Review

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# A roadmap toward clinical translation of genetically-modified stem cells for treatment of HIV

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**During the past decade, successful gene therapies for immunodeficiencies were finally brought to the clinic. This was accomplished through new gene therapy vectors and improved procedures for genetic modification of autologous hematopoietic stem cells. For HIV, autologous hematopoietic stem cell (HSC) gene therapy with 'anti-HIV genes' promises a functional cure for the disease. However, to develop such a therapy and translate it into a clinical application is rather challenging. The risks and benefits of such a therapy have to be understood, and regulatory hurdles need to be overcome. In this joint paper by academic researchers and regulators, we are, therefore, outlining a high level roadmap for the early stage development of HSC gene therapy as a potential functional cure for HIV.**

## The promise of gene therapy

Over the past decade, gene therapy has been able to bring long awaited treatments for immunodeficiency diseases to the clinic [1]. This was accomplished through the development of new gene therapy vectors, in particular, viral based vectors, and gene modified cell therapies [2]. As an example, in indications characterized by severe combined immunodeficiency (SCID), genetically modified hematopoietic stem cells (HSCs; see Glossary) led to the cure of several children suffering from adenoside-deaminase (ADA) SCID [1]. Genetic modification of autologous HSCs also avoids the high mortality and morbidity associated with allogeneic bone marrow transplantation [3], particularly in a setting of pediatric patients with SCID. In order to achieve a prolonged clinical benefit, however, high transduction efficiency into the CD34+-target cell population and durable expression of the transgene following stable integration of the therapeutic gene expression cassette, particularly in the hematopoietic progeny, is required. It is very encouraging that this has been achieved in recent clinical gene therapy trials [4]. For several years, stem cell and T cell gene therapies have also been contemplated for infectious diseases, particularly for those caused by HIV. So called 'anti-HIV genes' have been developed, and several of them have been utilized in combinations transferred by a single gene therapy vector into either T cells or HSCs [5]. HSC gene therapy for HIV does promise a functional cure from the disease, if most of these stem cells can be gene modified and successfully engrafted in the infected recipient [6].

In principle, however, gene therapy for HIV is rather challenging. In order to treat a large population of HIV infected individuals, risks of novel gene therapeutic approaches and the benefit of a possible cure have to be very well understood and properly weighed against standard therapy. Additionally, manufacturing and regulatory challenges need to be overcome to make the new therapeutic widely available. In both Europe [7] and the United States [8], regulatory requirements for gene therapy products, classified as advanced therapy medicinal products (ATMPs) by the European competent authorities in the different member states, are more complex than for the other two product classes within the ATMP category: namely somatic cell therapy medicinal products and tissue engineered products.

Currently, ATMPs are mainly produced by small companies and academic institutions [9]. Most ATMPs are also not licensed, marketed products; they are still in different phases of clinical development. Small companies and academic institutions have limited budgets and resources, and sometimes also limited experience of mastering regulatory requirements. As ATMPs are often very promising candidates to be translated into the clinic, there is a clear need for these enterprises to build regulatory competencies [10], and to initiate an early dialogue with regulatory agencies. Reflecting on the importance of linking these efforts, in this paper, we present a high level roadmap for the early stage

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## Glossary

**Antiretroviral therapy (ART):** small molecule combination therapy consisting of two or more individual compounds that act on different stages of the HIV life cycle. It is used to suppress HIV replication to undetectable levels and to stop HIV disease progression.
**CD25:** alpha chain of the IL-2 receptor. The wild type receptor is present on activated T and B cells.
**CD34:** a cell-surface antigen found on hematopoietic stem and progenitor cells, used to characterize and purify these cell populations.
**Cytomegaloviruses (CMV):** a human herpesvirus carrying double stranded DNA. Infection may be asymptomatic in immunocompetent individuals, but life-threatening in immunocompromised patients.
**Epstein–Barr virus (EBV):** a human herpesvirus associated with various types of malignancies such as Hodgkin's lymphoma and nasopharyngeal carcinoma.
**Hematopoietic stem cell (HSC):** a multipotent stem cell with asymmetric division ability. It gives rise to all blood cells by differentiating into progenitor cells and finally mature blood cells. At the same time, it can generate more HSCs to maintain the stem cell pool.
**Human immunodeficiency virus 1 (HIV-1):** a human lentivirus found within the subfamily of orthoretrovirinae, in the family of retroviridae. HIV-1 is the cause of AIDS.
**Induced pluripotent stem cell (iPSC):** a pluripotent stem cell can be generated by reprogramming a differentiated somatic cell using early acting transcription factors.
**Insertional mutagenesis (in transduced cells):** DNA integrated into a host cell's DNA can recombine with existing DNA and generate a recombinant mutant cell.
**Insertional oncogenesis (in transduced cells):** integrated DNA in a host cell's genome can upregulate the expression of an oncogene or inactivate a tumor suppressor gene, ultimately causing oncogenic transformation of the cell.
**Gene knockdown:** reduction or shutdown of gene expression often accomplished by RNA interference or RNA antisense molecules; can be performed *in vitro* or *in vivo*.
**Gene knockout:** deletion of a gene from a chromosome. This is often used to generate mice with a specific gene knockout to simulate a certain genetic disease in an *in vivo* model.
**Lentivirus:** a virus genus among the family of retroviridae, in the subfamiliy of orthoretrovirinae. HIV-1 belongs to this virus genus. Contrary to retroviruses which can only integrate into dividing cells, HIV can also integrate into non-dividing cells.
***LMO2* [*LIM domain only 2 (rhombotin-like 1)*]:** a gene that codes for a protein that is critical for early hematopoiesis, particularly red blood cell development. It is also found in leukemias of the myeloid and lymphoid lineages.
**Long terminal repeats (LTRs):** identical DNA sequences that may repeat hundreds or even thousands of times at each end of an integrated virus within the host cell genome. These LTRs are used by viruses to facilitate integration and also act as strong viral promoters.
**Murine leukemia virus (MLV):** a gamma-retrovirus causing leukemia in mice. A well-studied and often used retrovirus engineered as a gene transfer vector. The first type of retrovirus vector used in human gene therapy clinical trials.
**Retrovirus:** single stranded RNA virus which is enveloped. For replication, the RNA is reverse transcribed into double stranded DNA, which is integrated into the host cell DNA.
**Severe combined immunodeficiency (SCID):** a group of diverse disease manifestations involving the failure of T lymphocytes to develop or function normally. One of these disease manifestations is known as adenosine deaminase (ADA) deficient SCID.
**Short hairpin RNA (shRNA):** an engineered RNA molecule of approximately 60 nucleotides that can form a stem–loop structure. Inside a cell it can be cleaved into a short interfering RNA, able to induce gene silencing.
**Transduction:** gene transfer accomplished by the use of viral vectors. Gene transfer can be non-integrating or integrating into the target cell's genome.
**Transfection:** Gene transfer accomplished by non-viral methods, often used to generate retro- or lentiviral vectors.
**Transgene:** a gene that is stably integrated into a cell's genome and can be transmitted to any daughter cell and successive generations of cells during cell division.
**Vaccine:** a pharmaceutical or biologic used either orally or by injection for stimulation of an immune response in order to achieve protective immunity to a toxin or infectious agent.
**Viral vectors:** engineered viruses to transfer genes, often used for gene therapy purposes. Such vectors include integrating retroviruses and lentiviruses. Other non-integrating viral vectors are adenoviral vectors and adeno-associated viral vectors.

development of a gene-based approach aiming to cure HIV, and briefly detail some of the unique features of this process. This paper is a joint publication by an academic group and regulators, which could be seen as a paradigm of dialogue between both parties in order to advance translational medicine. The roadmap points out to researchers, both in Europe and the US, the major challenges for clinical translation of an anti-HIV stem cell gene therapy approach. It will also be discussed how to achieve a well-established safety and efficacy profile for such a gene therapy product that may speed up the regulatory approval process, and proceed toward a marketing authorization application. Finally, we reflect upon the importance of initiating an early collaboration between academic institutions and regulatory agencies to facilitate the process of providing access to a possible cure for HIV.

## A path toward curing HIV infection

HIV is part of the retrovirus family, and within this family, falls into the group of lentiviruses. It is a single-stranded RNA virus that carries all the required enzymes to reverse-transcribe its viral RNA into double-stranded DNA, which is then permanently integrated into the target cell genome. After HIV infects its host, the virus persists lifelong and causes AIDS. AIDS is characterized by the destruction of CD4-positive T helper cells, eventually causing the complete loss of immune function, resulting in the development of opportunistic infections, prolonged illness, and death [11]. Antiretroviral therapy (ART) based on small molecule drugs has helped to make HIV a manageable disease and has prevented countless deaths, but is unable to cure the disease [12]. Many attempts to eradicate HIV from the body of infected individuals using ART have failed [13–17]. Additionally, and in spite of 30 years of intense research, there is still no effective vaccine against HIV [18]. Many infected individuals long for a cure for the disease, not only to be able to stop taking daily medication that can develop long term toxicity, but also to be regarded as cured from an infection that has been carrying a social stigma since its first appearance in the late 1970s.

The largest driver of the economic burden of HIV is the life-long commitment to ART. The lifetime cost of care for an HIV infected individual (life expectancy of 24.2 years) is in excess of $600 000, with medication cost being 73% of the overall cost [19]. In many cases, taking daily anti-HIV medication is not just an inconvenience; it can also be associated with significant side effects [20]. Additionally, non-compliance with the daily dosing regimen may lead to the development of drug resistant HIV mutants [21]. Therefore, there is a need to develop alternative therapies for HIV, particularly treatments that could potentially be curative.

Experts will question: how will a cure for HIV infection be defined? There are two types of cures: a sterilizing cure, and a functional cure [6]. A sterilizing cure completely eradicates HIV from a patient. However, a sterilizing cure may be difficult to achieve with an integrating virus such as HIV. Silent reservoirs of integrated HIV need to be eliminated, as HIV could rebound from these when activated. A functional cure, in contrast, would not require the complete eradication of all integrated HIV, but rather ART independent suppression of viral replication by the immune system, accompanied by a lack of HIV transmissibility from person to person. Examples of this type of cure include the successful control of Epstein–Barr virus (EBV)