Integrating Vectors for Gene Therapy and Clonal Tracking of Engineered Hematopoiesis

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KEYWORDS

• Retroviral vector • Lentiviral vector • Transposon • Stem cells • Gene therapy
• Clonal tracking • Insertion site analysis • Hematopoietic dynamics

KEY POINTS

- In addition to the integration site preference, vector architecture (SIN vs LTR-driven vectors) and cargo have a major risk-benefit impact.
- The retrovirus- and transposon-based vector toolbox is indispensable for several applications and clinical trials.
- Integrating vectors create an integration site tag, which is used to monitor hematopoietic dynamics in healthy and malignant cells.

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INTRODUCTION

After some initial setbacks, gene therapy (GT) has experienced an unquestionable renaissance over the past decade. The therapeutic efficacy of an increasing number of strategies was demonstrated in clinical trials and some treatments have even recently received market authorization. Generally, the term GT refers to the genetic modification of target cells for therapy for inherited or acquired diseases. Of the various methodologies used for GT (discussed elsewhere in this issue), gene delivery tools are grouped into integrating vectors, such as retrovirus- and transposon-based vectors; and nonintegrating vectors, which do not result in the permanent modification of the genome and often consist of RNA or episomal/extrachromosomal DNA. Approximately 24% of the platforms currently used in GT clinical trials use integrating vectors (http://www.wiley.com/legacy/wileychi/genmed/clinical/), which are the focus of this review. The stable integration into the host cell chromatin offers long-lasting therapeutic possibilities to deliver genes and regulatory RNA and uniquely allows benign and malignant cell behavior to be tracked in healthy or diseased individuals, respectively.

GAMMARETROVIRAL AND LENTIVIRAL VECTORS

Retroviruses have coevolved with their hosts and are thus efficient carriers of genetic information. These viruses have the unique characteristic of reverse transcribing their plus-stranded RNA genome (by the virally encoded enzyme reverse transcriptase) into DNA that is subsequently integrated into the host cell chromatin (catalyzed by the virally encoded integrase).

Although retroviral vectors are derived from wild-type retroviruses, they differ substantially from their natural counterpart in terms of genome content and genetic payload. Indeed, GT vectors are completely, or to the largest degree possible, depleted of original retroviral sequences encoding structural proteins (*gag*), replication enzymes (*pol*), and envelope glycoproteins (*env*). Thus, GT vectors contain only the nucleic acid sequences essential for efficient transfer and expression of the therapeutic information. To improve the biosafety of vector production and to prevent replication-competent retrovirus formation, DNA sequences encoding for *Gag*, *Pol*, and *Env* are provided *in trans* and encoded on separate helper plasmids, a concept that is called split-packaging design.[1,2] Particle production is accomplished with human "packaging cell lines," from which retroviral vector particles (**Fig. 1**A) are harvested after transient or stable transfection of vector and helper plasmids. Modulation of the target cell tropism is possible by modification of the *env* sequences to achieve enforced and/or specific vector entry into the desired cell types or tissues.[3]

Over the past decades, retroviral vectors have been generated from a variety of retroviruses. Exhibiting the highest efficiency, vectors derived from the *Gammaretrovirus* murine leukemia virus (MLV),[4,5] *Lentivirus* human immunodeficiency virus type 1 (HIV1),[2,6,7] Foamy virus[8] and *Alpharetrovirus* avian sarcoma and leukosis virus (ASLV)[9] are most widely used.

The first retrovirus-based gene transfer approaches date back to the 1980s, where gammaretroviral vectors (gRV) were derived from MLV. These experiments provided proof-of-principle for the feasibility of GT in hematopoietic cells. Later work demonstrated that hematopoietic stem and progenitor cells (HSPC) could be genetically modified and could subsequently reconstitute the blood and immune system of transplanted animal models, creating the basis for the translation of GT into clinical trials. Vector improvements included the use of *gag*-free leader regions, intronic sequences, and stronger promoters in the long-terminal repeats (LTRs) to increase transgene expression levels.[10,11] However, gRV are dependent on mitosis for nuclear entry,