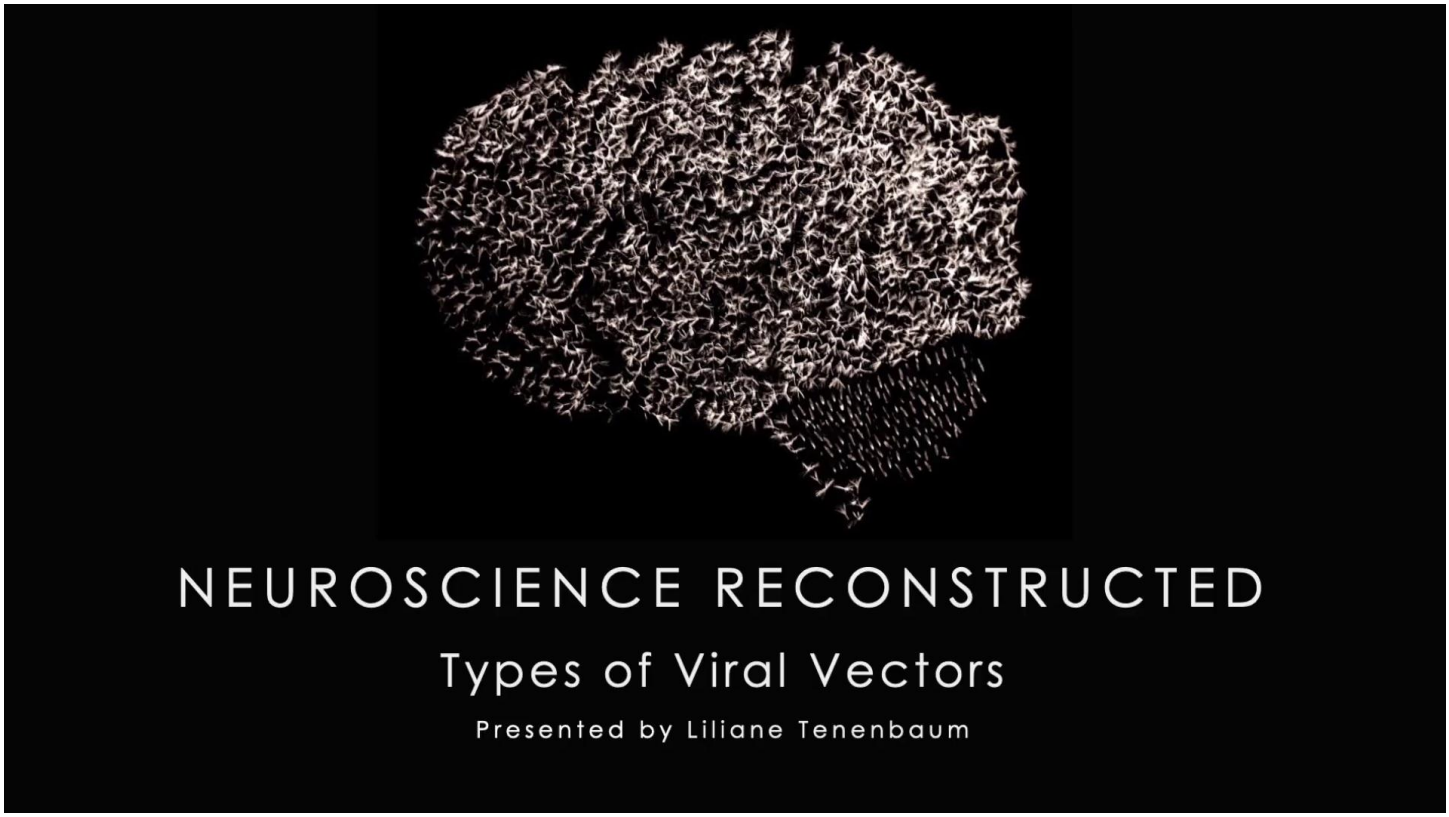

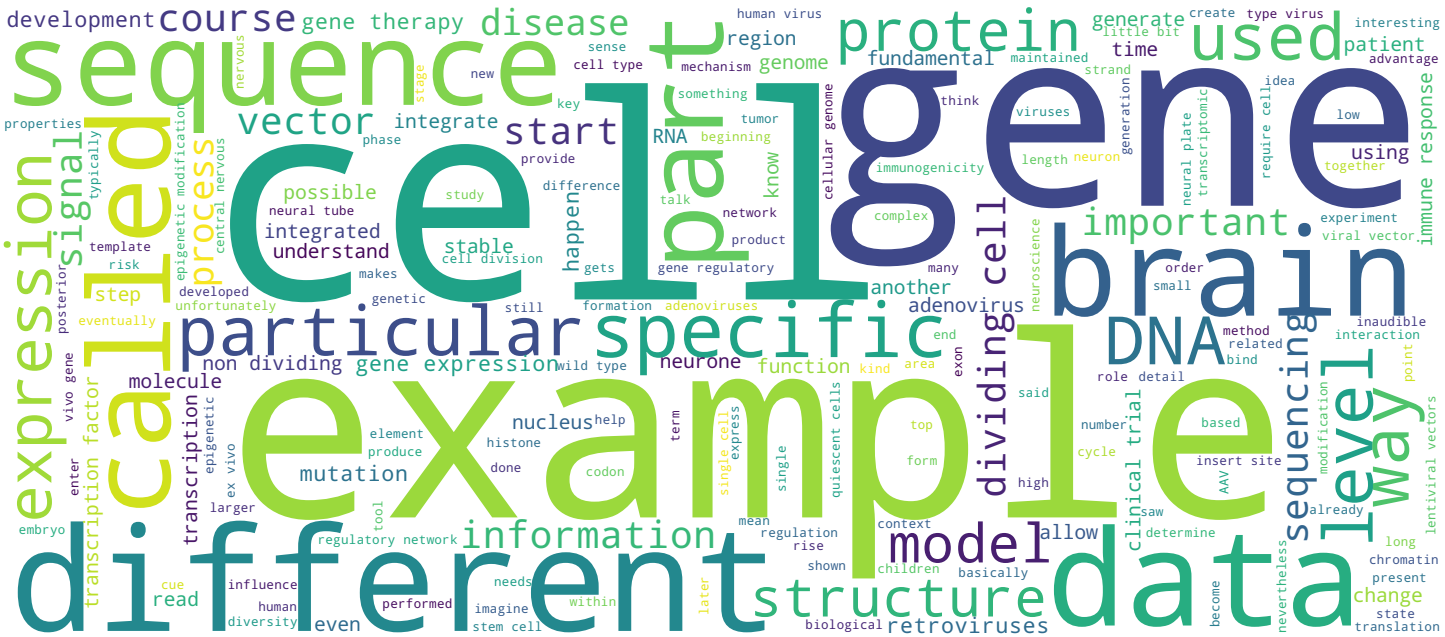

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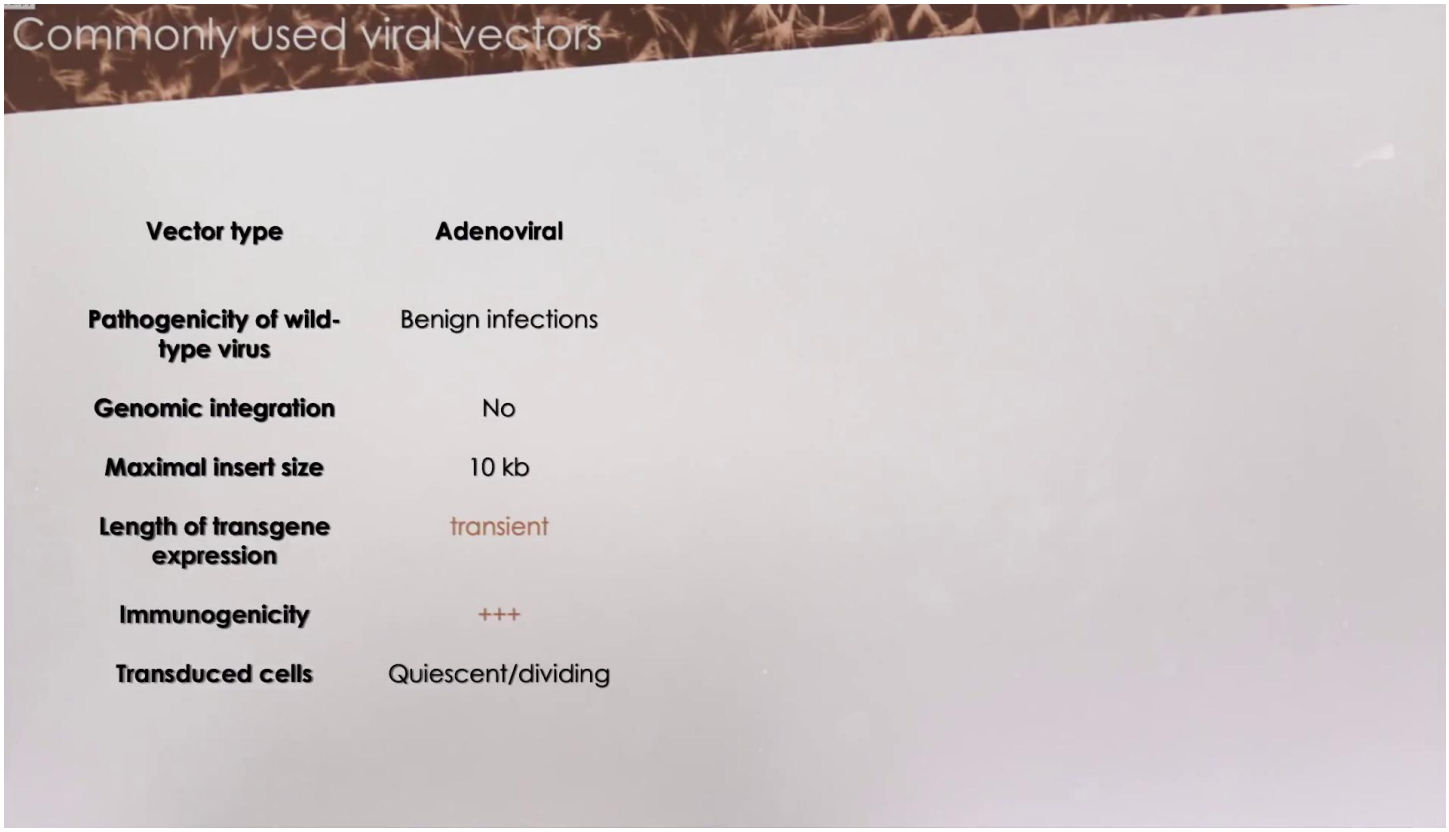
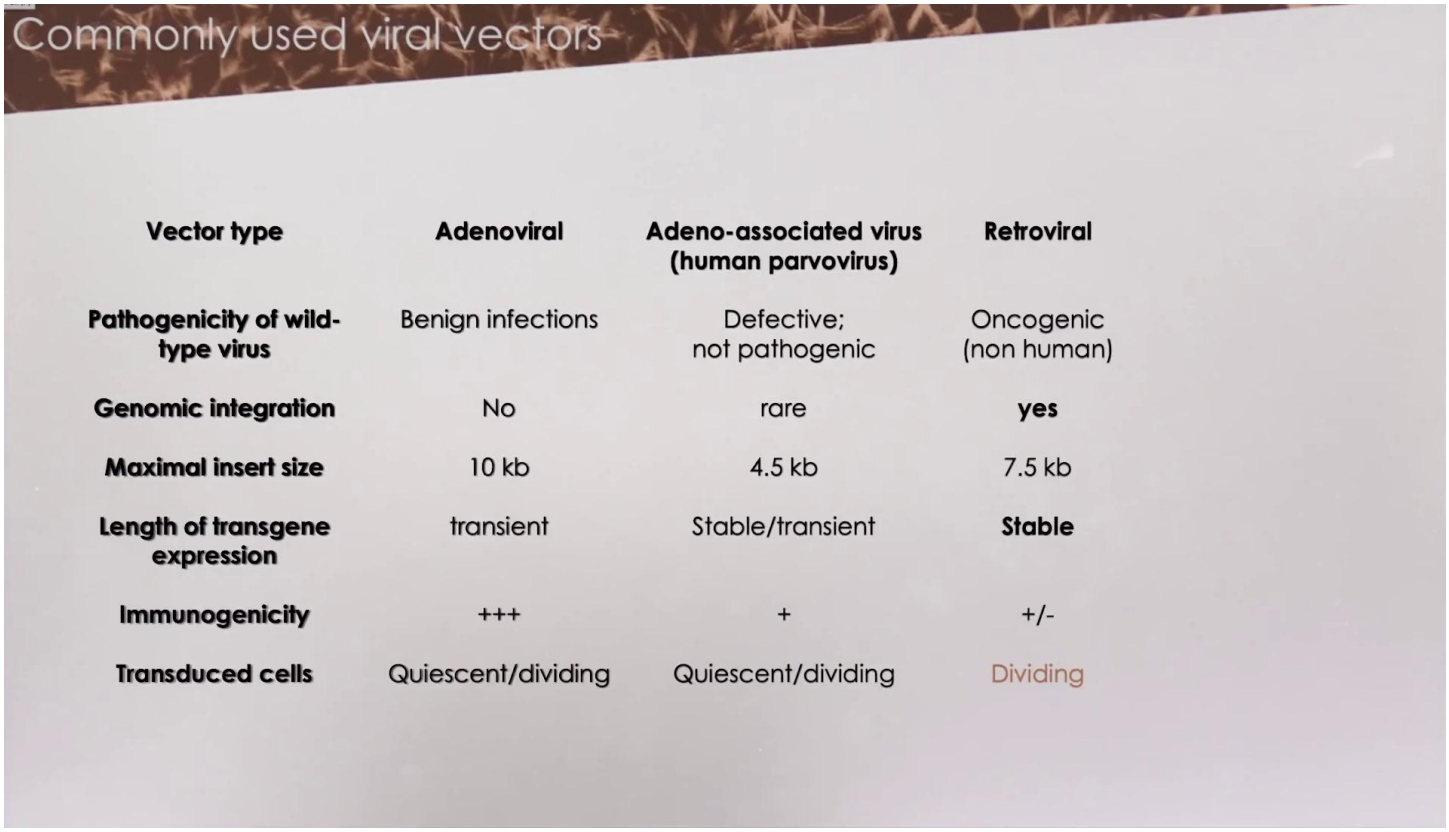


Let's look closer to the properties of the mainly-used viral vectors. Adenoviruses have high interest in the beginning because they are not very pathogenic. We all had benign infections by different serotypes of adenoviruses. These are respiratory infection or gastroenteric infection, but these are really benign. But you can immediately guess that since we all have been infected with adenoviruses, we have a memory immune response against these viruses. Thus, the immunogenicity of the vectors is very high. Due to that, the length of transgene expression will be short simply because the transduced cells will be eliminated by the immune system. The advantage of adenovirus is that the capacity is relatively large. They don't integrate their genome into the cellular genome, which makes them a bad candidate for ex vivo gene therapies. It will not be stable in dividing cells. In quiescent cells, in non-dividing cell, the expression can be maintained. But unfortunately in vivo, we are facing this immune response that it's really a problem to use these viruses. Even in the brain, which is considered immune-privileged sites, it has been shown that adenoviruses elicit an immune and inflammatory response, and that the expression of the transgene disappears after a few weeks.

Notes

Summary

0m 05s



Adeno-associated viruses are more and more used in gene therapy, and there are good reasons for that. First of all, it's a human virus, but it's not pathogenic. It's actually a defective virus that cannot replicate by itself. It needs an adenovirus or an herpes virus, another big virus to provide some function that AAV doesn't have and that cannot be given also by the cell. It was also developed because of this non-pathogenic aspect. But again, since it's a human virus, there is a risk of immune response. We'll see it's not as bad as adenovirus, but nevertheless, we have to deal with it. Unfortunately, the maximal insert site is only 4.5 kilobase and it's not integrated, so no risk of oncogene activation. In rare cases, it does integrate, but it's not part of its cycle. Most of the copies remain episomal. Episomal means that the viral genome is present in the nucleus but has a separate genome not integrated into the cellular genome. Due to the lack of integration in dividing cells, it will be diluted and lost. But in non-dividing quiescent cells like neurones, which is our focus, the genome will be maintained for very long periods. The third family are retroviral vectors.

Notes

Summary

2m 20s

Commonly used viral vectors

Vector type	Adenoviral	Adeno-associated virus (human parvovirus)	Retroviral
Pathogenicity of wild-type virus	Benign infections	Defective; not pathogenic	Oncogenic (non human)
Genomic integration	No	rare	**yes**
Maximal insert size	10 kb	4.5 kb	7.5 kb
Length of transgene expression	transient	Stable/transient	**Stable**
Immunogenicity	+++	+	+/-
Transduced cells	Quiescent/dividing	Quiescent/dividing	Dividing

These have been used and are still used in ex vivo gene therapy. For example, for these bubble babies, when the hematopoietic stem cells are harvested from patient and transduced by retroviral or lentiviral vectors before being reimplanted. The retroviruses are non-human virus. They have oncogenic potential. But the wild-type virus, it has been observed only in animals. It's not tumorogenic for human. The genomic integration, it's part of the viral cycle. All the retroviral genomes will be integrated. The insert site that it accepts is larger than that of AV vectors. The expression will be stable since it's integrated, the immunogenicity is low. But retroviruses have a particularity. It's that they can only infect dividing cells, because they require cell division to be able to enter the nucleus. They can be used for ex vivo gene therapy. They could be used to transduce tumors and preferentially destroy dividing cells in tumors. This is a strategy that has been envisaged. As I said, the wild-type virus has never been oncogenic for human. But in early clinical trials, the vectors have shown to be the cause of tumor of leukemia in some children that were part of the clinical trials.

Notes

Summary

4m 34s

Commonly used viral vectors

Vector type	Adenoviral	Adeno-associated virus (human parvovirus)	Retroviral	Lentiviral
Pathogenicity of wild-type virus	Benign infections	Defective; not pathogenic	Oncogenic (non human)	Usually pathogenic (ex. HIV)
Genomic integration	No	rare	yes	**yes**
Maximal insert size	10 kb	4.5 kb	7.5 kb	7.5 kb
Length of transgene expression	transient	Stable/transient	Stable	**Stable**
Immunogenicity	+++	+	+/-	+/-
Transduced cells	Quiescent/dividing	Quiescent/dividing	Dividing	**Quiescent/dividing**

Finally, lentiviruses. Lenti and retroviruses are part of the big family of retroviridae. They function similarly. They are both RNA viruses. But most of the vectors are derived from HIV, so the virus that causes AIDS. You can imagine that many demonstration of safety of these vectors have had to be performed before they could be used in clinical settings, but this has been done. Like retroviruses, they integrate, they accept 7.5 kb of foreign genetic material. But the difference with retroviruses is that they will be stable in both... They infect both dividing and non-dividing cells because they don't require cell division to enter the nucleus. They form a complex called the preintegration complex that has a nuclear localization site that allows it to pass the nuclear membrane. This lentiviral vectors are also interesting for our applications for gene delivery to the central nervous system because we mainly target non-dividing cells.

Notes

Summary

6m 50s