**What are viruses?**
Virus are obligate 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 viral or VIRAL.

### 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 or DNA damage, it is non-pathogenic and the cycle remains latent. When there is coinfection, it changes to lytic AAV Life Cycle.

- **Viral elements needed to convert the virus into a vector:**
  - Rep: Cap structural capsid proteins
  - ITRs: Irreversible terminal repeat

- **Genome:** AAVs includes 2 open reading frames (ORFs) and 3 promoters between the two 145bp-inverted terminal repeats (ITRs).

- **pseudotyping:** It is the practice of using adeno-associated virus vectors to deliver genetic material to target tissues. They are usually combined with a helper virus to deliver the vector to the tissue.

### 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.

**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.

**Limitations:**
- Small packaging capacity: only 4 kb of transgene can be packaged, which leaves many diseases such as muscular dystrophy or cystic fibrosis.
- 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 AA.V2. Furthermore, due to the high titer 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 AAV 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 to increase AAV’s 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 AA.V2: there are some cases in which pseudotyping is not enough, so a better solution should be developed.

**References:**

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**ADENO-ASSOCIATED VIRUS-BASED VECTORS**

- **Vector production:**
  - Lab scale: Triple transfection system:
    - AA.V2 Rep and Cap from the desired serotype
    - Transgene plasmid within the two AA.V2 ITRs
    - EIA, EIB, 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 systems: 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 is a promising future.