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Current progress and challenges in HIV gene therapy

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Abstract

HIV-1 causes AIDS, a syndrome that affects millions of people globally. Existing HAART is efficient in slowing down disease progression but cannot eradicate the virus. Furthermore the severity of the side effects and the emergence of drug-resistant mutants call for better therapy. Gene therapy serves as an attractive alternative as it reconstitutes the immune system with HIV-resistant cells and could thereby provide a potential cure. The feasibility of this approach was first demonstrated with the ‘Berlin patient’, who was functionally cured from HIV/AIDS with undetectable HIV-1 viral load after transplantation of bone marrow harboring a naturally occurring *CCR5* mutation that blocks viral entry. Here, we give an overview of the current status of HIV gene therapy and remaining challenges and obstacles.

Keywords

antibody mimetic; dominant negative mutant; gene therapy; hematopoietic stem cell; HIV-1; ribozyme; RNA decoy; RNAi; sequence-specific nuclease; T cell

HIV is a lentivirus, a subgroup of the *Retroviridae* family characterized by reverse transcription of the RNA genome as part of the replication cycle (depicted in Figure 1). There are two subtypes, HIV-1 that causes the majority of worldwide cases and HIV-2, which is more regionally confined to western Africa and southern/western India [1]. According to the WHO, in 2009, 33.3 million people were infected with HIV-1, with 1.8 million AIDS-related deaths. The current standard treatment option for AIDS is HAART, which consists of a cocktail of several drugs targeting multiple stages of the HIV-1 life cycle. Although HAART is effective in improving the quality of life and prolonging the survival of infected individuals, it is a lifelong therapy that does not cure but only slows down disease progression. In addition, HAART is often linked with severe side effects and rigid schedules with dietary restrictions that make patient compliance difficult. Furthermore, the high occurrence of viral escape mutants requires constant monitoring of viral loads and adjustment to therapeutic drug selection. Despite being on HAART treatment, patients often face increasing outbreaks of infection, such as tuberculosis [2], Kaposi’s sarcoma and fungal

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infections [3], resulting from a compromised immune system. These shortcomings motivate the development of a more efficient and less costly alternative treatment.

Gene therapy approaches to the treatment of HIV infection

Gene therapy is an increasingly promising alternative, as its goal is to reconstitute an HIV-resistant immune system, and it therefore has the potential of curing the disease. Not only can gene therapy slow down disease progression by interfering with HIV replication in a similar manner to HAART, but it could also prevent the initial infection or even eradicate the integrated virus from the genome. The feasibility of an effective HIV gene therapy was first demonstrated with the ‘Berlin patient’ who received an allogeneic bone marrow graft carrying homozygous *CCR5*Δ32 alleles (a naturally occurring 32-base pair deletion in *CCR5* gene) in conjugation with myeloablation for leukemia treatment, and who was functionally cured of HIV/AIDS, maintaining undetectable levels of the virus for several years, despite not being on standard HAART therapy [4]. However, this approach cannot be widely applied to treat HIV/AIDS because of the difficulty of finding allogeneic grafts that are homozygous for this relatively rare allele (frequency of 0.0808 in the Caucasian population) and also the absence of this allele in people of African or Asian descent who make up the majority of the population in many areas where HIV is most prevalent [5]. A more feasible approach is the use of autologous grafts utilizing isolated CD4⁺ T cells or CD34⁺ cells that are genetically modified with anti-HIV genes *ex vivo*, followed by reinfusion back into the patient. Because the progression of HIV/AIDS depletes CD4⁺ T cells and thereby impairs the immune system, the goal of T-cell gene therapy is to infuse autologous, gene-modified, HIV-resistant T cells to recover the immune system. The advantages of this approach include the relative abundance of CD4⁺ cells in peripheral blood and the high transduction efficiency of CD4⁺ cells. However, this strategy cannot protect other HIV-susceptible cell types. An alternative solution is gene therapy targeting CD34⁺ hematopoietic stem cells, which involves the reinfusion of autologous gene-modified cells that have the potential to reconstitute the immune system with disease-resistant leukocytes. Because hematopoietic stem cells are able to self-renew and to differentiate into any cells in the immune system, their progeny could theoretically provide a cure for HIV infection. This protocol currently makes it necessary to open niches in the bone marrow for the reinfused cells via myeloablation.

Potential strategies to interfere with the HIV life cycle

Both small RNAs and protein agents have been utilized to target HIV replication and host factors. There are many types of small RNAs with different mechanisms of action that can be employed to interfere with the HIV-1 life cycle. The first and the most common are siRNAs (recently reviewed in [6]) that direct post-transcriptional gene silencing in a sequence-specific manner. When the target mRNA contains a site that is perfectly complementary to the siRNA, this directs site-specific degradation of the message [7]; on the other hand, when the mRNA contains the ‘seed sequence’ in the 3′ untranslated region (UTR) that has partial complementarity to the siRNA, this can trigger translational repression or facilitate the transport of the message to cytoplasmic P bodies for degradation [8], similar to the endogenous miRNAs [9]. Not all siRNAs have the same efficiency in silencing their targets, since RNA interference (RNAi) is a multistep process. The efficiency of silencing can depend on loading and processing by the RNA-induced silencing complex and the selection of the correct strand in the RNA duplex in silencing the intended target. Moreover, the level of knockdown also depends on the abundance of the targeted mRNA and the stability of the protein. siRNAs can be expressed in the form of a shRNA or a miRNA-mimic. These RNAs differ in secondary structure: shRNAs usually contain a perfectly paired stem; however, the overall complementarity of the processed siRNA to the

target determines the mechanism of silencing [10,11]. In HIV gene therapy, it is often sought to multiplex shRNAs or miRNAs to target various stages of the viral life cycle, in addition to host factors [12,13]. Additionally, it is possible to design bifunctional siRNAs that pair to one gene with perfect complementarity to mediate message cleavage, while pairing only via the seed region of the 3' UTR of another gene similar to the miRNAs to mediate translational repression [14]. We have utilized RNA polymerase III (Pol III)-expressed sense and antisense siRNA targeting the HIV *Tat* and HIV *Rev* mRNAs to downregulate Tat and Rev protein expression, thereby successfully inhibiting HIV replication (Figure 1J–L) [15].

Another group of small RNAs used in HIV gene therapy is RNA decoys that function as 'sponges' to sequester viral proteins and thereby interfere with viral replication. Two examples of RNA decoys are the transactivation response element (TAR) decoy that sequesters the HIV Tat protein to block transcription activation (Figure 1J) and the Rev-binding element (RBE) decoy that sequesters the HIV Rev protein to block the export of intron-containing HIV transcripts (Figure 1K & 1L). In one design the TAR decoy contains the apical loop of the HIV TAR RNA, while the RBE decoy contains the stem-loop structure which is the minimal and high-affinity binding site for Rev within the Rev-response element (RRE) [16,17]. Because Tat and Rev are known to localize in the nucleolus [18–21] and reports have implicated the importance of the nucleolus during viral replication [22], RNA decoys have been designed to be nucleolar-localizing [23,24]. To achieve this, chimeras of the human U16 small nucleolar RNA (snoRNA) and the TAR or RBE RNA decoy were designed and their anti-HIV activities demonstrated [23–25]. It is worth noting that the nucleolar-localizing TAR decoy is far more potent than the cytoplasmic- and nuclear-localizing decoys, highlighting importance of the nucleolus during viral replication [24].

Finally, RNA hammerhead ribozymes have been used in HIV gene therapy. These molecules cleave their targets in a sequence-specific manner and are capable of multiple turnover reactions. Most often the effectiveness of the ribozyme depends on the ability to colocalize with the target as well as target accessibility [26]. Colocalization of ribozymes with their targets can be further optimized by careful consideration of the target cellular location. For example, a nucleolar ribozyme that targets a conserved region within the U5 region of all HIV transcripts [27,28] was efficient, as HIV-1 transcripts associate with the nucleolus during the later stages of viral replication [22]. Using the same principle, forced cytoplasmic localization of a *CCR5*-targeted ribozyme was shown to down-regulate the coreceptor expression and partially inhibit viral entry (Figure 1B) [29,30].

HIV therapy can also be accomplished via the use of proteins or peptides that interfere with viral replication or infection. For example, the prokaryotic carbohydrate-binding proteins Actinohivin and Cyanovirin-N bind to the HIV-1 gp120 protein and interfere with viral adsorption (Figure 1A) [31,32]. HIV-derived peptides and proteins can be equally potent in targeting viral entry; for example, gp41-derived peptides that inhibit the fusion of the virus with the cell membrane (Figure 1B) [33,34]. Endogenous genes that confer innate immune defense against retroviral infection have also been utilized in HIV gene therapy. The α -isolate of TRIM5 α provides species-specific retroviral protection as it directs degradation of the viral core via proteasome-dependent and proteasome-independent pathways (Figure 1C). Notably, TRIM5 α from rhesus macaques confers protection against HIV-1 while the human TRIM5 α does not, although single point mutations can be used to provide protection against HIV [35,36]. A combination therapy including TRIM5 α was successfully used to suppress HIV-1 replication in a humanized mouse model [37]. The integrase interactor-1 (INI1) is an endogenous human protein that interacts directly with the HIV-1 integrase and is packaged in the HIV virion [38,39]. A dominant negative human INI1 mutant was used to block HIV-1 integration (Figure 1g) [39]. Another dominant negative mutant, the RevM10 protein,

is also a potent inhibitor of HIV replication [40] as it disrupts the export function of wild-type Rev proteins. This mutant cannot bind to the nuclear export factor Crm-1 despite retaining its ability to bind to the RRE and to other Rev molecules (Figure 1K & 1L). A different strategy is the development of HIV-specific antibody mimetics, including antigen-binding fragments (Fab), single-chain variable fragments (scFv) and intrabodies, engineered to recognize and neutralize HIV proteins. Fabs are comprised of constant and variable domains of the antibody light and heavy chains that shape an antigen-recognizing paratope while remaining a heterodimer. In contrast, scFvs carry both chains fused with a spacer protein. An anti-Rev Fab and anti-integrase scFv have been developed and shown to have potent anti-HIV activity *in vitro* [41,42]. Intrabodies are intracellularly expressed proteins with antibody specificity. More recent designs are based on the unique minimal structure of camel antibodies with only single heavy chain domains [43]. A more conservative design targeting HIV-1 Vif was shown to neutralize viral infectivity [44]. Interference with the viral RNA is possible as well, as it can be cleaved by a chimera protein containing the TAR RNA binding domain of HIV-1 Tat and the ribonuclease (RNase H) domain of the HIV-1 reverse transcriptase [45].

While the previously mentioned approaches aim to interfere with the HIV life cycle, a different strategy is to direct DNA modification using zinc-finger nucleases (ZFNs) – fusion proteins of a zinc finger domain that recognizes a specific DNA sequence with an endonuclease domain [46]. ZFNs are unique in creating permanent disruptions in the target gene, even with transient expression, and therefore eliminating the need for persistent expression. CCR5-targeting ZFNs are currently under clinical evaluation for CD4⁺ T cells [47], while CXCR4-targeting ZFN has also been described recently [48].

A related but different approach is the excision of the complete proviral cDNA [49] by an evolved recombinase that recognizes asymmetric sequences within the HIV long terminal repeat (LTR) or the excision of proviral genes [50]. Finally an approach that utilizes homing nucleases has also been described; it creates double-stranded breaks in essential viral genes, which leads to mutation after misrepair by the cellular machinery [51].

Options for gene delivery using viral vectors

Various viral vectors have been utilized to deliver genes and regulatory RNAs in HIV gene therapy. Temporary expression of immunogenic proteins to prime or boost an immune response has been demonstrated by adenovirus [52] and vaccinia virus vectors [53]. Reinfusion of genetically modified T cells after transduction with adenoviruses is currently in clinical trial [201]. For long-term expression of anti-HIV proteins or RNAs, CD34⁺ cells from bone marrow have been transduced with retroviral vectors and have been reinfused into patients [54–57]. Recently, lentiviral vectors have received increased attention for use in clinical trials [58–60]. There are two major reasons for this: their low oncogenic potential upon integration (in contrast to gamma retroviruses) [61] and their ability to transduce non-dividing cells [62,63], which provides a wider range of target cells [64]. There is a broad spectrum of literature discussing the features, advantages and disadvantages of this approach, as well as preparation of various viral vector types (e.g., [65–67]). It is worth noting that current HIV-1-based lentiviral vectors are ‘self-inactivating’ as they harbor a deletion in the 3′ LTR rendering them replication deficient after integration. In addition, the lentiviral vectors have most of the viral genes removed, and therefore require cotransfection of these genes during packaging for efficient viral production. Several non-HIV-1 packaging systems (cross-packaging) have been tested for HIV-1 based vectors to avoid potential packaging of cis-acting factors into the virus particle. Examples of cross-packaging systems are feline immunodeficiency virus (FIV) [68], simian immunodeficiency virus (SIV) or HIV-2 [69]. While several non-HIV-based lentiviral systems are in use, for example for

neurological diseases [70], HIV-based platforms predominate for hematopoietic cell-based applications like HIV gene therapy.

Expression strategies for anti-HIV genes

HIV most prevalently infects hematopoietic cells, including the lymphoid lineage (T cells) and the myeloid lineage (macrophages). Two different classes of promoters have been employed for the expression of anti-HIV proteins or regulatory RNAs. The first type is driven by the RNA polymerase II (Pol II) usually used for expression of protein-encoding genes. The transcribed RNA is processed into a mature mRNA containing a 5' cap and 3' poly(A) tail. Export is performed by binding of PABP2 to the poly(A) tail of the message [71]. Once in the cytoplasm, the poly(A) tail forms a loop with the cap structure via PABP1 [72] and recruits the ribosomal 40S subunit to initiate translation. Translation without the cap structure is possible if an internal ribosome entry site is present in the mRNA, but the efficiency is impacted strongly by the existence of a poly(A) tail or PABP [73,74]. The most common Pol II promoter used in lentiviral vectors is the cytomegalovirus immediate early (CMVie) promoter. This promoter shows strong activity in several eukaryotic cells lines, but is prone to transcriptional silencing or suppression in certain types of cells [75,76]. The CMVie promoter can be trans-activated by NFkB expression to reverse silencing [77]. The human EF1 α promoter has been found to be persistently expressed and highly active in human hematopoietic cells [78,79], but its large size (1.3 kb) can negatively impact packaging and subsequent transduction efficiency [80,81]. The human PGK promoter is used for transgene expression, although its activity is somewhat lower than the EF1 α promoter in certain human hematopoietic and immune cells [78,79,82]. The human ubiquitin C promoter has been demonstrated to have strong activity in a wide variety of host cells as well [83]. Recently, the human small nuclear U1 Pol II promoter has been used to drive anti-HIV-1 gene cassettes [13]. This promoter has the advantage of being ubiquitously expressed and is of a small enough size (~400 base pairs) to facilitate cloning into lentiviral vectors.

RNA polymerase III (Pol III) promoters are typically smaller and have very defined start and termination signals. The resulting RNA has no cap structure and terminates in a string of U residues, and is therefore not suitable for protein expression. Export of Pol III-driven RNA short hairpin transcripts is mostly exportin-5-dependent and is initiated by 3' overhangs [84]. Commonly used Pol III promoters include the human H1 and U6 promoters, for which concerns have been raised about their activity being too high for safe application [85]. Additionally, human tRNA promoters have also been utilized recently [86].

Combinatorial approaches that involve coexpression of multiple anti-HIV RNAs and/or proteins to target both viral and cellular factors can reduce the likelihood of viral escape. This can be accomplished by expressing multiple anti-HIV RNAs, each with an independent promoter [30], or by coexpression from a single transcription unit, such as within an intron [13,87].

Challenges for successful HIV gene therapy

Although HIV gene therapy has shown much promise, there are several reasons why HIV gene therapy is not yet a routine form of therapy. A general problem is the emergence of escape mutants and subsequent disease progression [88,89]. Viral escape from siRNA therapy can be mediated by as little as a single point mutation in the target site [90–92] or the evolution of an alternative secondary structure that occludes the target site [93]. Approaches that could overcome these limitations include targeting highly conserved HIV sequences so that the escape variants would be less fit, or a combinatorial approach that includes multiple siRNAs with the possibility of some targeting viral escape mutants [88].

The challenge of designing an efficient RNA-based therapy is the balance between gene expression with potentially toxic side effects and the resulting therapeutic effect. Although one may presume higher expression should yield better HIV protection, this is not always the case. For example, when siRNAs are over-expressed, it can saturate the endogenous RNAi processing pathway leading to toxicity *in vivo* [85,94] and increase the likelihood of binding to seed sequences in 3' UTRs of other genes leading to off-target effects. Careful design of the RNA duplex and preferential incorporation of the intended RNA strand into RISC are also equally important for minimizing off-target effects of siRNAs. The sufficient level of expression for each type of anti-HIV RNA to reach a therapeutic threshold is dependent on the mechanism employed. For instance, the theoretically necessary expression levels by siRNAs and ribozymes (capable of multiple turnover reactions) should be less than for those required RNA decoys and miRNAs (which mainly function by sequestering their target in a stoichiometric manner). Because RNA decoys act by sequestration of the specific protein target and tend not to interfere with other host factors, overexpression of these agents has no known toxicities so far.

A potential risk associated with gene therapy is tumorigenesis, as a consequence of random genomic integration of the vector in order to sustain long term gene expression. Retroviral vectors preferentially integrate into transcriptional start sites and regulatory gene regions of proliferation-associated genes resulting in an increased likelihood of tumorigenesis. Lentiviral vectors, on the other hand, tend to integrate within transcriptionally active regions without bias toward proliferation-associated genes and transcriptional start sites, and are therefore the safer alternative for gene therapy [95].

An additional concern is that an immune response against vector-expressed foreign peptides can abrogate any therapeutic effect. This has been shown to be especially problematic when the peptide is expressed from antigen-presenting cells (APCs), such as B cells, dendritic cells and macrophages. Because APCs express MHCII and the necessary coreceptors on the cell surface, they are capable of mounting a complete immune response, including priming of immune cells. Modulating the level of transgene expression via inclusion of endogenous APC-specific miRNA target sites in the transcripts extends the stability of the transgene expression *in vivo* [96–98]. On the other hand, over-expression of anti-HIV proteins could interfere with cellular pathways, creating undesired side effects. For example, ZFNs could cleave unspecifically in the genome, resulting in toxicity, and the frequency of such events should be closely monitored *in vivo* to ensure safety [99,100].

The duration and levels of therapeutic gene expression are important concerns given the knowledge that cells infected by HIV-1 exhibit differences in HIV gene expression over maturation and differentiation [101]. There is no single promoter that could provide sufficient expression in all stages or cell types. Furthermore, potential silencing of the promoters used for gene therapy can be a problem as well, which motivates the current use of ubiquitously active and very strong promoters, such as the Pol III U6 promoter, for gene expression. Using multiple copies of strong promoters to express shRNAs is risky since overexpression of sh/siRNAs could saturate the endogenous RNAi processing pathway [85]. A possible solution to address these issues is the use of cell-type specific lentiviral vectors, which would limit the repertoire of cells that they can transduce [102].

While the transduction of T cells has reached an efficacy of therapeutic relevance [103], this is not the case for transplantation of CD34⁺ cells in humans. A major obstacle of the latter is the low engraftment of gene-modified CD34⁺ cells [104,105]. Current purification methods in humans lead to a heterogeneous CD34⁺ population, with only a small percentage capable of engraftment and transduction with a lentiviral vector, which yields an even smaller percentage of gene-modified cells with long term therapeutic potential [58]. A recent

publication reports that the CD49f⁺ subset of CD34⁺CD38⁻CD45RA⁻Thy1⁺ is the selection marker for human repopulating cells at least in the mouse model [106]. This purification marker could overcome the hurdle of low transduction and engraftment rates when treating the whole CD34⁺ cell pool. Another approach is the chemical selection of gene-modified cells, either by conferring drug-inducible growth advantage or resistance to toxic drugs, to lower competition for engraftment from untransduced cells [107–109]. This is especially promising in the case of *in vivo* selection, as results indicate that the culture media and growth factors used to maintain cells *ex vivo* can decrease the repopulating potential of hematopoietic stem cells [110,111].

Conclusion

The ‘Berlin patient’ has proven the feasibility of a gene therapy approach for inhibition and perhaps even eradication of HIV. Nevertheless, there remain technical obstacles that need to be overcome before HIV gene therapy becomes the standard treatment. The major current roadblock is the low engraftment of modified hematopoietic stem cells when providing long term anti-HIV gene expression for a clinical benefit in patients. In a recent study, gene marking frequencies between 0.02 and 0.32% (200–3200 copies per 10⁶ cells) in peripheral mononuclear blood cells were found [58]. Nevertheless promising approaches to overcome these difficulties have been reported or are in development. While there is steady progress being made in the development of HIV gene therapy, it has yet to reach the status of a generally accepted modality for treatment of HIV infection.

Future perspective

Current properties of HIV gene therapy still hinder its application as a routine treatment. In order to obtain substantial numbers of gene-modified lymphocytes resistant to HIV infection, it is still necessary to use myeloablation for stem cell transplants or repeated infusions of modified differentiated T cells for T-cell therapy. There is the added difficulty of efficiently transducing stem cells to provide a sufficient population of gene-modified stem cells that can give rise to HIV-resistant progeny in the long term. Additionally, the large-scale production of viral vectors required for current gene therapy protocols can be costly. Thus, there is a need for better stem cell isolation and expansion protocols that do not result in loss of pluripotency.

Results from early stage clinical trials have shown that gene-modified stem cells can engraft and reconstitute the hematopoietic niches. This is promising in combination with reports about naturally cycling repopulating cells. It is possible that in the future the approach of permanent HIV gene therapy will change based on these findings. For example, it is conceivable that future protocols will comprise a continuous treatment with only few but highly pure repopulating cells that have been genetically modified prior to infusion. Repeated infusions of small numbers of cells could eventually lead to filling marrow niches with gene-modified progenitors. It may also be feasible to reconstruct the ‘Berlin patient’ results using ZFNs to disrupt the *CCR5* gene in hematopoietic stem cells, followed by mini-transplants.

Improvements in combinatorial approaches involving anti-HIV small RNAs and proteins with low immunogenic profiles and identification of promoters better suited for continuous gene expression are expected to enhance anti-HIV resistance of individual cells. With the advances in our knowledge of HIV-1 biology, novel cellular targets can be identified, thereby expanding the repertoire of potential targets for HIV-1 gene therapies while reducing the likelihood of viral escape. As these obstacles are overcome, expect to see wider applications of gene therapy for the treatment and perhaps ‘curing’ of HIV-1 infection.

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Executive summary

Current status of HIV gene therapy

- The 'Berlin patient' has shown that the re-engraftment of bone marrow stem cells with certain genetic traits can cure AIDS and at least inhibit HIV infection in the long term to below detectable levels.
- Gene-modified CD4⁺ T cells and CD34⁺ hematopoietic stem cells with anti-HIV genes have been reinfused into patients and shown to be safe and well-tolerated.
- Anti-HIV small RNAs with different mechanisms of action, including siRNAs, decoys and ribozymes, have been utilized and shown to have potent antiviral activity.
- Protein-based therapy involving anti-HIV peptides/proteins, dominant negative mutants, antibody mimetics and sequence-specific nucleases are efficient in reducing viral replication.
- Multiple viral vectors based on adenovirus, vaccinia virus and retroviruses are used for different purposes, with self-inactivating lentiviral vectors a promising development in the last few years.
- Combinational therapies or multiplexing anti-HIV RNAs and/or proteins targeting various viral and crucial host factors has been demonstrated to decrease the likelihood of viral escape.

Ongoing challenges & protocol requirements

- Designs need to confer to long-term expression at therapeutically sufficient levels for a wide variety of cell types and differentiation stages.
- Avoid toxicity from transgene expression, tumorigenesis from integration and activation of immune response.
- Avoid the likelihood of viral escape, by combinatorial approaches for example, and eliminate if possible.
- Overcome the low frequency of gene marking in the reconstituted immune system for a beneficial clinical outcome.

Conclusion

- Much progress has been made over recent years in understanding the details of technical HIV gene therapy demands, roadblocks and potential solutions, and recent developments are promising to overcome the remaining obstacles.
- While progress is steady, HIV gene therapy has not yet become a general therapy option.

Figure 1. The HIV-1 life cycle

DNA is shown in blue while RNA is shown in pink. **(A)** Adsorption. The HIV-1 gp120 on the surface of the virion binds to the CD4 receptor on helper T cells, macrophages and dendritic cells, and either the α -chemokine receptor CXCR4 (T cell-tropic) or the β -chemokine receptor CCR5 coreceptor (macrophage-tropic). **(B)** Fusion. **(C)** Uncoating. **(D)** Reverse transcription of the viral RNA genome into cDNA. **(E)** Formation of the pre-integration complex. **(F)** Nuclear import of pre-integration complex. **(G)** Integration of viral cDNA into the host genome to form the provirus. **(H)** Transcription of the proviral DNA. Although the HIV promoter embedded in the 5' long terminal repeats is functional and able to recruit the host's transcription machinery, the elongation efficiency is very low, resulting in production of short and early-terminated transcripts. Additionally, most of the mRNA transcripts are spliced multiple times at this stage by the cellular machinery, and as a result, mRNAs encoding Tat and Rev proteins are produced. **(I)** Translation of Tat and Rev. **(J)** Import of Tat and Rev into the nucleus. The HIV Tat enhances transcription elongation by interacting with the transactivation response element in the 5' end of HIV transcripts to increase the number of full-length transcripts. **(K)** Rev facilitates the export of full-length HIV-1 RNA genome for packaging. **(L)** Rev exports unspliced and singly spliced HIV-1 transcripts to the cytoplasm by interacting with the Rev-response element for the translation of late gene products, including Gag and Gag-Pol (which later cleaves into viral enzymes, including protease and reverse transcriptase), Env, and accessory proteins Vpu, Vpr and Vif. **(M)** Assembly. The assembly of a new HIV virion takes place at the plasma membrane of the host cell, involving two copies of the viral RNA genome and the Gag and Gag-Pol polyproteins. The proper selection of the viral genome for packaging depends on interaction of the packaging signal, the ψ locus, on the RNA, with the nucleocapsid domain of the Gag polyprotein. **(N)** Budding. **(O)** Maturation. The viral protease cleaves the HIV polyproteins into functional protein and enzyme components during maturation to form fully infectious virions. Vif: Viral infectivity factor; Vpr: Viral protein R; Vpu: Viral protein U.