# ADENO-ASSOCIATED VIRUS
## From Defective Parvovirus to Effective Gene Therapy Vector

Universitat Autònoma de Barcelona

**Clàudia Arasa Cuartiella Tutor: PhD Antonio Villaverde**

claudia.arasa@e-campus.uab.cat

Degree in Biochemistry

Autonomous University of Barcelona, June 2015

## What are virus?

**Virus are obligated intracellular non-living parasites whose size goes from 20 to 300nm in diameter. They are composed of whether an RNA or single- or double-stranded DNA core, surrounded by a protective protein coat and in more complex types, an additional lipid envelope. Their main function is to deliver their genome into the host cell.**

## Gene therapy – a fast developing field

**Gene therapy consists in the gene transfer into cells or tissues in order to cure or prevent diseases. This type of therapy goes directly to what is causing the disease, aiming to target the underlying molecular abnormalities. This gene transfer is possible thanks to a "vehicle" with the genetic material. This vehicle can be no-viral or VIRAL**

### 1st: RETROVIRAL VECTORS

**Integrative virus and vectors**

**RNA nucleus**

**Advantages: they don't have any viral gene, so they are not immunogenic. Stable expression**

**Disadvantages: Used in ex vivo therapies, as they can only infect dividing cells**

### 2nd: LENTIVIRAL VECTORS

**Retroviral subfamily**

**RNA nucleus**

**Advantages: they can infect both dividing and non-dividing cells**

**Disadvantages: mostly derived from the HIV virus**

### 3rd: ADENOVIRAL VECTORS

**Extrachromosomal virus and vector**

**dsDNA nucleus**

**Advantages: the gutless vectors can package up to 37kb genes. High expression levels**

**Disadvantages: first generation vectors were immunogenic: short-time expression**

### 4th:ADENO-ASSOCIATED VIRUS

**Integrative virus, extrachromosomal vector**

**ssDNA nucleus**

**Advantages: high level protein production, long-term expression of the transgene**

**Disadvantages: small size, as they genome has only 4.7kb**

## ADENO-ASSOCIATED VIRUS (AAVs)

- Infection: the AAV is an integrative virus with only one integration site in all the human genome. If there is no coinfection with a helper virus nor DNA damage, it is no-pathogenic and the cycle remains latent. When there is coinfection, it changes to lytic

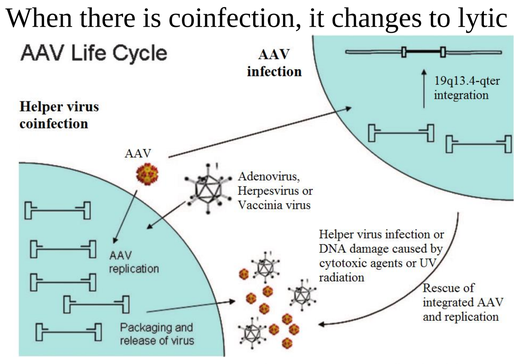



**Figure 2. AAV genome.** Rep and Cap genes are flanked by ITRs
Image from: Daya, S. & Berns, K. I. Gene therapy using adeno-associated virus vectors. *Clin. Microbiol. Rev.* **21,** 583–593 (2008).

- Genome: AAVs includes 2 open reading frames (ORFs) and 3 promoters between the two 145bp-inverted terminal repeats (ITRs). Encoded genes:
  - Rep: non-structural replication proteins
  - Cap: structural capsid proteins

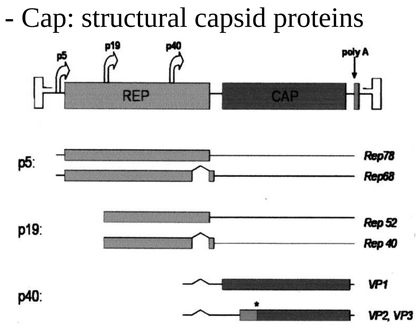



**Figure 2. AAV genome.** Rep and Cap genes are flanked by ITRs
Image from: Daya, S. & Berns, K. I. Gene therapy using adeno-associated virus vectors. *Clin. Microbiol. Rev.* **21,** 583–593 (2008).

## ADENO-ASSOCIATED VIRUS-BASED VECTORS

- Viral elements needed to convert the virus into a vector:
  - Rep
  - Cap
  - ITRs

  ITRs are the only indispensable part for the packaging and the use of the AAVs as vectors. They leave 4'3kb free

- Vector design:
  1. Deep study of the pathology
  2. Know which gene needs to be corrected
  3. Find a good promoter and/or virus serotype to get to the desired tropism

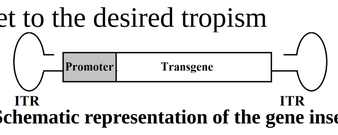



**Figure 3. Schematic representation of the gene inserted into a vector**

- Vector production:

  Lab scale: Triple transfection system:

  - AAV2 Rep and Cap from the desired serotype
  - Transgene plasmid within the two AAV2 ITRs
  - E1A, E1B, E2A, E4 genes and VA RNAs from Adenovirus in a plasmid

  Hek 293 cells are transfected with this 3 preparations and then lysed and purified using a Iodixanol gradient

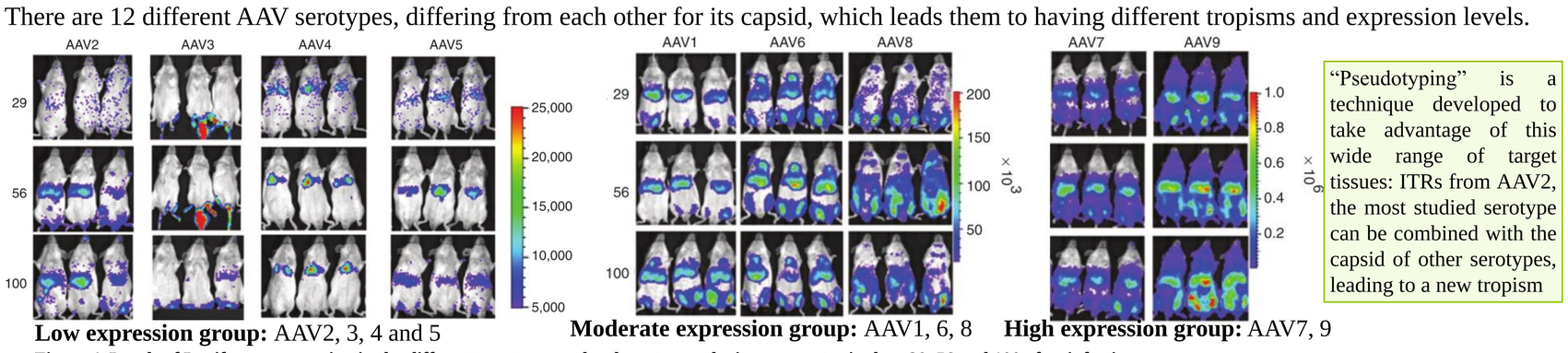
  Higher production titers:

  - Based on the infection with baculovirus
  - Single- or two-vector based system: one for Rep and Cap, the other for the transgene, or both together due to the large capacity of the baculovirus
  - No need for serum or medium additives, as insect (natural hosts for baculovirus) cells grow easily in suspension

  This system still needs to be developed, but it has a promising future

## VIRUS AND VECTOR

There are 12 different AAV serotypes, differing from each other for its capsid, which leads them to having different tropisms and expression levels.




"Pseudotyping" is a technique developed to take advantage of this wide range of target tissues: ITRs from AAV2, the most studied serotype can be combined with the capsid of other serotypes, leading to a new tropism

**Low expression group:** AAV2, 3, 4 and 5 **Moderate expression group:** AAV1, 6, 8 **High expression group:** AAV7, 9

**Figure 4. Levels of Luciferase expression in the different serotypes under the cytomegalovirus promoter in days 29, 56 and 100 after infection.**
Images from Zincarelli, C., Soltys, S., Rengo, G. & Rabinowitz, J. E. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. *Mol. Ther.* **16,** 1073–1080 (2008).

## AAVs' applications in Gene therapy:

AAV vectors, for their characteristics, are becoming the vector of choice for a wide range of gene therapy approaches during the last years, not only for the treatment of monogenic pathologies, in which it is clearly characterized what is wrong, but also in other kind of illnesses, such as polygenic or no heritable diseases that usually have high prevalence, such as cancer

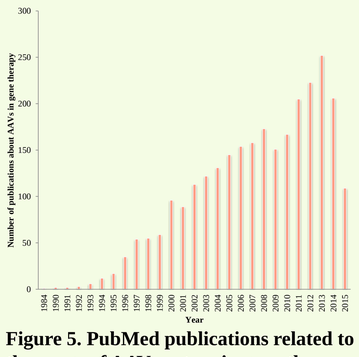



**Figure 5. PubMed publications related to the usage of AAV vectors in gene therapy**

## Limitations

- Small packaging capacity: only 4.3bp of transgene can be packaged, which leaves many diseases such as muscular dystrophy or cystic fibrosis. This could be solved with capsids containing different parts from the gene, although with low efficiency

- Immune response either against the vector or the transgene: it is not a frequent event due to the non-pathogenic nature of the wild type virus, but 50-80% of the adult population is seropositive for neutralizing antibodies against AAV2. Furthermore, due to the high titers needed for a gene therapy treatment, the capsid of the vector is able to create neutralizing antibodies against it, so gene therapy treatments should be administered in a single dose

## Conclusions and future prospects

Even though adeno-associated viral vectors represent a promising tool for gene therapy, with characteristics that no other viral vectors can achieve, they are not the key to cure all genetic diseases.
Despite it is usually thought that the major inconvenient in AAVs is its limited genome capacity, it has been proved that with small genes this vector gives good results, so further studies should be developed in order not to increase AAVs' capacity but to find another vector displaying the same features and admitting bigger genomes.
It has some other drawbacks that need to be taken into account, such as the high percentage of population with neutralizing antibodies against AAV2: there are some cases in which pseudotyping it is not enough, so a better solution should be developed.

**References**

[1] Verma, I. M. & Weitzman, M. D. Gene therapy: twenty-first century medicine. *Annu. Rev. Biochem.* **74,** 711–738 (2005).
[2] Gonçalves, M. a F. V. Adeno-associated virus: from defective virus to effective vector. *Virol. J.* **2,** 43 (2005).
[3] Daya, S. & Berns, K. I. Gene therapy using adeno-associated virus vectors. *Clin. Microbiol. Rev.* **21,** 583–593 (2008).
[4] Muramatsu, S., Mizukami, H., Young, N. S. & Brown, K. E. Nucleotide sequencing and generation of an infectious clone of adeno-associated virus 3. *Virology* **221,** 208–217 (1996).
[5] Afione, S. a., Wang, J., Walsh, S., Guggino, W. B. & Flotte, T. R. Delayed expression of adeno-associated virus vector DNA. *Intervirology* **42,** 213–220 (1999).
[6] Zincarelli, C., Soltys, S., Rengo, G. & Rabinowitz, J. E. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. *Mol. Ther.* **16,** 1073–1080 (2008).
[7] Wu, Z., Yang, H. & Colosi, P. Effect of genome size on AAV vector packaging. *Mol. Ther.* **18,** 80–86 (2010).
[8] Grieger, J. C. & Samulski, R. J. Packaging capacity of adeno-associated virus serotypes: impact of larger genomes on infectivity and postentry steps. *J. Virol.* **79,** 9933–9944 (2005).
[9] Mingozzi, F. *et al.* CD8(+) T-cell responses to adeno-associated virus capsid in humans. *Nat. Med.* **13,** 419–422 (2007)