

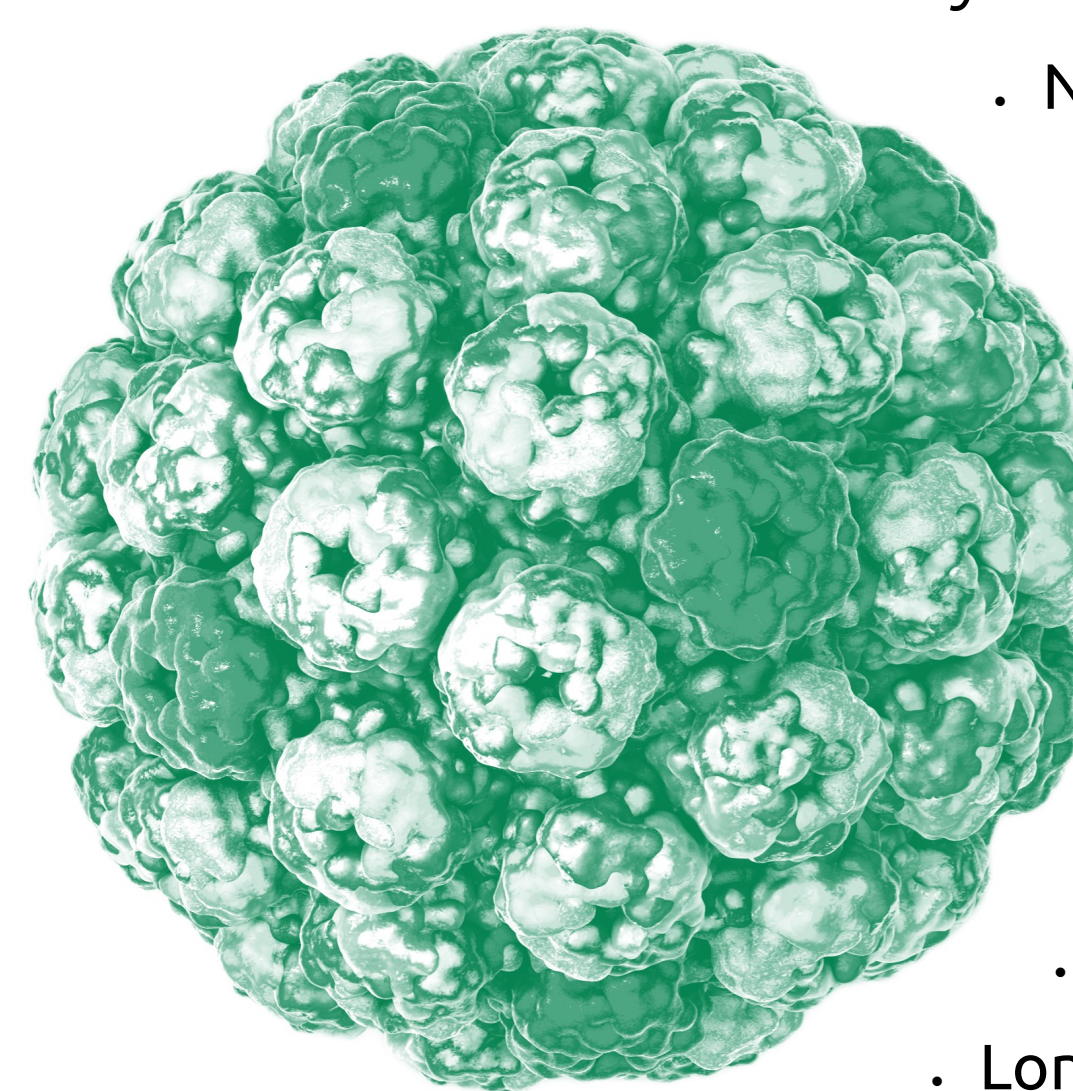
Overcoming the limits of current gene therapies

Viral vector-mediated gene therapy has come of age with the first products that have reached the market. The currently most used gene delivery vectors are those derived from adeno-associated viruses, AAV. Despite initial successes, clinical studies using AAV vectors revealed that administration of vector particles elicits innate and adaptive immune responses against the viral and transgene-encoded proteins in most patients. These responses lead to decreased transgene expression levels over time and elimination of the transduced cells from the body. Additionally, these vector-specific immune responses compromise the ability to re-administrate viral vectors. Attempts to improve efficacy have resulted in the use of extremely high vector doses in clinical studies. These high doses consequently can result in serious inflammation and adverse effects. Furthermore, the ability of AAV to cause genomic integration, which can result in cancer, further compromises long-term health.

To improve the safety and efficacy of *in vivo* gene therapies, an advanced gene transfer method is required. For this, both viral-based methods and non-viral methods have been studied. Non-viral methods seem attractive due to their high productivities, however effective delivery of the transgene to a nucleus of a target cell remains challenging. We developed a new replication-defective viral gene delivery vector platform named Nimvec, which is derived from the macaque polyomavirus Simian Virus 40, SV40. Since humans can be considered naive to SV40, it is expected that Nimvec is non-immunogenic or tolerogenic when applied in clinical settings.

Moreover, because of these properties it is expected that patients can be re-dosed with the same vector to increase or restore transgene expression. With such a non-immunogenic vector, gene delivery will be safer, more effective, and new applications are within reach such as the induction or restoration of immune tolerance to transgene proteins for the treatment of autoimmune diseases.

SV40 as new basis for *in vivo* gene delivery



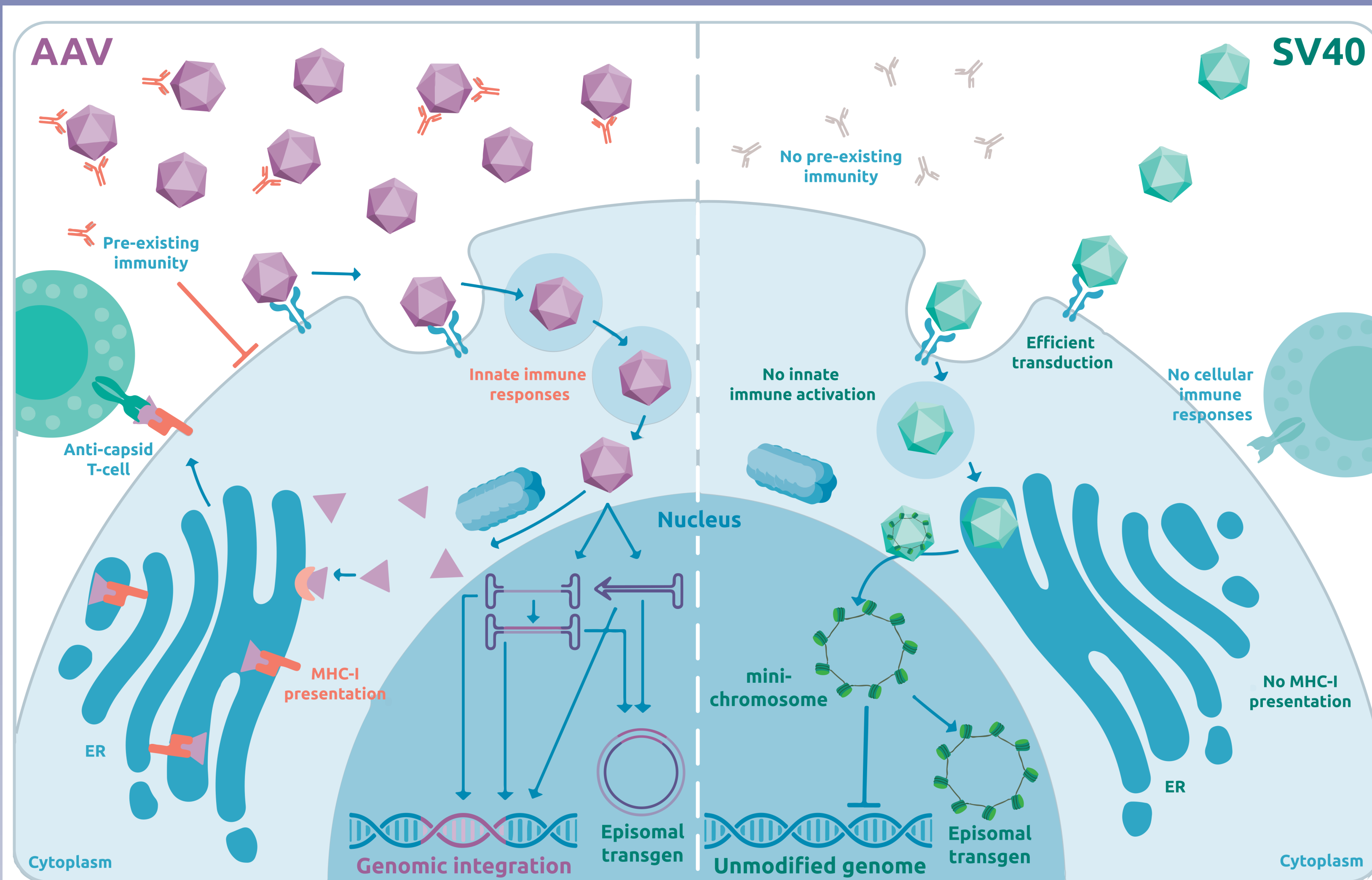
- Polyomavirus with 45nm capsid
- No pre-existing capsid immunity
- Replication defective vector
- 5.2 kb transgene capacity
- Non-integrating, circular mini-chromosome
- Increased gene transfer efficiency allowing lowered dosing
- No induction of immunity towards the vector nor the transgene
- Allowing repeated administration
- Long term stable transgene expression
- Proprietary, scalable production

Improved *in vivo* gene delivery

SV40 vectors for gene transfer have been studied almost directly after the discovery of the virus in the 1960s. Replication defective SV40 vectors have been developed but its use was limited due to the contamination with replication competent particles. A new packaging cell line has been developed at Amarna Therapeutics derived from Vero cells, allowing production of 100% replication defective vector particles.¹

SV40 cell entry is mediated by binding to GM1 expressed at the cell surface. Liver-based expression is obtained by intravenous injection of mice, as shown by the luciferase based delivery of Nimvec encoding the luciferase transgene (SVLuc) in mice. Beyond this single dose, re-administration of the vector is possible using SV40 vectors. In an early study in Gunn rats by Sauter et al, re-administration up to a total of 7 injection showed to be possible and resulted in high transduction efficiencies of liver cells.³ Moreover, one animal was treated with reporter gene containing SV40 vector after having received 3 doses of therapeutic SV40 vectors earlier. The observed reporter gene expression showed that no neutralizing responses against SV40 are generated during the first injections. Re-administration of viral vectors is highly favorable as it allows for personalized treatment to reach an optimal therapeutic effect and for re-treatment of the patient in case the therapeutic effect decreases due to target cell renewal.

Current gene replacement therapies are limited to a single-dose strategy since immune responses are generated against the capsid, thereby limiting the effectivity of repeated dosing. In combination with the low moiety of transduction of current AAV vectors, a single AAV administration requires extremely high dosing. These doses are frequently in the range of 10^{14} VG/kg. *In vivo* experiments with SV40 showed that likely lower doses can be used due to its higher moiety of transduction and favorable immunological profile. Combined with the possibility of re-administration, SV40 promises severely improved *in vivo* gene delivery.



Current AAV-mediated gene transfer compared to SV40 as non-immunogenic vector. Observed immunological barriers are pre-existing capsid immunity, innate immune responses, and antigen presentation on MHC-I (shown in red). Under the immune radar gene delivery by SV40 is possible due to absence of pre-existing immunity, no innate immune activation and no MHC-I based antigen presentation (green)².

A non-immunogenic viral vector

There are three main immunological barriers to AAV gene transfer:

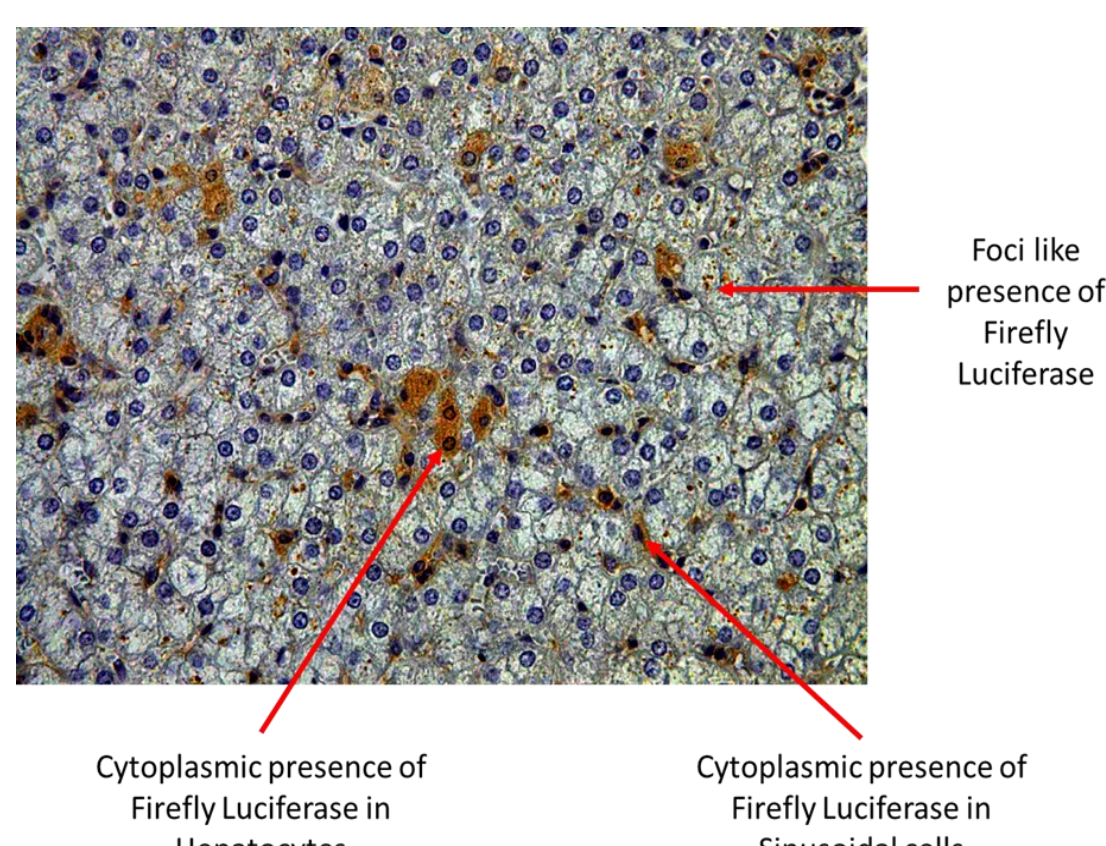
- Pre-existing capsid neutralizing antibodies.
- Vector capsid/DNA recognition by toll-like receptors (TLRs), inducing an inflammatory response.
- Attracting T-cells that will enable the activation of a cellular immune response

SV40 is able to circumvent these immunological barriers. Humans are not a host for SV40 and the virus does not replicate in humans, therefore the human population is considered naïve to SV40. In addition, SV40 particles prevent activation of TLRs and escape from endolysosomes into the ER before they can be broken down and loaded on MHC-I molecules. Therefore, the host's immune system is not activated and immunity to SV40 does not arise, allowing for effective re-dosing.

Efficient gene delivery

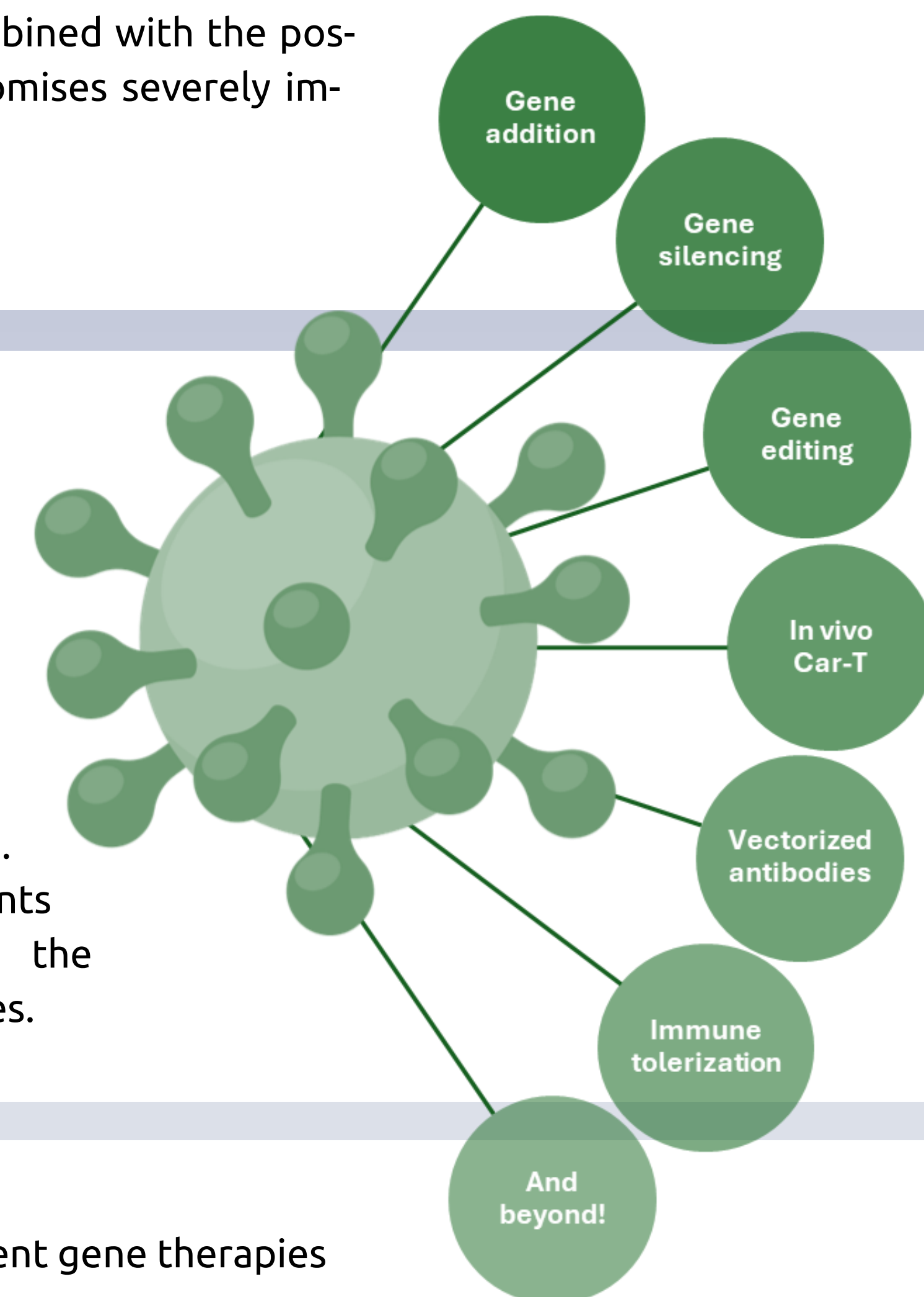
Increasing the gene delivery efficiency assists in overcoming the immunological barriers, as detection of gene delivery particles becomes more difficult for the immune system. Here we transduced marmosets with low dose levels of purified SV40 vectors expressing Firefly Luciferase. Various blood biomarkers related to liver inflammation (e.g. albumin, CRP) and general health (e.g. glucose, cholesterol) were measured before and 14 days after Nimvec administration. None of the measured markers deviated significantly from healthy marmoset blood levels, indicating that there is no apparent adverse response to the treatment.

Immunohistochemistry staining on liver tissues for firefly luciferase. All images shown are auto-levelled using Paint.net. A single marmoset was IV administered and immunohistochemistry against firefly luciferase was performed on liver slides (brown colour) isolated 14 days post IV administration.



Unlocking new applications

Employing the power of the variety of available viruses unlocks multiple new classes of gene therapy. The integrating potential of Retroviruses makes them especially useful for applications requiring long-term gene transfer. Moreover, the immune silent delivery of SV40 allows the development of gene therapies raising immune tolerance to a specific transgene. This approach allows the development of treatments for autoimmune diseases, possibly reverting the damaging effects by self-targeting immune responses.



Conclusion

- Novel viral vector systems overcome limits of current gene therapies
- Improved gene delivery requires immune silent gene transfer
- Increased gene transfer efficiency results in lowered dosing
- Replication deficient SV40 vectors have all characteristics for improved gene delivery

References

1. Toscano MG, van der Velden J, van der Werf S, Odijk M, Roque A, Camacho-Garcia RJ, Herrera-Gomez IG, Mancini I, de Haan P. Generation of a Vero-Based Packaging Cell Line to Produce SV40 Gene Delivery Vectors for Use in Clinical Gene Therapy Studies. *Mol Ther Methods Clin Dev.* 2017 6:124-134.
2. Gerritzen MJH, Viral vector gene delivery: balancing safety, efficacy and dose, *Cell & Gene Therapy Insights* 2021; 7(8), 1119–1123
3. Sauter BV, Parashar B, Chowdhury NR, Kadakol A, Ilan Y, Singh H, Milano J, Strayer DS, Chowdhury JR, A replication-deficient rSV40 mediates liver-directed gene transfer and a long-term amelioration of jaundice in Gunn rats, *Gastroenterology* 2000, 119(5), 1348-1357

