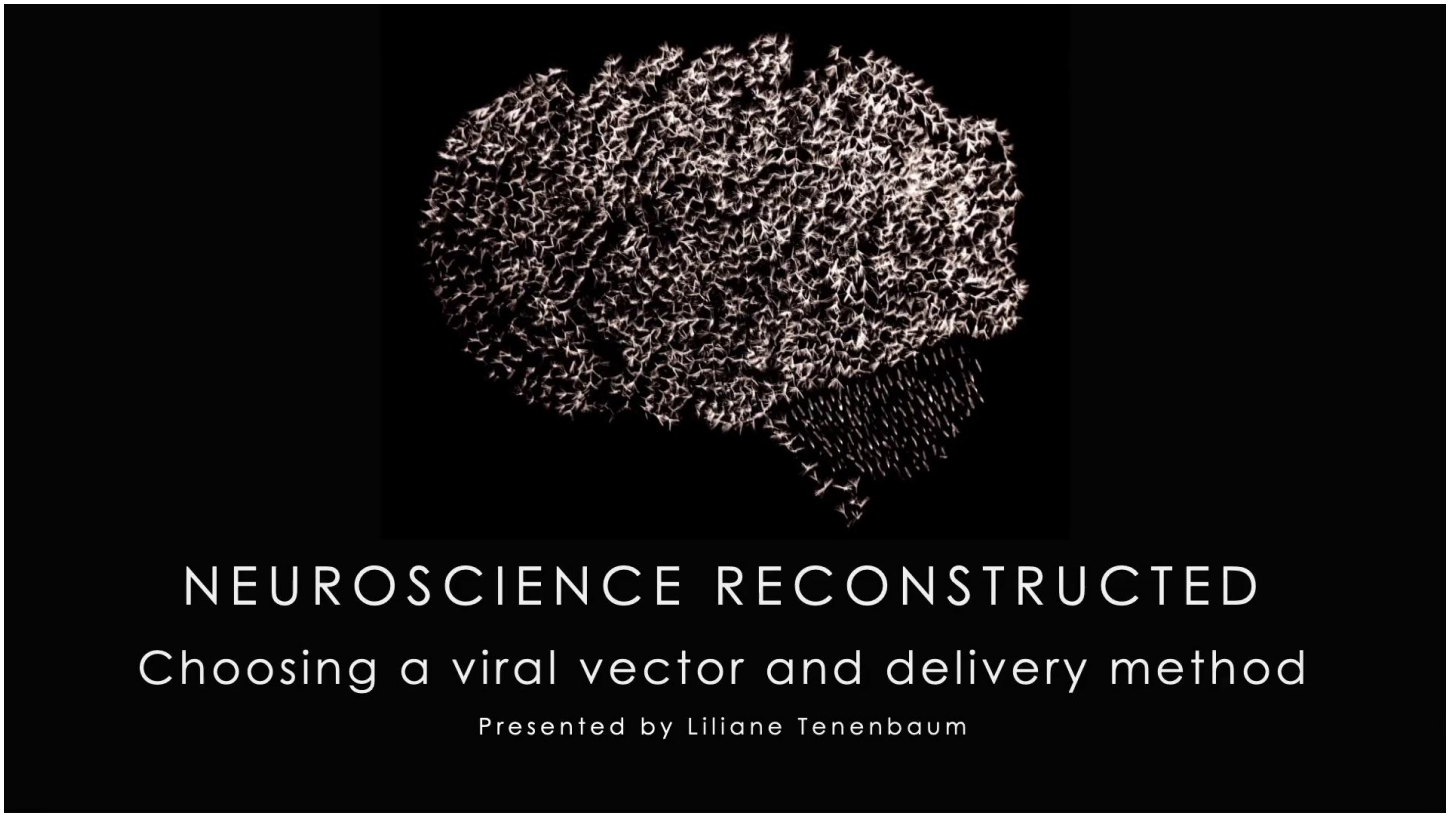
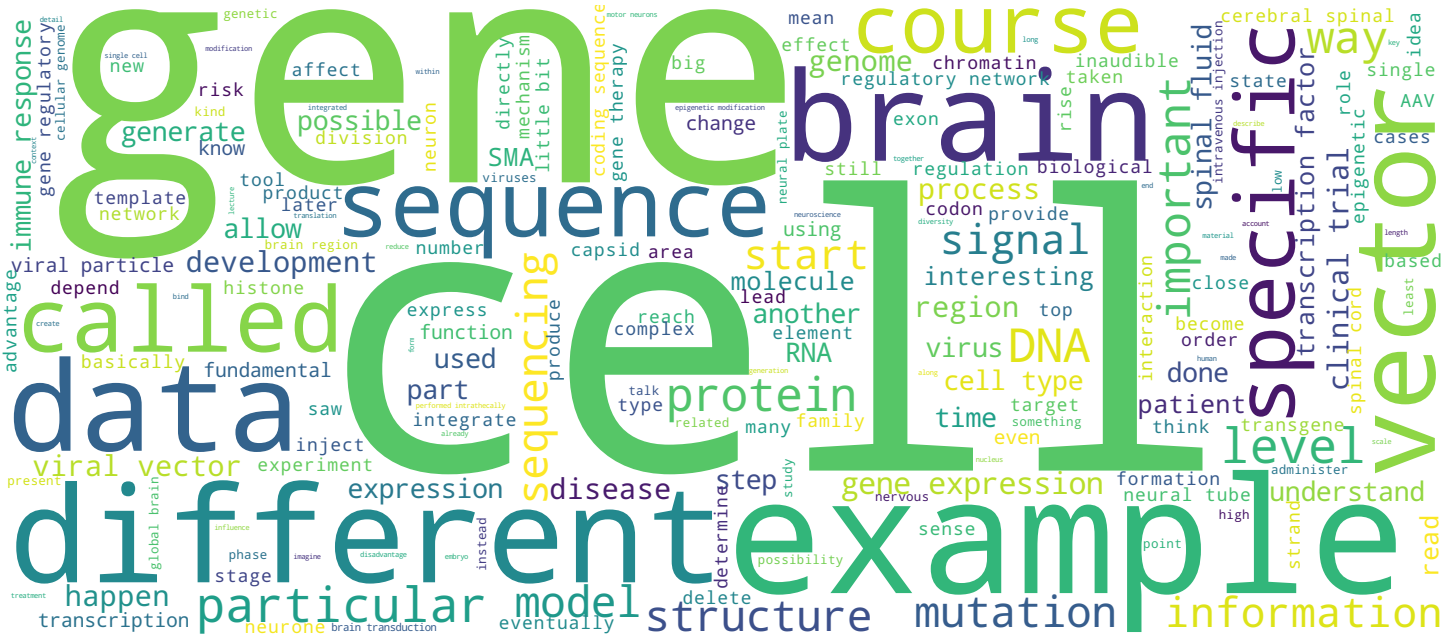


NEUROSCIENCE RECONSTRUCTED

Choosing a viral vector and delivery method

Presented by Liliane Tenenbaum



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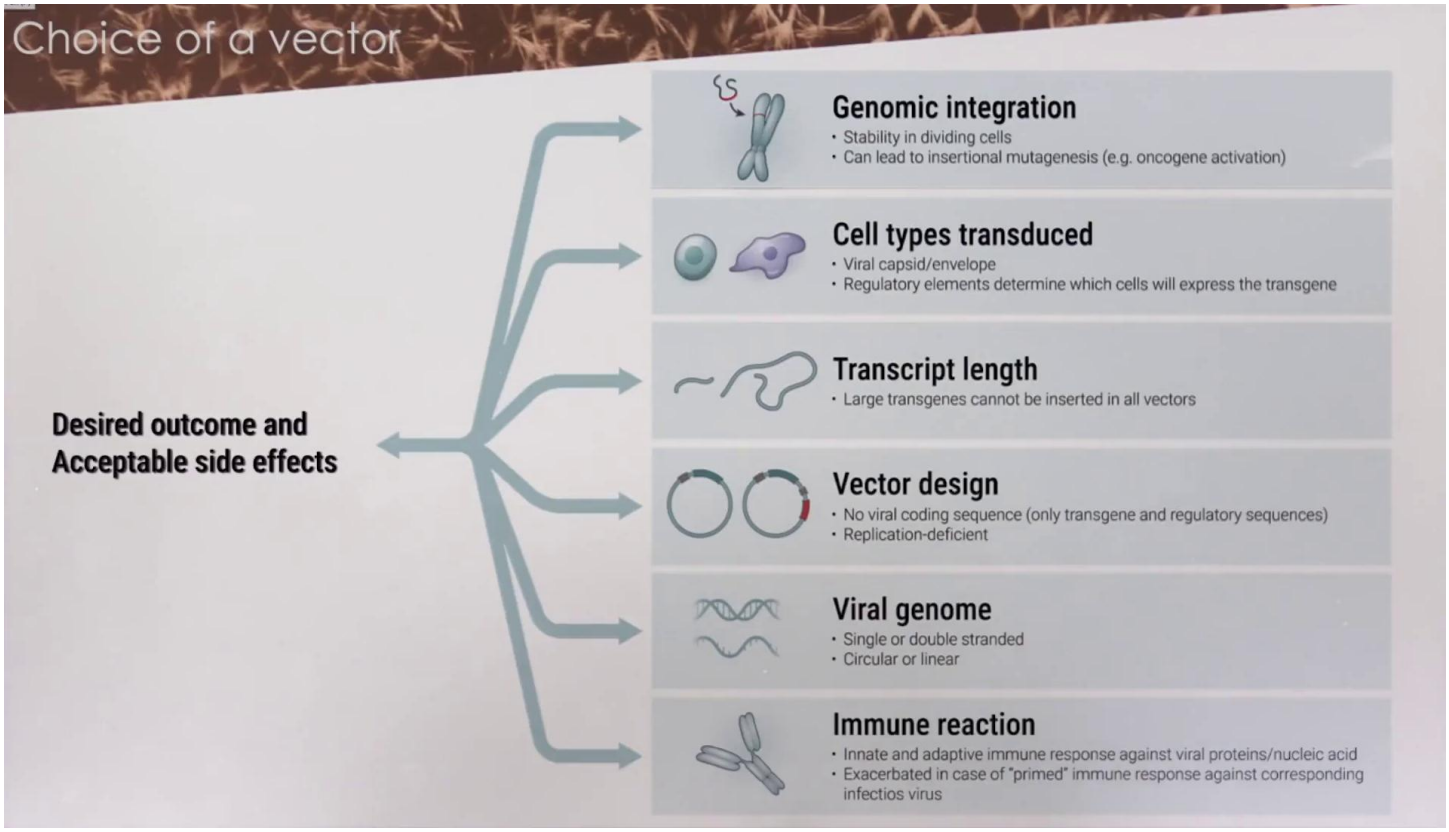
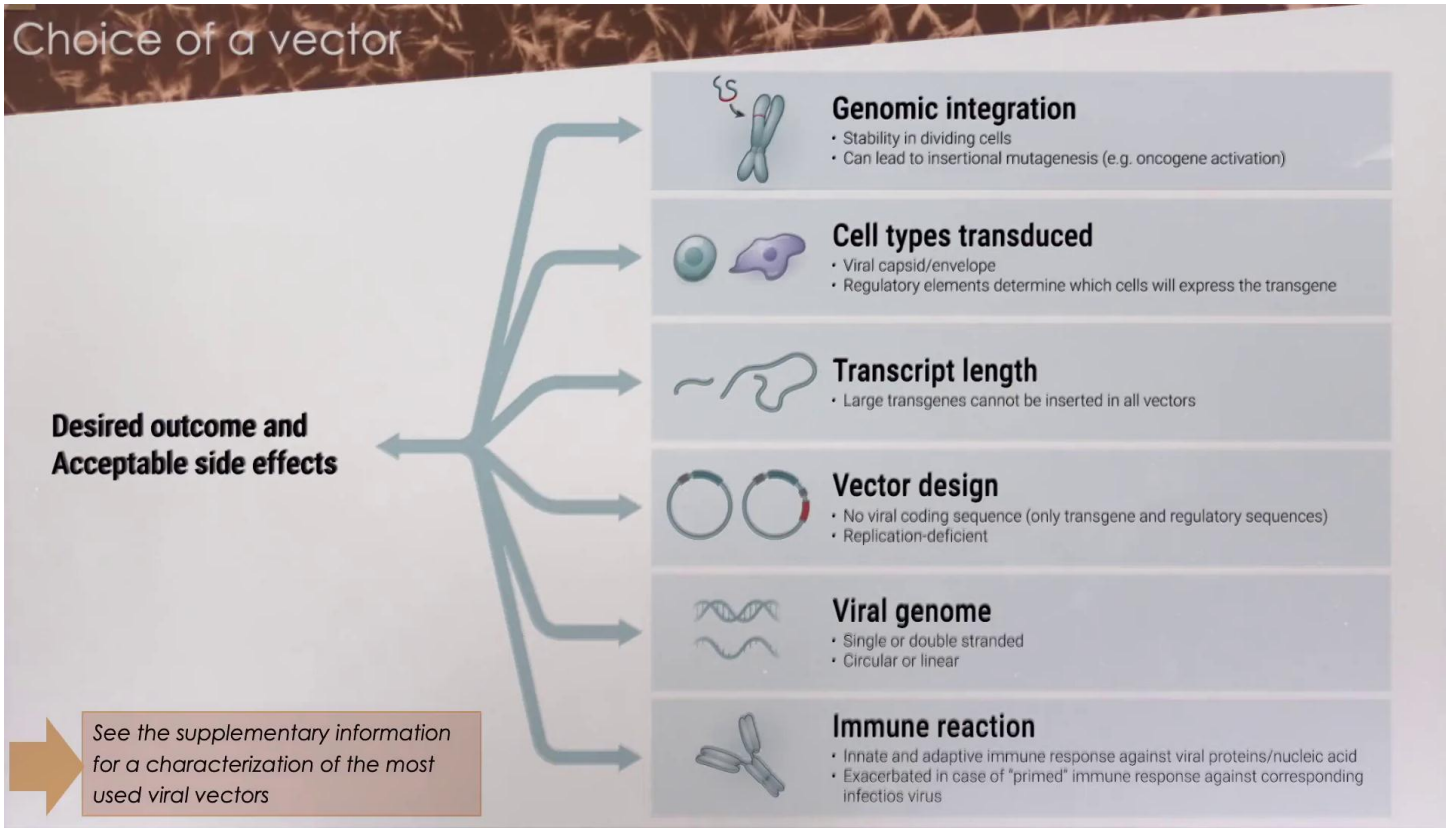


While choosing a viral vector, we have to think ahead of what is designed, what we want to do. The application will be very important to decide which viral vector to use. And the criteria that have to be taken into account is first of all, do we want a vector that will integrate its genome into the cellular genome, or do we want a vector that transfer its material to the nucleus but that will not integrate into the cellular genome? The advantage of integration is the stability of the genome. If the cells are dividing, you can understand that if the viral genome is not integrated, at each division it will be more and more lost. And after a certain number of division, it will disappear. So the effect of the transgene will be transient. But the disadvantage is that it can lead to insertional mutagenesis. Then you have to consider which cell type you want to transduce because all the viruses don't infect all types of cells. This depends on the capsids or depending on the family. But it also depends on the regulatory element that you will use to express your therapeutical gene. The promoter might be cell-specific and in some cases, there are also three prime untranslated region of genes that can be introduced downstream to the coding sequence.

Notes

Summary

0m 06s



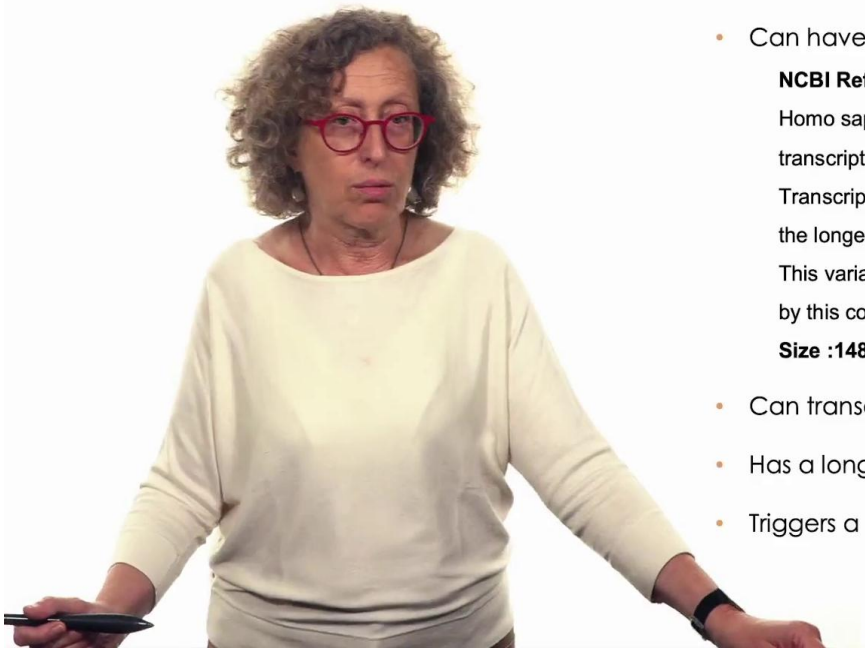
These elements can affect the cell type specificity of transduction. Of course, the length of the coding sequence is very important. Large tangent cannot be inserted in all vectors. Of course, the construction of the vector in which you want to delete all the coding region, this is sometimes difficult to achieve with some large viruses that will lose their replication ability to produce the viral vector when you delete all genes. And finally, an important factor is the immune reaction. When we choose a virus that infect humans, the serological status of the patient has to be first evaluated. Patient having a strong immune response, again, the virus from which the vector derive, are going to be not eligible for the clinical trial.

Notes

Summary

2m 14s

Viral vector for SMA1 therapy



In the case of SMA1, we need a vector that:

- Can have an insert the size of the SMN1 cDNA:

  NCBI Reference Sequence: NM_000344.4;

  Homo sapiens survival of motor neuron 1, telomeric (SMN1), transcript variant d, mRNA

  Transcript variant d represents the longest transcript and encodes the longest isoform (d).

  This variant is thought to be the predominant transcript produced by this copy of the gene.

  Size :1482 bp

- Can transduce motoneurons (non-dividing cells)
- Has a long-term gene expression
- Triggers a low immune response and has a low toxicity

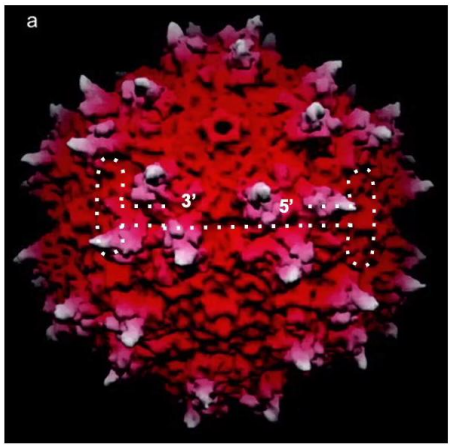
What do we need for SMA? The target population was SMA Type 1. We need first a vector that is large enough to accept SMN1 cDNA. This is an extract from the gene bank database. We need a vector that can transduce motoneurons, so non-dividing cells. And finally, we need a vector that will provide a long-term gene expression. Because the ideal would be to administer the treatment only once and have a lifelong expression of the SMN1 gene. Last but not least, we need a vector that will trigger as low as possible immune response and that is not toxic, of course.

Notes

Summary

3m 33s

Viral vector for SMA1 therapy



Wild-type adeno-associated virus (AAV)

- Already used in clinical trials for other pathologies
- Safe and well tolerated by children and adults

Xie Q, Bu W, Bhatia S, et al. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proc Natl Acad Sci U S A*. 2002;99(16):10405-10410. doi:10.1073/pnas.162250899

See the supplementary information to learn about the properties of AAV-based vectors and how viral vectors are produced

The researcher decided to turn to adeno-associated virus that had been already used in some pioneering clinical trials, both in small children with severe genetic diseases and also for Parkinsonian patients, so adults. And in both cases, the safety and tolerability of the vector have been demonstrated.

Notes

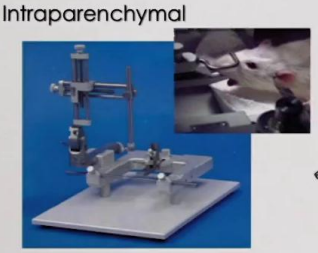
Summary

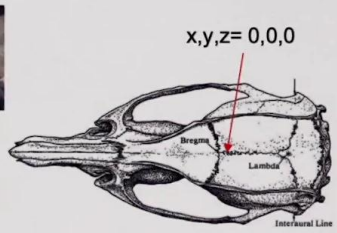
4m 33s

AAV vectors for the CNS

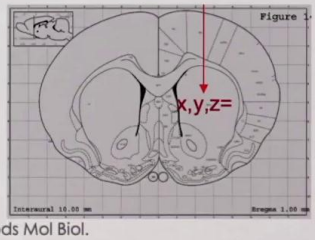
Different routes of administration result in different efficiencies, specificities, and adverse effects

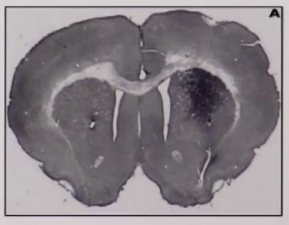
- Intraparenchymal





Brain Atlas





Bockstael O et al. Recombinant AAV delivery to the central nervous system. Methods Mol Biol. 2011;807:159-77. doi: 10.1007/978-1-61779-370-7_7

- Breakthrough: AAV serotype 9 passes the Blood-Brain barrier

  Global brain transduction

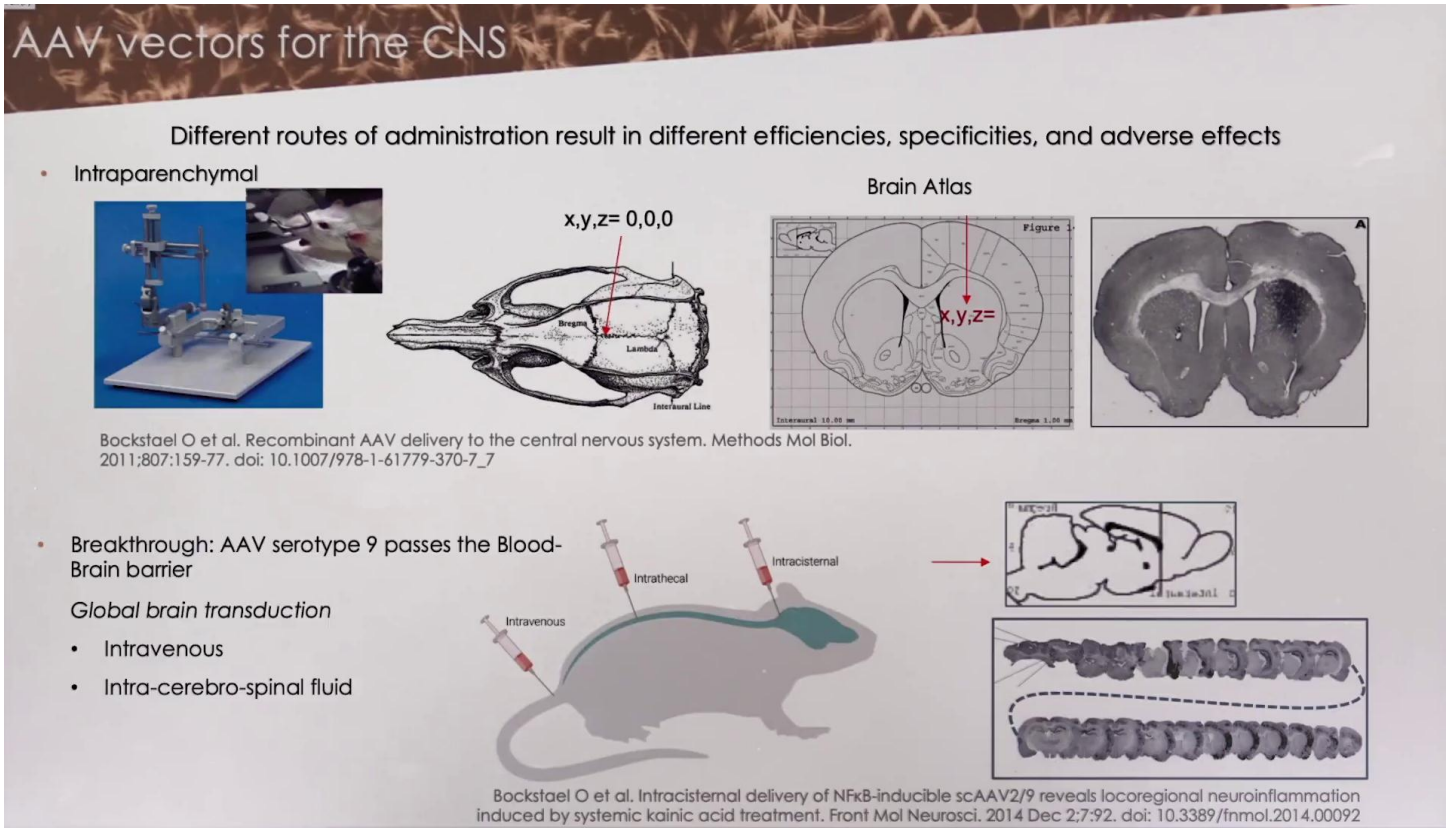
  - Intravenous
  - Intra-cerebro-spinal fluid

Now that we have these viral particles, we have to introduce them in the brain, and there are several ways to do that. The most obvious is to administer it directly into the brain and this in addition allows to target specific regions of the brain. And to do that, we have brain atlases. This is interesting when you want to target a precise region. As you see here, the product of the transgene has been revealed by immunohistochemical method using antibodies. And you see that the protein is located in the region that we targeted. There is no transgene expression in other regions. The viral particles are able to diffuse from the injection site, but not too far. We can have a relative targeting. In 2009, there was a big breakthrough in AAV vectorology. French group discovered that the serotype nine of AAV is able to pass the blood-brain barrier. This is very interesting because instead of making this brain surgery, which can have some risk, it would be possible to inject the viral particle in the blood circulation and it will reach the brain. A little bit later, they also discovered that the AAV9 particles are able to cross the barrier between the cerebral spinal fluid and the brain.

Notes

Summary

5m 10s



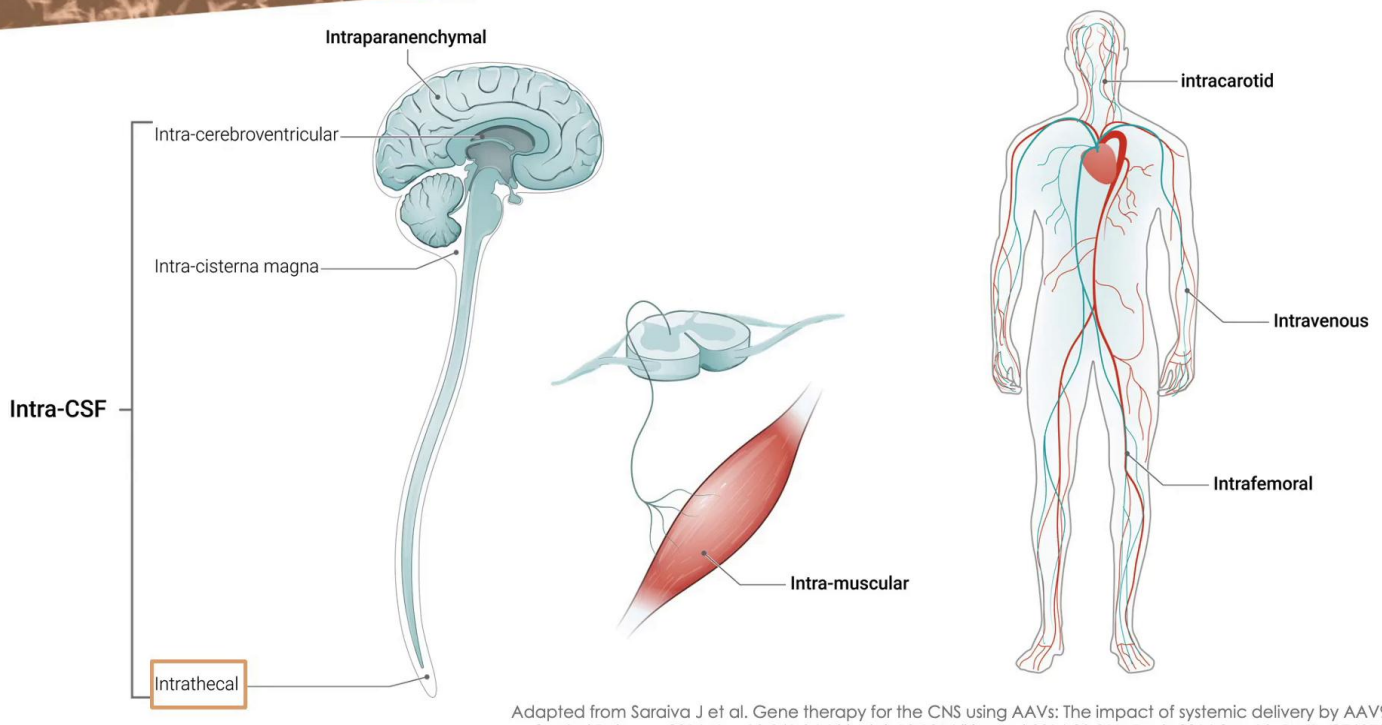
That's a second possibility. This when it's done in rodents, the intravenous injection is done in the tail vein and the intracerebral spinal fluid injection can be made in different way. Here in blue, you have the cerebral spinal fluid You see that it can be injected close to the brain in the cisterna magna. It's a reservoir of CSF behind the brain. But it can also be injected intrathecally, so close to the spinal cord. When you do that, you don't target a specific brain region, but you obtain a global brain transduction. You see here after the intracisternal injection, that from the posterior part of the brain to the anterior part, transduction is visible through the whole brain, but without the possibility of targeting.

Notes

Summary

7m 18s

Gene delivery to motoneurons



Adapted from Saraiva J et al. Gene therapy for the CNS using AAVs: The impact of systemic delivery by AAV9. J Control Release. 2016 Nov 10;241:94-109. doi: 10.1016/j.jconrel.2016.09.011. Epub 2016 Sep 13. PMID: 27637390

What was done for SMA? For SMA, we want a global brain transduction since the mutation is, of course, everywhere in all cells, in all motor neurons. Specifically, we would like to correct this mutation. There are several delivery methods that could allow to reach this goal. It could be injected in the cerebral spinal fluid, so in the cisterna magma or along the spinal cord. A particularity of some viral vectors is that they are able to perform a retrograde transport. Another possibility is to inject the vector intramuscularly, and it will be taken up by the terminals of the neuron, and be retrogradely transported to the motor cortex. That's also a non-invasive way to do it. But unfortunately, it's not efficient enough. Finally, it can be injected intravenously, and in some pre-clinical models into the femoral artery or into the carotid. But for SME, the first clinical trial were using an intravenous injection of the vector. Later on, actually, in order to reduce the risk of immune response, it was performed intrathecally. The latest clinical trials, which I will not describe because it's really recent were performed intrathecally.

Notes

Summary

8m 41s