Nonintegrating Gene Therapy Vectors

Takis Athanasopoulos, PhD[a], Mustafa M. Munye, PhD[a],
Rafael J. Yáñez-Muñoz, PhD[b,*]

KEYWORDS

- Nonintegrating • Gene therapy • Adenovirus vectors
- Adeno-associated virus vectors • Poxvirus vector
- Integration-deficient lentiviral vectors (IDLVs) • Plasmid • Genome editing

KEY POINTS

- Host cell genome integration of first-generation gene therapy vectors may result in various effects on cellular genes (knockout, overexpression, altered splicing), variegated levels of transgene expression, or transcriptional silencing, as well as clonal expansion and oncogenic transformation.
- Nonintegrating gene therapy vectors can be viral and nonviral. Viral vectors can be nonintegrating like their parental organisms (adenovirus, herpesvirus, poxvirus, Sendai) or engineered to minimize integration (adeno-associated virus, retro-lentivirus).
- Nonintegrating vectors can provide stable transgene expression in quiescent cells and transient or stable expression in proliferating cells.
- Variants of nonintegrating vectors carrying suitable payloads (transposons, site-specific recombination cassettes, genome editing cassettes) are suitable platforms for genetic modification of the cellular genome by transposition, site-directed integration, and genome editing.
- Successful clinical trials have already been reported using adenoviral vectors (genome editing of *CCR5* for AIDS), herpesvirus vectors (cancer), and adeno-associated virus vectors (hemophilia).

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[a] Cell and Gene Therapy Discovery Research, Platform Technology and Sciences, GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK; [b] AGCTlab.org, Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK
* Corresponding author.
E-mail address: rafael.yanez@royalholloway.ac.uk

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BRIEF HISTORICAL OVERVIEW OF GENE THERAPY

The concept of gene therapy arose during the 1960s and early 1970s. Rogers and Pfuderer[1] carried out the first genetic modification of a virus (Tobacco mosaic virus [TMV]), and proposed in 1970 that so-called good DNA could be used to replace defective DNA in people afflicted by genetic disorders. In 1972, Friedmann and Roblin[2] assessed the requirements and risks and called for a moratorium. An unsuccessful early attempt at gene therapy was reported in the scientific literature in 1975.[3] The first subject with some degree of long-term transgene persistence from a gene therapy clinical trial was in adenosine deaminase severe combined immunodeficiency (ADA-SCID), following an autologous transplant of T-cells treated *ex vivo* with an integration-proficient retroviral vector, initiated in 1990 and reported in 1995.[4] The first clear success was published by the group of Fischer in 2000, describing the treatment of X-linked severe combined immunodeficiency (SCID-X1) patients with autologous hematopoietic stem or progenitor cells (HSPC) genetically modified with similar retroviral technology.[5] Efforts with developing therapies with integrative vectors were ultimately crowned with success in 2016 when Strimvelis was approved in Europe for the treatment of patients with ADA-SCID for whom no suitable human leukocyte antigen (HLA)-matched stem cell donor is available. This represented the first autologous ex vivo stem cell gene therapy. It was developed in collaboration between GSK together with the Telethon Foundation and the Hospital San Raffaele, acting through their joint San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), a world-leading research centre for stem cell gene therapy in Milan, Italy.

Despite the initial success with integrating vectors, nonintegrating viral vectors were the first approved products for cell and gene therapies in China and Europe. Onyx-015[6] (originally named Ad2/5 dl1520), an experimental oncolytic adenoviral (Ad) vector trialed as a possible treatment of head and neck cancer, was the first gene therapy product licensed, in China in 2006, under the name *H101*.[7] An adeno-associated viral (AAV) vector, Glybera (uniQure/Chiesi),[8] was subsequently approved by european medicines agency (EMA) in 2012 as the first gene therapy in Europe, for lipoprotein lipase deficiency. An oncolytic herpes simplex virus (HSV)-1 (IMLYGIC, Amgen),[9] was approved in 2015 for the treatment of advanced melanoma by both the US Food and Drug Administration (US FDA) and EMA.

NONINTEGRATING VECTORS

Optimal vectors for gene delivery should exhibit high payload capacity, cell tropism for specific target cell types, high transduction efficiency, little or no genotoxicity and cytotoxicity, and should elicit minimal or no immune response. Nonintegrating vectors specifically share reduced risk of genotoxicity, offering a safer profile *in vivo* and *in vitro*, and expression can be retained for long periods in postmitotic tissues. However, unless they have been specifically genetically engineered for replication and segregation, nonintegrating vectors will dilute progressively in proliferating cells. If stable expression in dividing cells is required, repeated administration of nonintegrating vectors is an option, provided that an immune response can be avoided or managed.

This article provides an overview of the main nonintegrating viral vectors: Ad, AAV, integration-deficient lentiviral vectors (IDLVs), poxviral, and others. Nonviral vectors (plasmid, artificial chromosomes) are also discussed. The structure of the main nonintegrating viral vectors is illustrated (**Fig. 1**) and the use of nonintegrating vectors in clinical trials is summarized (**Table 1**). Different vector systems provide a variety of advantageous properties and challenges (**Table 2**). Customarily, viral vectors are