NEXT-GENERATION VECTORS

SPOTLIGHT

EDITORIAL

Next-generation gene therapy vectors: alternative approaches to vector design and application

Richard P Harbottle

> An ideal vector for human gene therapy should provide sustainable and therapeutic levels of gene expression without compromising the viability of the host.

This issue of *Cell and Gene Therapy Insights* will focus on alternative next-generation vectors for gene therapy and the genetic modification of cells. Rather than discussing the use of attenuated viruses, which are the most commonly used vector system, this issue comprises reviews and commentary on a range of alternative vectors that are being developed and which provide properties, capabilities and utility not available with typically used retrovirus and lentivirus, adenovirus or adeno-associated virus (AAV) vectors. Antonio Marchini from the German Cancer Research Centre in Heidelberg will share his insight into the use of infectious and replicating viral vectors and describe the development and clinical application of this vector system in anti-cancer treatment. We also have insightful expert reviews on the advances in the application and

improvements to our understanding of the Sleeping Beauty transposon system from the labs of Zsuzsanna Izsvák (Max Delbrück Center for Molecular Medicine, Berlin) and Zoltán Ivics (Paul Ehrlich Institute, Langen) as well as an overview from Laurence Cooper's lab at MD Anderson Cancer Center, Houston describing the use of this non-viral vector system for the genetic modification of T-Cells for human anti-tumour immunotherapy. We also have an insight into the utility and use of minimally sized Nanoplasmid DNA vectors from the team at Nature Technology Corporation (Lincoln, NE) and Minicircle vectors from PlasmidFactory (Bielefeld).

BACKGROUND

Despite decades of discussion and development, clinical trials and tribulation, the successful application of gene therapy to patients using viral vectors has become a reality. The initial treatment of SCID-X1 patients using modified retroviruses is deservedly celebrated as a success that is tempered only by the vector-mediated genotoxicity, which led to oncogenesis in several of the treated patients [1]. Subsequent modifications to the vector and protocols have led to a steady stream of refinements and improvements [2]. Since these first successful clinical studies that utilized integrating viral vectors, the clinical application of non-integrating AAV vectors in a multitude of trials has greatly increased the scope of successful gene therapy treatments in patients. AAV has been effectively applied in a variety of clinical trials, which were discussed in a recent issue of this journal [3]. AAV has been used to treat a range of conditions from the

treatment of retinal diseases such as Leber congenital amaurosis [4], Leber hereditary optic neuropathy [5] and choroideremia [6] to neurological disorders (aromatic L-amino acid decarboxylase deficiency) [7] to blood disorders such as hemophilia B [8] and lipoprotein lipase deficiency [9]. With an increasing number of clinical trials currently underway and an array of biotech companies and financiers investing in the field, there might seem to be no avenue worth exploring for alternative vector systems. But, AAV vectors do have some fundamental limitations that cannot be simply overcome.

DEVELOPMENT OF ALTERNATIVE VECTORS

One of the principle barriers that limits the clinical use of AAV vectors is the significant number of people who have pre-existing cross-reactive antibodies to one or more of the commonly used AAV serotypes [10]. Even low levels of this anti-AAV antisera can cross-react and neutralize a range of AAV, limiting their therapeutic potential and repeat administrations [11]. The second drawback of AAV vectors is their limited capacity to accommodate transgenic DNA. This not only limits the range of genes that can be engineered into the vector, but also reduces the potential for complexity in design to drive these genes with cell or tissue specificity; typically, smaller and ubiquitous promoters are used to drive transgene expression of small gene sequences in AAV vectors. Finally, one of the biggest drawbacks to utilizing AAV vectors is their inability to provide persistent and sustained expression in dividing cells and

this fundamentally limits their application to non-dividing cells and tissues.

INFECTIOUS VIRAL VECTORS

Typically, an ideal vector for human gene therapy should provide sustainable and therapeutic levels of gene expression without compromising the viability of the host. In this issue, we have articles describing such systems; sophisticated non-viral DNA vectors that are designed to provide long-term and safe transgene expression in cells. We also have a rather contrasting discussion on the use of oncolytic viruses for the treatment of cancer. Antonio Marchini describes his lab's development of new anticancer strategies based on autonomous oncolytic parvoviruses. This is an application of viruses for the treatment of disease as nature designed them – self-replicating biological weapons that can specifically infect and propagate in cancer cells before killing them and spreading their viral progeny further into a tumor mass. An additional benefit of this viral-mediated cancer cell killing is the release of tumor-associated antigens and their activation and stimulation of the host immune system, which can act against the treated tumor and disseminated metastases.

NEXT-GENERATION NON-VIRAL DNA VECTORS

Despite the considerable success of viral vectors for gene therapy, many researchers continue to work on the development of alternative non-viral gene therapy vectors. A typical

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limitation, which restricts the clinical application of these vectors, is the transient nature of the transgenic material in dividing cells. Our bodies can defend themselves from the effects of genetic damage or infection and, just like viruses, many non-viral gene therapy vectors can also activate these defence mechanisms and are subsequently destroyed or rendered silent. It has become apparent that, without refinement or considered design, the clinical utility of a typical non-viral vector is fundamentally limited due to the transient nature of its transgene expression and the vector itself.

Following successful transfection there may be several mechanisms for the loss or silencing of expression, such as the reduction in the copy number of the vector or by the removal of cells that have been lethally damaged during the gene delivery process. There may be immune or inflammatory responses against cells expressing the transgene and innate immune responses intracellularly against DNA that has been recognized as foreign by the cell. The loss of expression may also be due to epigenetic events, where the transgenic DNA still exists in the nucleus but its expression has been silenced perhaps by positional effects of the chromatin status. By considering the mechanisms that may lead to the loss of expression,

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the design of non-viral DNA vectors can be developed rationally to enhance their gene expression profile and improve their safety.

IMMUNE RESPONSES

Although DNA vectors are typically less immunogenic than viruses, a substantial immune response against this class of vectors still provides a barrier against efficient gene transfer and sustained expression. DNA prepared from bacteria can elicit immunostimulatory responses. Compared with mammalian DNA, bacterial sequences comprise relatively high levels of non-methylated CpGs [12]. Dendritic cells, macrophages and antigen-presenting cells actively detect this conspicuous presence of unmethylated DNA and this can elicit a widespread immune response including the maturation, differentiation and proliferation of monocytes, macrophages, T cells and natural killer cells against the transgenic DNA and transfected cells [13,14]. Additionally, the presence of expressed transgenic proteins in the circulation can also provoke antibody responses, which also results in the suppression of transgene expression and the removal of transfected cells [15]. There is also data that suggests that cells can detect integrated transgenic DNA and this can also lead to the down-regulation of transgene expression [16].

MINIMALLY SIZED DNA VECTORS

Modifying non-viral vectors by reducing their unmethylated CpG content can reduce or avoid immune responses against them. Several strategies have been developed to produce minimally sized DNA vectors, which comprise only the mammalian expression cassette thereby removing any extraneous elements not required for transcription of the transgene of interest in eukaryotic cells. These methods typically utilize site-specific recombination of integrase systems such as phiC31 [17], λ intergrase [18], Flp recombinase [19] or Cre recombinase [20], which require post-production purification of the minimal DNA vectors. In this issue, Hodgson *et al.* describe advances their company Nature Technology Corporation has made over the past 15 years to develop an alternative methodology to produce a class of minimally sized DNA vectors known as NanoPlasmids. In an alternative approach to generating smaller, optimized and minimally sized vectors Shankar *et al* from PlasmidFactory discuss their development of protocols to generate clinically relevant High Quality Grade minicircle DNA.

INTEGRATING NON-VIRAL DNA VECTORS

In dividing cells, most non-viral DNA vector systems are expressed for a transient period due either to the shut down of transcription or more likely the loss of the DNA molecule from the cell. Several different strategies have been followed in the attempt to produce a stably integrated non-viral vector. Possibly

the most well understood and investigated is the *Sleeping Beauty (SB)* transposon, which is perhaps the most active *Tc1/mariner*-type transposable element in vertebrates. In this issue, we have reviews from the laboratories of Zsuzsanna Izsvák and Zoltán Ivics who provide an insight into the progress their teams have made in the development of the *SB* vector system and their understanding of the challenges that lie ahead. We also have a review of the ground-breaking clinical trial performed by Laurence Cooper's lab using this vector system for adoptive T cell immunotherapy for the treatment of hematologic malignancies and solid tumors.

FUTURE PERSPECTIVE

The genetic treatment of human disease is almost certainly going to require a range of technologies and approaches. One single vector system or approach will not cure all. Despite the continued development and advances made in the field of attenuated viral vectors, there remains great opportunity and prospects for the development and application of alternative vector systems. The progress described in this issue clearly illustrates the improvements that are being made in the development and utility of next-generation gene therapy vectors.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The author has no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.
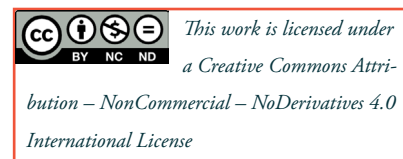
REFERENCES

1. Hacein-Bey-Abina S, Garrigue A, Wang GP *et al.* Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* 2008; 118(9): 3132–42.

2. Booth C, Gaspar HB, Thrasher AJ. Treating Immunodeficiency through HSC Gene Therapy. *Trends Mol. Med.* 2016; 22(4): 317–27.

3. Clément N. AAV vector and gene therapy: en route for the American dream? *Cell Gene Ther. Insights* 2016; 2: 513–9.

4. Jacobson SG, Cideciyan AV, Ratnakaram R *et al.* Gene therapy for leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. *Arch. Ophthalmol.* 2012; 130(1): 9–24.

5. Koilkonda RD, Yu H, Chou TH *et al.* Safety and effects of the vector for the Leber hereditary optic neuropathy gene therapy clinical trial. *JAMA Ophthalmol.* 2014; 132(4): 409–20.

6. MacLaren RE, Groppe M, Barnard AR *et al.* Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet* 2014; 383(9923): 1129–37.

7. Hwu WL, Muramatsu S, Tseng SH *et al.* Gene therapy for aromatic L-amino acid decarboxylase deficiency. *Sci. Transl. Med.* 2012; 4(134): 134ra61.

8. Nathwani AC, Reiss UM, Tuddenham EG *et al.* Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N. Engl. J. Med.* 2014; 371(21): 1994–2004.

9. Gaudet D, Stroes ES, Methot J *et al.* Long-Term Retrospective Analysis of Gene Therapy with Alipogene Tiparvovec and Its Effect on Lipoprotein Lipase Deficiency-Induced Pancreatitis. *Hum. Gene Ther.* 2016; 27(11): 916–25.

10. Calcedo R, Vandenberghe LH, Gao G, Lin J, Wilson JM. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. *J. Infect. Dis.* 2009; 199(3): 381–90.

11. Boutin S, Monteilhet V, Veron P *et al.* Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. *Hum. Gene Ther.* 2010; 21(6): 704–12.

12. McLachlan G, Stevenson BJ, Davidson DJ, Porteous DJ. Bacterial DNA is implicated in the inflammatory response to delivery of DNA/DOTAP to mouse lungs. *Gene Ther.* 2000; 7(5): 384–92.

13. Rothenfusser S, Tuma E, Endres S, Hartmann G. Plasmacytoid dendritic cells: the key to CpG. *Hum. Immunol.* 2002; 63(12): 1111–9.

14. Zhou HS, Liu DP, Liang CC. Challenges and strategies: the immune responses in gene therapy. *Med. Res. Rev.* 2004; 24(6): 748–61.

15. Sarukhan A, Camugli S, Gjata B, von Boehmer H, Danos O, Jooss K. Successful interference with cellular immune responses to immunogenic proteins encoded by recombinant viral vectors. *J. Virol.* 2001; 75(1): 269–77.

16. Baer A, Schubeler D, Bode J. Transcriptional properties of genomic transgene integration sites marked by electroporation or retroviral infection. *Biochemistry* 2000; 39(24): 7041–9.

17. Chen ZY, He CY, Ehrhardt A, Kay MA. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression *in vivo*. *Mol. Ther.* 2003; 8(3): 495–500.

18. Darquet AM, Rangara R, Kreiss P *et al.* Minicircle: an improved DNA molecule for *in vitro* and *in vivo* gene transfer. *Gene Ther.* 1999; 6(2): 209–18.

19. Nehlsen K, Broll S, Bode J. Replicating minicircles: Generation of nonviral episomes for the efficient modification of dividing cells. *Gene Ther. Mol. Biol.* 2006; 10: 233–44.

20. Bigger BW, Tolmachov O, Collombet JM, Fragkos M, Palaszewski I, Coutelle C. An araC-controlled bacterial cre expression system to produce DNA minicircle vectors for nuclear and mitochondrial gene therapy. *J. Biol. Chem.* 2001; 276(25): 23018–27.

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