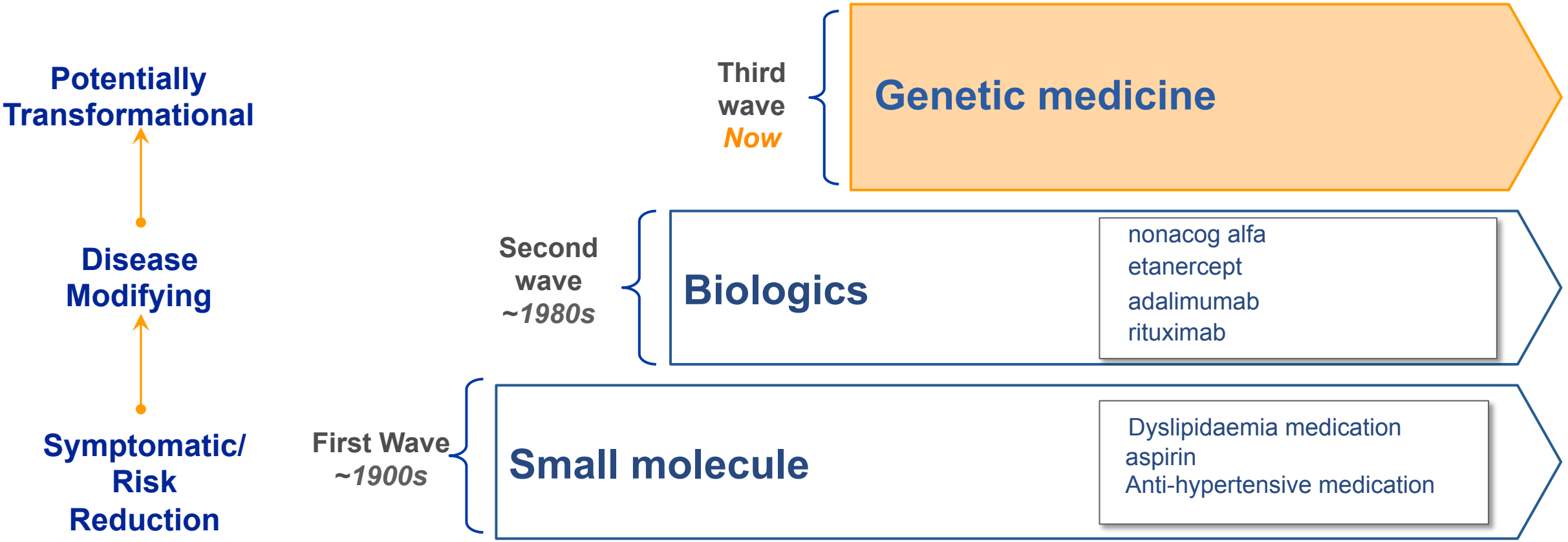
- As of March 2019, there were **3831 "Gene Therapy" trials** registered on ClinicalTrials.gov[1]

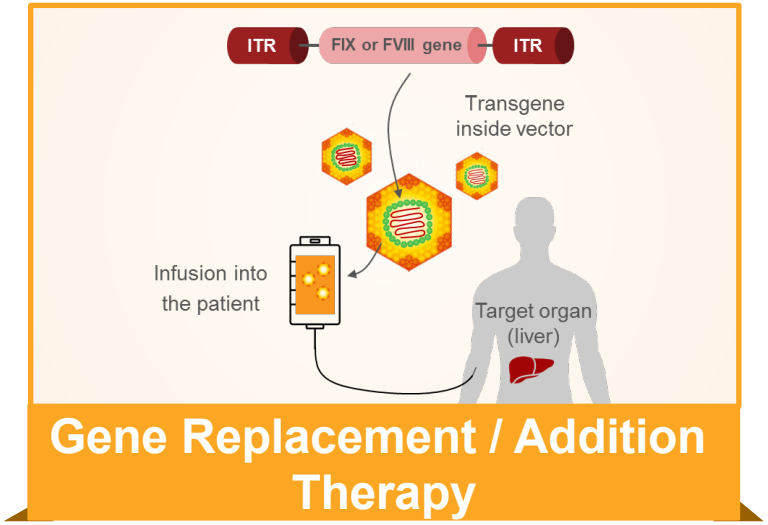
1. ClinicalTrials.gov. Search of gene therapy. https://clinicaltrials.gov/ct2/results?cond=&term=gene+therapy&cntry=&state=&city=&dist= (Accessed April 2019). 2. Beitelshees M, et al. *Discov Med* 2017;24(134):313–22.

- **1972** : Friedmann and Roblin publish "Gene therapy for human genetic disease?" in Science
- **1990** :First patient to be treated with gene therapy – 4 yr old girl with adenosine deaminase (ADA) deficiency
- **1999** : Following Jesse Gelsinger's death the FDA suspended several clinical trials pending the reevaluation of ethical and procedural practices.
- **2012** : Glybera became the first treatment to be approved for clinical use in Europe
- **2017**: Kymriah approved in US for certain pediatric and young adult patients with a form of acute lymphoblastic leukemia.
- **2017** : Luxterna (Spark therapeutics) approved for retinal dystrophy due to a mutation of the RPE65 gene

Genetic Medicine Represents a Third Wave of Innovation

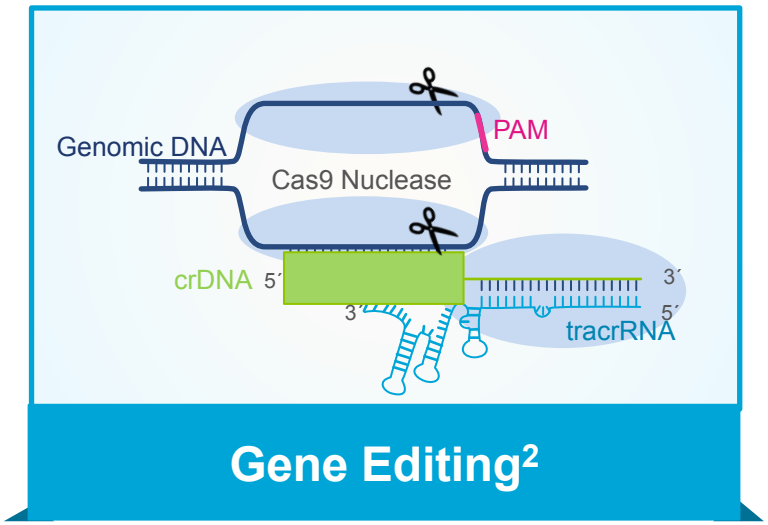


Genetic Medicine: Approaches



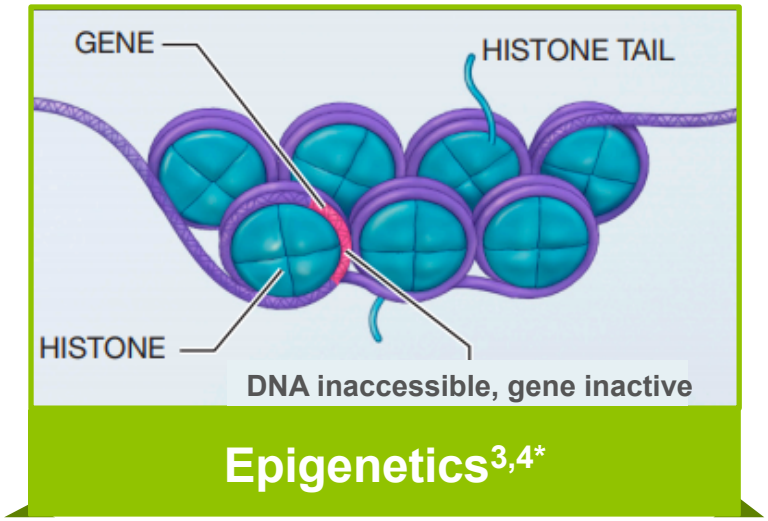
Gene Replacement / Addition Therapy

Add a functioning gene



Gene Editing[2]

Permanently remove, modify, or add a gene



Epigenetics[3,4*]

Modify gene expression

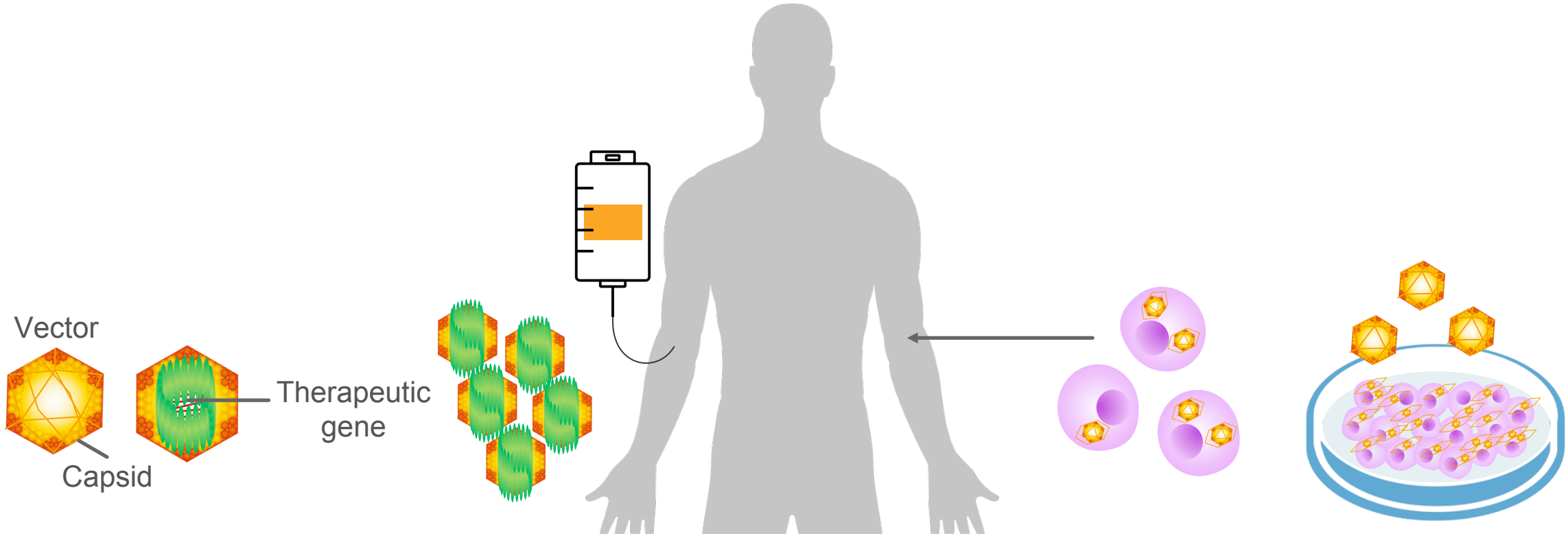
*Figure from National Institutes of Health: https://commonfund.nih.gov/epigenomics/figure

1. Kumar SR, et al. *Mol Ther Methods Clin Dev* 2016;3:16034. 2. Cox DBT, et al. *Nat Med* 2015;21(2):121–31. 3. Lomberk GA, et al. *Epigenomics* 2016;8:831–42. 4. Epigenetic Therapy as Cancer Treatment. https://www.slideshare.net/janelle_leggere/efficacy-of-epigenetic-therapy-as-cancer-treatment?from_action=save (Accessed April 2019).

Gene Replacement / Addition Therapy

In vivo

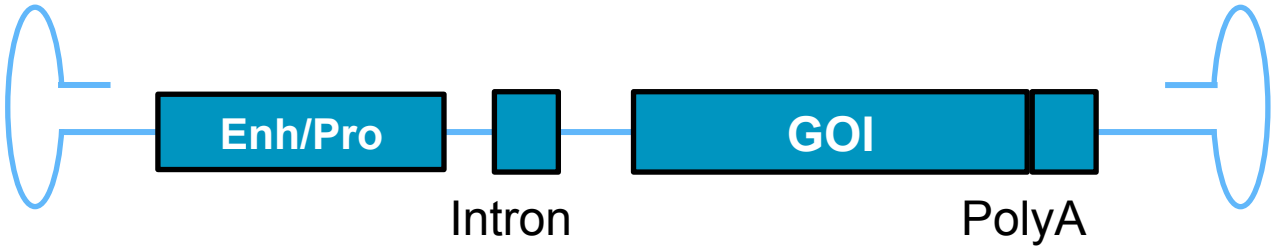
Ex vivo

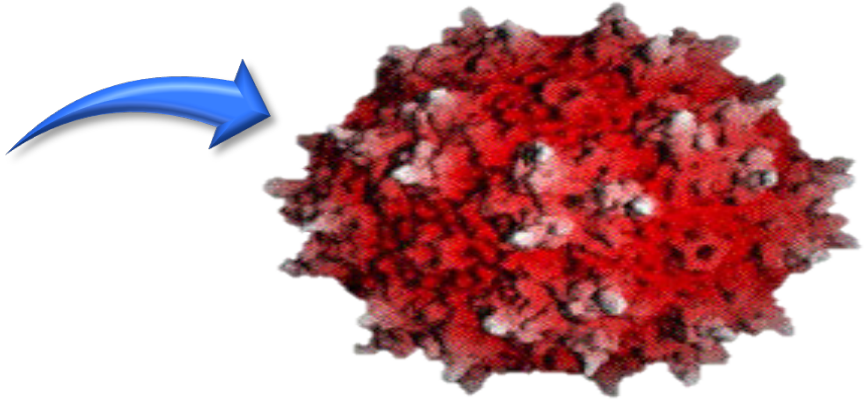


Adapted from: Kumar SR, et al. 2016
Kumar SR, et al. *Mol Ther Methods Clin Dev* 2016;3:16034.

AAV Vector Background

AAV Vector Consists of Two Components





- Single-stranded genome containing gene of interest (GOI), transcriptional regulatory elements (enhancer and promoter) and AAV-ITR (AAV-inverted terminal repeats)
- Size limit about 4.8-5.0 kB
- Extrachromosomal

- Genome is encapsulated in a shell consisting of a viral capsid (cap) protein
- Different capsid serotypes can direct virus to specific tissues (e.g. AAV9 for muscle)

Delivery: Vectors

The success of gene therapy critically depends on effective vehicles for gene transfer, which are based on viral platforms (but are not viruses)

RETROVIRUS

Adeno-associated virus (AAV)

LENTIVIRUS

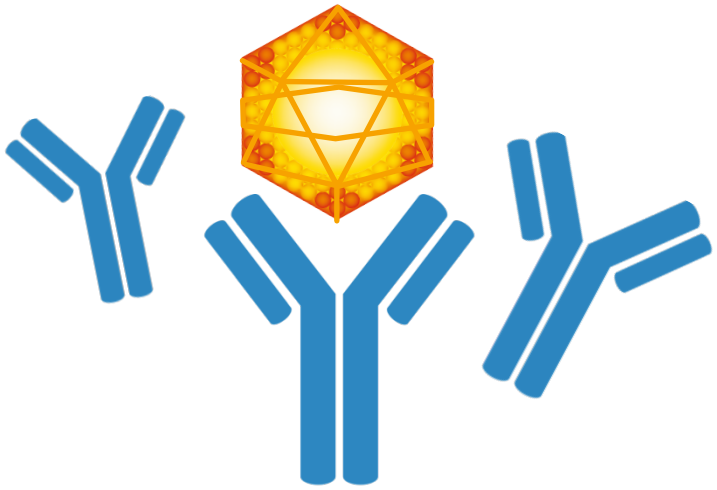
	Retrovirus	Lentivirus	AAV
Genetic material	ssRNA	ssRNA	ssDNA
Tropism	Dividing cells only	Dividing and nondividing cells	Dividing and nondividing cells
Vector genome forms	Integrated	Integrated	Nonintegrated (Primarily episomal)
Carrying capacity	8 kb	8 kb	<5 kb

Thomas CE, et al. *Nat Rev Genet* 2003;4:346–58.

Immunologic Challenges in Gene Therapy

Humoral Immunity

- Pre-formed **neutralizing antibodies** to vector capsid[1]
- Humoral immunity **may prevent retreatment**[2,3]



Cellular Immunity

- Pieces of capsid displayed on transduced cells[4]
- T cells respond to vector capsid[1,4]
- T cells attack / kill transduced cells[4]
- **Loss of cells expressing the donated gene and producing clotting factor**[3,4]

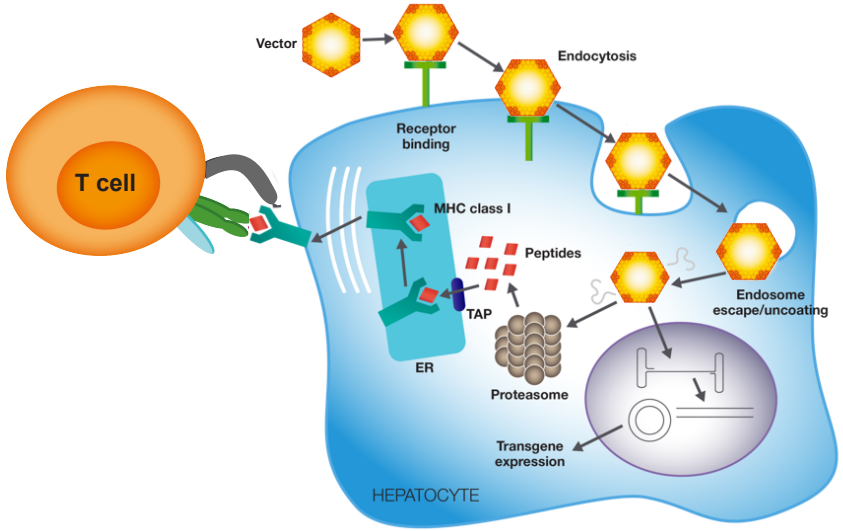


Figure adapted from Mingozzi F, High KA. (2013).[5]

1. Ohmori T, et al. *J Thromb Haemost* 2015;13(Suppl 1):S133–S142. 2. Rapti K, et al. *Mol Ther* 2012;20:73–83. 3. Nathwani AC, et al. *N Engl J Med* 2014;137(21):1994–2004. 4. George LA. *Blood Adv* 2017;1(26):2591–2599. 5. Mingozzi F, High KA. *Blood* 2013;122:23–36.

Future considerations

- **Durable efficacy and long-term safety**
- **Minimizing Immune response**

- **Optimizing viral vectors**
- **Multigene disorders.**

- **Germ line vs somatic cell line** gene therapy
- **Insertional mutagenesis.**

1. George LA, et al. *N Eng J Med* 2017;377:2215–2227. 2. Nathwani AC, et al. *N Engl J Med* 2014;371(21):1994–2004. 3. Kattenhorn LM, et al. *Hum Gene Ther* 2016;27(12):947-961. 4. Baruteau J, et al. *J Inherit Metab Dis* 2017;40(4):497–517. 5. Grieger JC, et al. *Mol Ther* 2016;24(2):287–297.

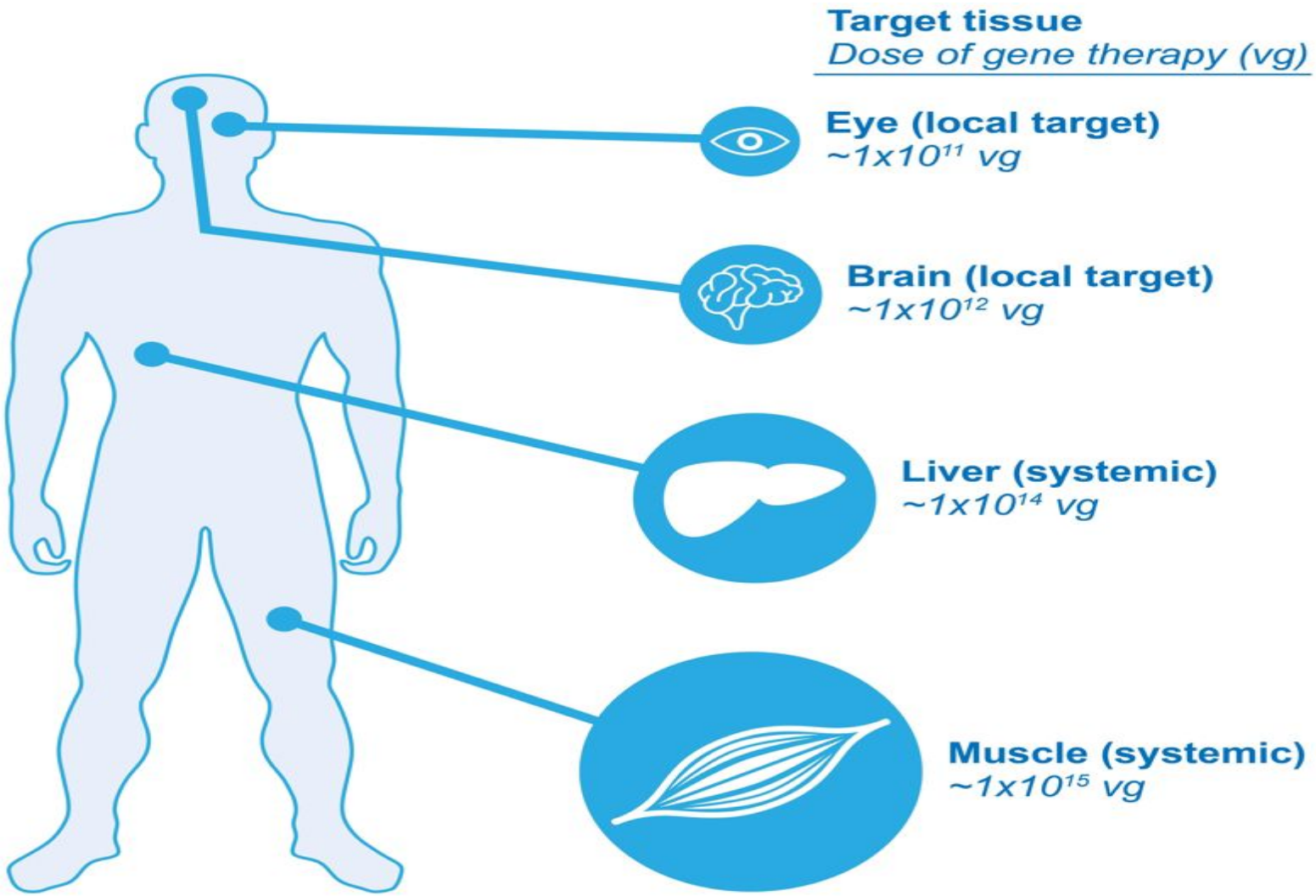
Other considerations

- **Capsid and DNA engineering**
  - Viral tropism for selection of target organ
  - DNA optimization (highly expressing gene variants, optimized codons, strong promotors)
  - Reduce immunogenicity; allow for broader treatable populations, possibly allow repeat infusions

- **Manufacturing**
  - Scalability from experimental studies to clinical supply

1. George LA, et al. *N Eng J Med* 2017;377:2215–2227. 2. Nathwani AC, et al. *N Engl J Med* 2014;371(21):1994–2004. 3. Kattenhorn LM, et al. *Hum Gene Ther* 2016;27(12):947-961. 4. Baruteau J, et al. *J Inherit Metab Dis* 2017;40(4):497–517. 5. Grieger JC, et al. *Mol Ther* 2016;24(2):287–297.

Dosing and Immunological response



One Hundred Billion

One Trillion

100 Trillion

One ‘Quadrillion’
(1000 Trillion)

Importance of Transforming AAV Production Capabilities *Enable Broader Gene Therapy Application*

From: Lab-Scale Culturing

To: Contemporary, Scalable Processes

40,000x Roller Bottles

~1 x 10^{12} vg/roller bottle

~4x iCELLis 500 Attached Cell Reactor

~1 x 10^{16} vg/iCELLis 500 cell reactor

~1x 250 L Cell Suspension Bioreactor

~4 x 10^{16} vg/250 L cell suspension bioreactor

AAV=adeno-associated virus; vg=vector genome.

Potential to Transform Therapy for Monogenic Rare Diseases

Liver
Hemophilia B, Hemophilia A

Disorder associated with lifelong need for 1x to 3x weekly infusions

Muscle
Duchenne Muscular Dystrophy

Incurable disorder with shortened life expectancy

CNS and LSDs
GAN, ALS, MPS's

Progressive, degenerative disorders leading to incapacitation and, potentially, early death

Gene therapy offers the potential to provide one-time, transformational treatment