

HIV Gene Therapy Strategies and Safety: What do we know from the Recent Publications?

Silvere D. Zaongo^{1,2}, Huan Xia^{1,3}, and Ping Ma^{1,3,4*}

¹Department of Infectious Diseases, Tianjin Second People's Hospital; ²International School of Medicine, Tianjin Medical University; ³Tianjin Association of STD/AIDS Prevention and Control; ⁴School of Medicine, Nankai University, Tianjin, China

Abstract

Almost 40 years ago, the world was noticing the emergence of one of the major public health threats it has ever known: HIV. Facing the cost-effectiveness and the health-related issues encountered with antiretroviral treatments, scientists have imagined and conceived gene therapies to tackle HIV infection. The success of such an approach was proved with the “Berlin” patient then recently reiterated in the “London” patient. In fact, the recent progress made in HIV gene therapy could provide a rapid emergence of powerful strategies to treat and totally cure the infection. Based on their principles, these approaches can be separated in three strategies that are (1) engineering HIV target cells to render them resistant to HIV replication, (2) generating gene-modified cells able to secrete antiviral proteins that interfere with HIV entry, and (3) modifying cytotoxic T cells to selectively target and eliminate infected cells. Herein, we proposed to review these approaches, their safety and their benefits as reported in recent publications. (AIDS Rev. 2020;22:195-202)

Corresponding author: Ping Ma, mapingtianjin@163.com

Key words

Gene therapy. HIV. AIDS. Safety. Strategy.

Introduction

Officially discovered in 1983, HIV is one of the major public health concerns of the world¹. Previously considered as a death sentence, the discovery and implementation of antiretroviral therapy (ART) changed HIV/AIDS into a chronic disease². However, HIV management through ART needs lifelong treatment³, which is potentially cost effective⁴ and toxic for vital organs^{5,6}. To date, only two patients are declared functionally cured. Well-known as the “Berlin” and “London” patients; both received allogeneic bone marrow transplants from a naturally mutated CCR5 gene (CCR5 delta 32) donor^{7,8}. Both were reported (several months later) having a

healthy CD4 count derived from the HIV-resistant transplanted stem cells (100% chimerism), no evidence of active viral infection in their blood, and remnants of integrated HIV-1 DNA remained (considered as fossils by researchers) in tissue samples. In 2011, the Berlin patient was the first HIV patient to be reported cured of the virus 3½ years after undergoing treatment including two rounds total-body irradiation, two rounds of stem cell transplant from a CCR5 delta 32 donors, five rounds of chemotherapy, and two rounds of immunosuppressive therapy. Indeed, while the transplant replaces the patient's immune cells by those of the donor that is capable of blocking the virus replication, the body irradiation and chemotherapy target any residual HIV virus. In 2019, the London patient also experienced a

*Correspondence to:

Pig Ma
E-mail: mapingtianjin@163.com

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less toxic and aggressive intervention with only one stem-cell transplantation and a reduced-intensity chemotherapy drug regimen, without whole-body irradiation. Bone marrow transplants from a naturally mutated CCR5 delta 32 donor are certainly replicable, but it is worth noting that it is a high-risk method (50% chance of success) only used as a last resort for patients with HIV who also have life-threatening hematological malignancies. In both cases, the HIV-infected patients were also suffering from acute myeloid leukemia and Hodgkin's lymphoma, respectively. Besides, despite the benefits of such an approach, it is further limited as bone marrow transplant is expensive, painful, and complicated⁹. In addition, such donors are difficult to find. In fact, it was reported that only 1% of the Caucasian¹⁰ and 1.44% the West African¹¹ population harbor the mutation delta 32 inherited from both parents. The mutation prevents the receptor CCR5 from appearing at the surface of the cells⁹. In such circumstances, HIV cannot penetrate the target cells. The prevalence of delta 32 gene defects in other communities has to be estimated in the future¹², but in regard of its very low distribution in communities, other options are investigated. Altogether, curing HIV-infected people through a bone marrow transplant is just not a viable option on any kind of scale.

Several researchers are developing more approaches to tackle HIV/AIDS in the absence of ART. Based on their principles, these approaches can be separated in three strategies¹³ that are (1) engineering HIV target cells to render them resistant to HIV replication, (2) generating gene-modified cells able to secrete antiviral proteins (AVPs) that interfere with HIV entry, and (3) modifying cytotoxic T cells to selectively target and eliminate infected cells. Majorly hypothesized, at first, the genetic approaches are pre-tested *in vitro* before their applications *in vivo*¹⁴. In other terms, only those that were showing strong evidence in lab context are taken for the tests on living organisms under organized and strict monitoring. Since the first approach using bone marrow transplants from an HIV resistant donor was concluded with success in Berlin then recently repeated in London, there are increasing numbers of trials initiated to develop or test the safety and efficacy of potential HIV treatments. Herein, we reviewed the three strategies aforementioned and highlighted the most recent publications realized in each to narrow the HIV research scope. Moreover, a summary of the risks-benefits of each strategy was provided.

Tackle HIV infection with engineered HIV resistant cells

Also refer to as conventional HIV gene therapy, the principle relies on rendering cells non-permissive to HIV replication. In general, CD4 or CD34 hematopoietic stem and progenitor cells (HPSCs) are extracted from a patient, modified *ex vivo* then infused back (Fig. 1). Briefly, through vectors presented in Table 1, the cells are engineered to be safely producing RNAs or proteins able to block at least one key step of the viral replication¹³. The targeted steps are: (1) receptor binding (CD4), (2) coreceptor binding (CXCR4 or CCR5), (3) membrane fusion¹⁵, (4) uncoating¹⁶, (5) reverse transcription¹⁷, (6) integration¹⁸, (7) transcription¹⁹, (8) RNA export²⁰, (9) translation²¹, (10) assembly²², (11) budding²³, and (12) maturation²³. Due to HIV genetic diversity²⁴, it is assumed that for better efficiency, the engineered cells should be able to act on two²⁵, three²¹, and ideally at least four steps²⁶ of the virus replication.

In general, the trials in which the conventional HIV gene therapy approach was tested are safe²⁷⁻³⁰. However, targeting the receptor CXCR4 presents a concern. In fact, this receptor is essential during embryonic development and plays an important role in the tissue recruitment on immune cells in adults³¹. Removing or modifying it from the surface of target cells could be a major threat to the patient's life. Therefore, some teams prefer to target the CCR5 receptor. To date, small hairpin³², zinc-finger nucleases³³, transcription activator-like effector nuclease³⁴, and clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR/CAS9)³⁵ have been employed to safely induce a mutation in the concerned gene. Fig. 2 illustrates the application of CRISPR/CAS9 to induce modifications rendering cells reluctant to HIV replication. Concretely, that technology uses a guide RNA (gRNA), a complementary sequence to the interested site, recruiting a nuclease (CAS9) that can break the viral or the host cell genetic material. Then, deletions, insertions, or substitutions are generated by cellular repair mechanisms resulting in the formation of inactivate specific gene loci leading to loss of function. The potential use of that genome-editing method for HIV gene therapy covers all steps of the viral infection cycle, from inhibition of cell invasion, through viral replication and integration inhibition, to excision of the latent provirus.

Finally, CRISPR/CAS9, the recently discovered and revolutionary bio-engineering tool³⁶ was used to edit CCR5 gene from HPSCs of a Chinese HIV positive

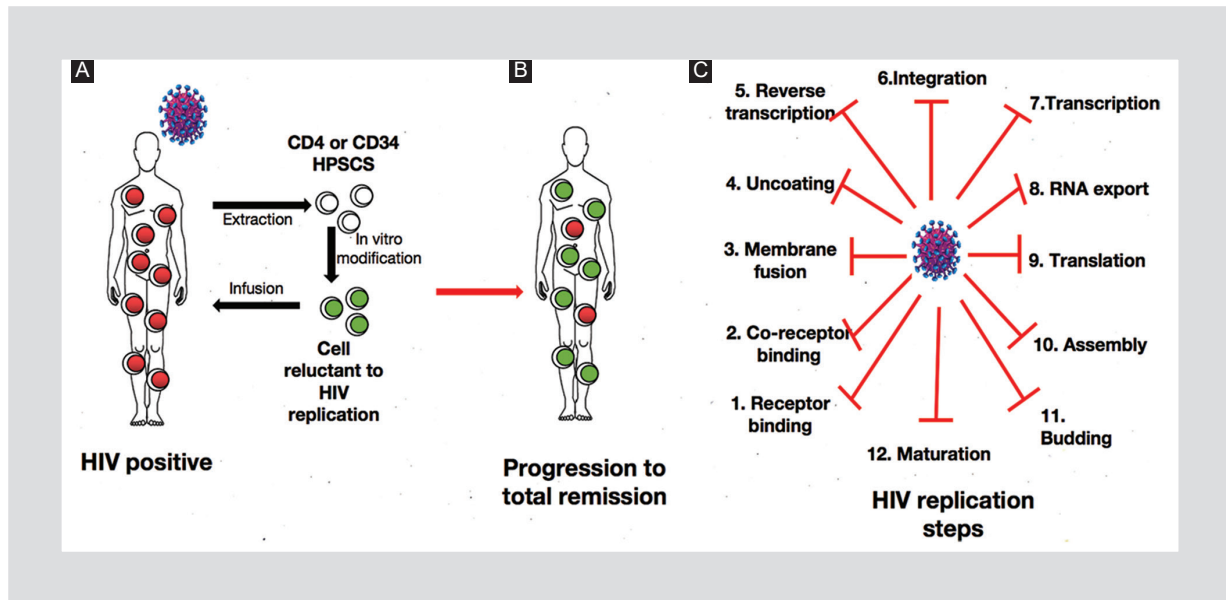


Figure 1. Conventional HIV gene therapy **A:** represents the step at which the CD4 or CD34 HSPCs are extracted, modified *ex vivo*, and then infused back to the HIV positive patient. The cells are modified to be producing RNAs or proteins able to block the HIV replication steps. **B:** are the progression to the total remission due to a positive selection of gene-modified HIV target cells (green). The engineered cells are successfully replacing the sensitive cells (red). **C:** HIV virus replication steps are blocked by the engineered cells. One, two, or several steps can be blocked simultaneously. HSPCs, hematopoietic stem, and progenitor cells.

Table 1. Examples of vector used in HIV gene therapy trials

Vector	Approach and targeted gene	References	Safe?	
Gammaretrovirus	RNA-based	U5 ribozyme and pol ribozyme	67	Yes
		Tat/vpr	27,68	
	Protein-based	RRE decoy RNA	69	
		RevM10	70	
		TdRev	71	
		maC46	28	
Combination (RNA+Protein)	TAR antisense RNA, dominant-negative Rev	72		
Lentivirus	RNA-based	<i>env</i> antisense RNA (VRX-496)	29	Yes
	Combination (RNA+Protein)	Tat/rev shRNA, TAR decoy RNA, CCR5 ribozyme	21	
		CCR5 shRNA, maC46 (Ca-1)	NCT01734850 (ClinicalTrial.gov)	
Adenovirus	Protein-based	CCR5 zinc-finger nuclease (SB-728)	30	Yes
		CCR5 zinc-finger nuclease (SB-728)	NCT01044654 (ClinicalTrial.gov)	
		CCR5 zinc-finger nuclease (SB-728)	NCT01252641 (ClinicalTrial.gov)	
		CCR5 zinc-finger nuclease (SB-728)	NCT01543152 (ClinicalTrial.gov)	
Gold particle	Protein-based	RevM10	73	Yes

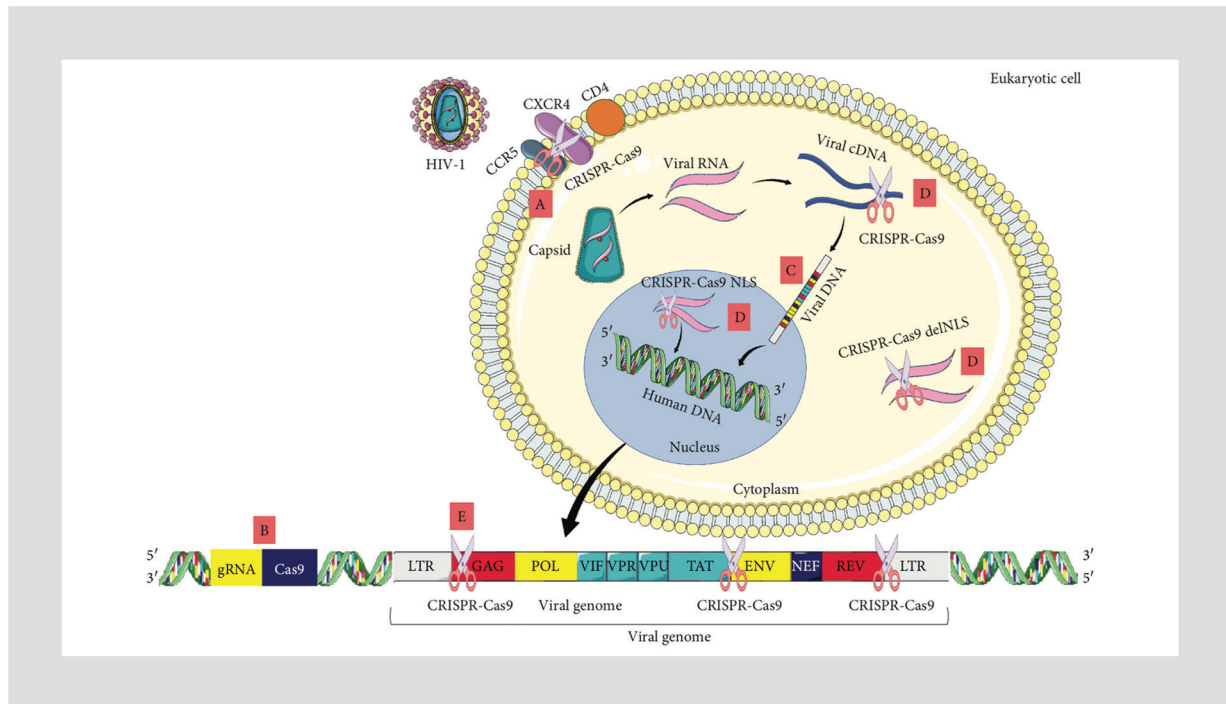


Figure 2. Overview of the CRISPR-Cas strategy to interfere on the HIV-1 infection cycle. **A:** Use of the CRISPR-Cas system to introduce loss-of-function mutations in the CCR5 and/or CXCR4 coreceptors in several cell types; **B:** inhibition of virus-cell invasion, reverse transcription, and integration by Cas and gRNA stable expression from the host cell genome; **C:** inhibition of viral replication through targeting gRNAs to different sites in the HIV-1 genome, including LTR, gag, pol, tat, and rev; **D:** inactivation of viral genetic material before integration into host DNA by transductions with Cas9-NLS or Cas9-delNLS and gRNA, whose targets are the R and U5 regions of LTR; **E:** rupture of the proviral genome from latent reservoirs with the LTR region as the main target, or by targeting other viral genes, thus modulating several HIV-1 characteristics and its infectious capacity. From Sanches-da-Silva et al., 2019.⁷⁴

patient in whom acute lymphoblastic leukemia developed similarly to “Berlin” and “London” patients^{7,8}. The HPSCs were isolated and engineered to suppress the CCR5 gene then use for bone marrow transplant. The method was reported to be stable and safe³⁷. In fact, the ablated CCR5 receptor cells persisted for 19 months without gene editing-related adverse event. Furthermore, a small increase in the number of edited cells was noted for 2 weeks after interruption of ART, suggesting that the persistence of such cells could provide – to the long run – a full resistance to HIV infection. These observations provide preliminary support for the gene-editing approach. However, further investigations on how to increase the rate of modified cells which could, in turn, improve the efficiency of this method should be undertaken. It is assumed that the low efficiency of gene editing in patient may be due to the competitive engraftment of the coinfecting HPSCs in CD34-depleted and the persistence of donor T cells³⁸. This said, it is essential to note that their approach is promising only in a context of CCR5 viral tropism. In fact, half of all circulating HIV isolates are capable of

utilizing CXCR4 as coreceptor³⁹, and in that case, other strategies should be investigated.

Production of cells able to secrete anti-HIV proteins

The principle of this alternative approach to the conventional HIV gene therapy is based on generating cells able to secrete anti-HIV proteins that bind to HIV-Env or proteins on the surface of the target cells. Basically, cells of the immune system such as CD4, CD8, or HSPCs to list a few can be modified *in vitro* then subsequently transferred to a living organism where their secreted AVPs neutralize HIV fixation and penetration in target cells. Another option is to use non-hematopoietic cells to secrete AVPs. Hence, liver or muscle cells can be modified *in vivo*. Since these organs are highly vascularized, the produced AVPs are easily released in the bloodstream, where they will be neutralizing HIV particles. Both methods aim to block the HIV virus at one or several point of these steps: (1) receptor binding (CD4), (2) coreceptor binding (CXCR4

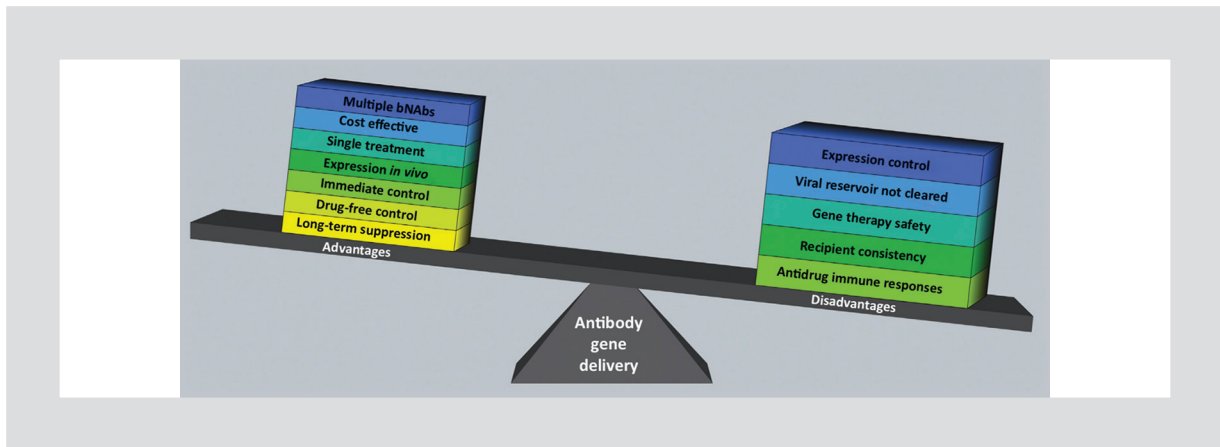


Figure 3. Illustration of advantages and disadvantages of AVPs approach through antibody gene delivery method. HIV-1 broadly neutralizing antibody (bNAbs) gene delivery has a number of potential advantages in preventing and treating infection, but these are countered by disadvantages that currently limit development. A functional cure based on this strategy may be possible if a balance can be reached to make the approach safe, feasible, and consistent among human recipients. From Haigwood and Hessel, 2019.⁷⁵

or CCR5), and (3) membrane fusion¹⁵. To be successful, the cells are transformed with vectors able to harbor and induce the expression of specific genes coding for AVPs such as soluble receptor (sCD4)⁴⁰, monoclonal antibody (mAb) b12-IgA⁴¹, mAb 2G12⁴², Simian scFv-IgG1 (4L6 or 5L7)⁴³, Simian mAb (4L6 or 5L7)⁴⁴, Siminized mAb VRC07⁴⁵, Rhesus sCD4-IgG1⁴³, and Rhesus eCD4-Ig⁴⁶ to list a few. Multiple vectors are known but adeno-associated virus (AAV) are the most commonly used for *in vivo* expression of proteins, as they are generally non-integrating⁴⁷.

To date, the AVPs approach has promising and encouraging results from *in vivo* studies majorly conducted on mice and macaques⁴⁰. Further, no apparent negative side effects subsequent to AVPs production in living animals were noted. For instance, a research team recently published an article in which they present the results of this approach. Concretely, through AAV⁴⁸, they transferred multiple genes expressing HIV-1 mAbs into rhesus macaques infected with a virus similar to HIV called SHIV. Actually, SHIV bears the HIV-1 envelope (env) protein to which these mAbs bind. The high titers of the virus in animals' bloodstream were rapidly lowered in two while one presented a rapid and 3 years viral suppression⁴⁹.

AVP approach, through Abs production *in vivo*, represents a good alternative to ART cost-effectiveness and toxicity as a single treatment is sufficient to induce an immediate and long-term viral suppression⁵⁰. From these promising results completing the long list of investigations on animal models, further research should be initiated to ensure the safety and efficacy of this

approach on humans. To date, passive administration of several different HIV-1-specific broadly neutralizing antibodies (bNAbs) has been rigorously tested in humans with no detectable toxicities even at high doses, and with repeated administration⁵¹. bNAbs are Env-directed mAbs that are very potent in their ability to neutralize across a broad range of HIV isolates worldwide. However, the major weakness of the AVPs approach in general and Abs production particularly stands in the fact that this strategy does not seem to be efficient on HIV reservoir clearance. Moreover, the AVPs expression was reported to be animal or host dependent⁴⁹ and generate anti-drug antibodies (ADA) against AVPs⁵². A summary of the advantages and disadvantages of this approach is presented in Fig. 3.

Modification of cytotoxic cells to selectively target and eliminate HIV-infected cells

The gene therapy using engineered CD8 cells to recognize and kill HIV-infected cells represents the third and last, thus far developed, concept. The process requires the use of CD8 or HPSCs that are modified *in vitro* to express HIV-specific T cells receptors (TCRs) or chimeric antigen receptors (CARs) (Fig. 4). It was reported that HIV-positive individuals naturally expressing HIV Gag-specific TCR (A2-SL9) display lower viremia⁵³. Thus, gene-modified CD8 cells expressing A2-SL9 was tested with success in mouse model as the process reduced the HIV infection. Therefore, Leibman et al. initiated to test the safety and efficacy of such approach

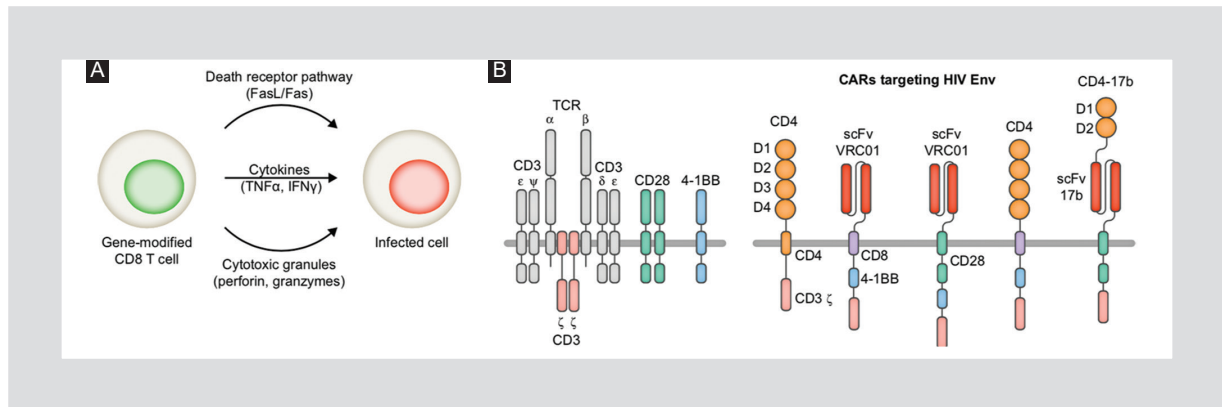


Figure 4. Gene Therapy Using Engineered CD8+ T Cells. **A:** CD8+ T cells are modified to express HIV-specific TCRs or CARs. On recognition of an infected cell, gene-modified CD8+ T cells mediate the destruction of the infected cell. **B:** Examples of HIV-specific CARs. The structure of the TCR/CD3 complex with costimulatory receptors CD28 and 4-1BB is shown in the left panel. The structure of HIV-specific CARs is depicted in the right panel. From Falkenhagen et al., 2018.¹³

in humans. However, the rapid onset of severe adverse effects leads the investigators to cancel the trial⁵⁴. Actually, these severe adverse events were explained by off-target effects observed and reported in other trials testing affinity-enhanced TCRs⁵⁵.

Since the approach of modifying CD8 shown severe adverse effects, another approach using CD4 cells to engineer an HIV-specific CAR was proposed. By inducing expression of the CD4 CAR, researchers imagined and proved *in vitro* that CD8 cells can selectively kill infected cells and suppress viral replication⁵⁶. However, few trials demonstrated that despite the persistence of the gene-modified cells, no remarkable benefit – except decreases in infected peripheral blood monocytes as well as HIV DNA isolated from rectal mucosa – was reported *in vivo*⁵⁷. A long-term follow-up (11 years) revealed that gene-modified cells are persistent⁵⁸, but HIV CAR T-cell therapy requires a persistent activity; otherwise, no clinical benefit is noted⁵⁹. Several factors such as *ex vivo* culture conditions of the T cells, the treatment administered to the patient, immune response, and design of CARs could explain the results⁶⁰. Furthermore, recent improvements of the CD4 CARs approach lead to fatal cases after the infusion of engineered cells⁶¹. Severe adverse events such as neurologic toxicity and cytokine release syndrome, not related to the vector used for the gene encoding CAR transfer, were also reported.

Challenges of HIV gene therapy

A total remission from HIV infection depends on the reservoir of latently infected cells. They harbor an

integrated HIV provirus but generally does not express viral proteins. Consequently, they become invisible to the immune system and, therefore, difficult to eliminate⁶². Several approaches simultaneously integrating at least two of the concepts aforementioned are developed to efficiently target and neutralize the viral reservoirs. For instance, it was recently demonstrated that an adaptable CAR-T cell platform based on cytotoxic lymphocytes engineered to bind a variety of broadly neutralizing anti-HIV antibodies can effectively kill HIV-infected primary cells. In addition, this hybrid approach can reduce viral reservoirs (by 50%–60% in 48 h) in the blood of infected individuals on ART⁶³. Despite these promising findings, the method needs to be tested on infected patients to ensure its safety and efficacy. Another fundamental challenge to consider is the cost of HIV gene therapy. In assuming that ART lifetime treatment combines with direct and indirect related expenses cost ~\$1 million⁶⁴; T cell-based therapy for the treatment of pediatric and young adult patients with acute lymphoblastic leukemia (~\$400,000)⁶⁵ and HSPC-based gene therapy (~\$660,000)⁶⁶, for instance, clearly demonstrate that HIV gene therapy is not an affordable option for a larger population.

Conclusion

Although colossal investments in HIV research for more than 30 years, a potent cure for HIV has been elusive. Fortunately, HIV gene therapy is on a fast-moving track with relevant results in recent years. In analyzing them, we conclude that conventional gene

therapy is safe, in general, with notable benefits that should be improved in the future. On the other hand, despite their relative safety and benefits observed on animal models, AVPs have proven rapid efficacy and long-term HIV suppression when passively administered to humans. Their productions in humans are, however, not explored yet, which keep the question about their safety rose. Finally, the TCRs and CARs approach in humans were reported either dangerous or not benefiting when they were safely induced.

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Supplementary 1. Critical appraisal overview.

Authors	Year of publication	Study Participation								Study Attrition					Prognostic Factor Measurement					
		Source of target population	Method used to identify population	Recruitment period	Place of recruitment	Inclusion and exclusion criteria	Adequate study participation	Baseline characteristics	RISK OF BIAS	Proportion of baseline sample	Attempts to collect information on participants who dropped out	Reasons and potential impact of loss to follow-up	Outcome and prognostic factor information on those lost to follow-up	RISK OF BIAS	Definition of PF	Valid and reliable measurement of PF	Method and setting of PF measurement	Proportion of data on PF available for analysis	Method used for missing data	RISK OF BIAS
Abad et al.	2000	Yes	Yes	Yes	Yes	Yes	Unsure	Partial	Moderate	Yes	Yes	Yes	Yes	Low	Partial	No	No	No	Unsure	High
Alagna et al.	2014	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	No	No	No	No	Unsure	High
Bor et al.	2013	Yes	Yes	Yes	Yes	Partial	Partial	Partial	Moderate	Yes	Yes	Yes	Yes	Low	No	Partial	No	Partial	No	High
Bouza et al.	2001	Yes	Partial	Yes	Yes	Unsure	Unsure	Yes	Moderate	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Yes	Low
Chong et al.	2003	Yes	Yes	Yes	Yes	Partial	Partial	No	Moderate	Partial	No	No	Unsure	High	No	Partial	No	Yes	No	High
Cicalini et al.	2001	Yes	Yes	Yes	Yes	Partial	Yes	Partial	Low	Yes	Unsure	Unsure	Unsure	High	Yes	Yes	No	Yes	No	Moderate
Cicalini et al.	2006	Yes	Yes	Yes	Yes	Partial	Yes	Yes	Low	Yes	Unsure	Unsure	Unsure	High	Yes	Yes	Yes	Yes	No	Low
De Rosa et al.	2007	Yes	No	Yes	Yes	Yes	No	Yes	Moderate	Yes	Yes	No	Yes	Low	No	Yes	Unsure	Unsure	Unsure	High
Fern.Guer.et al.	2009	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	Yes	Yes	No	No	No	High
Ferraris et al.	2013	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Unsure	Unsure	Moderate
Gansera et al.	2016	Partial	Partial	Yes	No	No	Unsure	No	High	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Unsure	Low
Gebo et al.	2006	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Unsure	Unsure	Unsure	High	Yes	Yes	Yes	Yes	No	Low
Losa et al.	2003	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Yes	Low
Martin-Dávila et	2005	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Partial	Yes	Yes	Low	Yes	Yes	Yes	Yes	No	Low
Meel et al.	2014	Yes	Yes	No	Yes	Partial	Unsure	Partial	High	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Yes	Low
Meel et al.	2018	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	No	Yes	Low	Partial	Partial	Yes	Yes	No	Moderate
Mestres et al.	2003	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Unsure	Unsure	Unsure	High	Yes	Yes	Yes	Yes	Yes	Low
Nel et al.	2014	Yes	Partial	Yes	Yes	Yes	Partial	Yes	Low	Yes	Yes	Yes	No	Low	Yes	Yes	Yes	Yes	No	Low
Pulvirenti et al.	1996	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	No	No	No	High	Yes	Yes	Yes	Yes	No	Low
Ribera et al.	1998	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Partial	Yes	Low
Robinson et al.	2000	Partial	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Yes	Low
Sambola et al.	2010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Unsure	Yes	Low	Yes	Yes	Unsure	Unsure	No	High
Smith et al.	2004	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Low	Partial	Yes	Unsure	Yes	Yes	Moderate

6 yes / 5 yes + 2 partial	Low	3 yes	Low	4 yes	Low
4 yes / 3 yes + 4 partial	Moderate	2 yes	Moderate	3 yes / 2 yes + 3 partial	Moderate
Less than 4 yes	High	Less than 2 yes	High	Less than 3 yes	High

Supplementary 1. Critical appraisal overview (continued).

Authors	Outcome Measurement				Study Confounding							Statistical analysis and reporting						
	Definition of outcome	Valid and reliable measurement of outcome	Method and Setting of outcome measurement	RISK OF BIAS	Important Confounders Measured	Definition of the confounding factor	Valid and Reliable Measurement of Confounders	Method and Setting of Confounding Measurement	Method used for missing data	Appropriate Accounting for Confounding	RISK OF BIAS	Presentation of analytical strategy	Model development strategy	Reporting of results	RISK OF BIAS			
Abad et al.	Yes	Yes	Yes	Low	Partial	No	No	No	No	No	High	No	No	Yes	High			
Alagna et al.	Yes	Yes	Yes	Low	Partial	No	No	No	No	No	High	Yes	Yes	Yes	Low			
Bor et al.	Partial	Unsure	Unsure	High	No	No	No	No	No	No	High	Yes	Yes	Yes	Low			
Bouza et al.	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Yes	Unsure	Low	Partial	No	Yes	High			
Chong et al.	Yes	No	Yes	Moderate	No	No	No	No	No	No	High	No	No	Yes	High			
Cicalini et al.	Yes	Yes	Yes	Low	No	No	No	No	No	No	High	Partial	No	Yes	High			
Cicalini et al.	Yes	Yes	Yes	Low	Yes	Partial	Partial	Yes	No	Partial	High	Yes	Partial	Yes	Low			
De Rosa et al.	Yes	Unsure	Yes	Moderate	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Low			
Fernandez et al.	Yes	Yes	Yes	Low	Partial	No	Partial	Partial	No	Partial	High	Yes	Partial	Yes	Low			
Ferraris et al.	Yes	Yes	Yes	Low	Partial	Yes	Yes	Partial	Unsure	Partial	High	Yes	Yes	Yes	Low			
Gansera et al.	Yes	Yes	No	Moderate	No	No	No	No	No	No	High	No	No	No	High			
Gebo et al.	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	No	No	Moderate	Yes	Yes	Yes	Low			
Losa et al.	Yes	Yes	Yes	Low	Partial	Partial	Partial	Partial	Unsure	No	High	Yes	No	No	High			
Martín-Dávila et al.	Yes	Yes	Yes	Low	No	No	No	No	No	No	High	Yes	Yes	Yes	Low			
Meel et al.	Partial	Yes	Yes	Low	Partial	No	No	No	No	No	High	No	No	No	High			
Meel et al.	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Unsure	Yes	Low	No	Yes	Unsure	Moderate			
Mestres et al.	Yes	Yes	Yes	Low	Yes	Yes	Yes	Yes	Unsure	No	Moderate	Partial	No	Yes	High			
Nel et al.	Yes	Yes	Partial	Low	No	No	No	No	Unsure	No	High	Yes	Yes	Yes	Low			
Pulvirenti et al.	Partial	Yes	Yes	Low	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Yes	Low			
Ribera et al.	Yes	Unsure	Partial	High	Yes	Yes	Yes	Yes	Yes	Yes	Low	Yes	Yes	Partial	Low			
Robinson et al.	Yes	Yes	Yes	Low	No	No	No	No	No	No	High	Yes	Yes	Yes	Low			
Sambola et al.	Yes	Yes	Yes	Low	No	No	Partial	Partial	Partial	Partial	High	Yes	Yes	Yes	Low			
Smith et al.	Yes	Yes	Yes	Low	Partial	No	Yes	Yes	No	No	High	Yes	No	Yes	Moderate			
		3 yes / 2 yes + 1 partial		Low						5 yes / 4 yes + 2 partial		Low				3 yes / 2 yes + 1 partial		Low
		2 yes / 1 yes + 2 partial		Moderate						3 yes / 2 yes + 4 partial		Moderate				2 yes / 1 yes + 2 partial		Moderate
		Less than 2 yes		High						Less than 3 yes		High				Less than 2 yes		High

Authors	Study design; Country	Cohort	Sample characteristics											Mortality						
			Comparison group or objective	Number of patients	Age	Sex (% male)	IV drug users (%)	HIV+ (%)	Viral load	Average CD4 count/ul	ART Use	Type of ART	Duration of ART use	Overall Mortality	HIV+				HIV-	Significance
															All Pt	<200	>200	Unknown CD4 Count		
Abad et al. (2000)	Retrospective case series; Spain	1991-1999	Case presentation of HIV infected patients in need of cardiac surgery	4	Median 44	100%	75%	100%				No			25%	25%			N.A.	
Alagna et al. (2014)	Prospective 1-year cohort; International	2000-2006	Risk factor and 1-year mortality of patients with repeat IE compared to single-episode IE	1874	Unknown	68%	9.2%	2.1%				No			9.5%					
Bor et al. (2013)	Retrospective chart review; USA	1998-2009	Evolution of IE in the US in the 21st century	382.153	Mean 59.7	57.7%	7.8%	2.8%				No			14.5%				Mortality not notably affected by HIV	
Bouza et al. (2001)	Prospective in-hospital case-study; Spain	1994-1996	Describing characteristics of IE episodes in HIV-infected patients and nosocomial acquired cases	101	Mean 50	73%	35.6%	33%		41% had >200-500/mm3; 47% had <200/mm3		No			25.7%					
Chong et al. (2003)*	Retrospective case series; USA	1990-1999	Early and late mortality and morbidity of valve replacement among HIV-infected patients	22	Mean 37.5	68%	73%	100%				No			45%	45%			N.A.	
Cicalini et al. (2001)	Retrospective case series; Italy	1984-1999	Clinical features, sites of involvement, bacteriological findings, treatment, complications, and outcome of IE in HIV-infected patients	105	Mean 30.1	73.3%	94.3%	100%		3 groups: 38% had 90; 25.7% had 260; 8.6% had 520; Rest is missing		No			17.8%	17.8%	72.2%*	27.8%*	N.A.	Higher mortality rate in patients with CD4 count <200 (no p given)
Cicalini et al. (2006)	Retrospective cohort; Italy	1980-2003	Characteristics of IE in IVDU and non-IVDU and factors associated with increased risk of death	283	Mean 38.6	67.1%	59.7%	30.4%		142-282 (during hospital stay); >50% less than 200		No			Unknown					Higher risk of death was observed among HIV+ patients (p=0.079)
De Rosa et al. (2007)	Retrospective cohort; Italy	1986-1999	IE in HIV-positive vs -negative patients	257	Mean 28	76%	100%	38%				No			16%	8.5%			14.5%	No significant difference in mortality in HIV- vs HIV+
Fernandez Guerrero et al. (2009)	Retrospective cohort; Spain	1985-2006	Characteristics of right-sided vs left-sided IE and HIV+ vs HIV- caused by S. Aureus	133	Unknown	77%	47%	38.3%		443; 27.4% had <200		No			18%	9.8%*			28.9%*	Lower mortality in HIV positive patients (no p given (OR 0.27 (0.08-0.85))
Ferraris et al. (2013)	Retrospective chart review; Italy	2003-2010	IE in a single center Hospital in Italy	166	Median 57	63%	26%	19%	40% had <10.000; 14% had 10.000-100.000; 11% had >100.000; 34% Unknown	Median 268		Yes (40%)	Unknown	Unknown	17%					
Gansera et al. (2016)	Retrospective case series; Germany	2013	Surgery in patients with IV abuse and recurrent IE	3	Mean 29.7	66.7%	100%	33.3%				No			33%	100%			0%	1 out of 3 patients died
Gebo et al. (2006)	Retrospective cohort; USA	1990-2002	HIV infected IE patients before vs after HAART era	58	Median 40	65.5%	84.5%	100%	Median 78.288; 19.6% had <10,000	Median 68; 44.8% had <50		Yes (31%)	3 or more NRTI's OR PI/NNRTI + 2 NRTI's OR PI + NNRTI	Unknown	52%	52%			N.A.	Significant mortality in 1-year HIV infected patients (no p); HAART not significant associated with mortality or recurrence (OR 1.67 (0.41-6.84) no p)
Losa et al. (2003)	Retrospective case-study; Spain	1979-1999	HIV infected IE patients not related to IVDU	8	Mean 44	100%	0%	100%		Median 22		No			12.5%	12.5%			N.A.	

Supplementary 2. Baseline Table (continued).

Authors	Complications			Requiring surgery		Recurrent IE		Hospital stay		Rehospitalization (%)		Effect of HIV on IE
	HIV+	HIV-	Significance values	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-	
Abad et al. (2000)	25% septic pulmonary embolism	N.A.		100%	N.A.							IE patients with HIV are frequently drug abusers or homosexual. Hospital morbidity and mortality are higher than usual in this group
Alagna et al. (2014)	x	Stroke 15.8%; Embolization 24%; Heart failure 28.2%; Intracardiac abscess 15%		x	50.3%	x	4.9%					Patients with repeated IE were more likely to have HIV
Bor et al. (2013)	x	Stroke 9.0%; CNS infection 2.1%; Encephalopathy 2.2%; Acute renal failure 18.0%	Incidence of IE among HIV+ fell					x	15.3			The proportion of HIV among IE patients fell. Mortality was not notably affected by HIV
Bouza et al. (2001)	Respiratory symptoms 92%; Anemia 83%; Pulmonary embolism 70%	Cohort: Pulmonary embolisms 27.5%; systemic embolisms 18.3%; congestive heart failure 31%; acute renal failure 17.4%	No clinical differences, only big difference between pulmonary embolisms in HIV+ and HIV-	x	42%			x	31			No differences in clinical presentation or outcome due to endocarditis in HIV positive/negative patients
Chong et al. (2003)*	Heart failure 59%; Sepsis 91%				N.A.	20%	N.A.					HIV-infected patients have low operative risk. HIV patients have poor prognosis because of immunocompromise and continued high-risk behavior. All patients with recurrent IE were IVDU
Cicalini et al. (2001)	Congestive heart failure 16.9%; Septic pulmonary emboli 51.8%; Stroke 4.8%; Acute renal failure 2.4%; Other systemic emboli 6%	N.A.	The characteristics of IE in patients with HIV infection have not been defined completely	5.9%	N.A.	2.9%	N.A.					The mortality rate among patients with IE and advanced HIV infection is significantly increased. Severe immunosuppression in HIV-infected patients with IE was associated with poor prognosis
Cicalini et al. (2006)	IVDU: None 32.5%; Heart failure 11.2%; Pulmonary emboli 43.2%; Cerebral emboli 10.1%; Other 3.0%.	Non-IVDU: None 54.4%; Heart failure 20.2%; Pulmonary emboli 0.9%; Cerebral emboli 12.3%; Other 12.3%		x	11.7%							HIV prevalence among IVDUs significantly decreased
De Rosa et al. (2007)	x	Cohort: Cardiac complications 14%; Pulmonary embolisms 36.1%; CNS emboli 8.3%; Spleen emboli 3.4%; Retina emboli 0.7%; Acute renal failure 1.9%	Clinical characteristics of IE were similar in HIV+ and HIV-	x	16.7%	x	2.3%					No significant association between mortality and HIV infection. IE incidence seems to be increasing in HIV positive IVDUs
Fernandez Guerrero et al. (2009)	Cardiac failure 1.9%; Neurologic signs 1.9%; Cutaneous lesions 5.8%	Cardiac failure 39.4%; Neurologic signs 34.2%; Cutaneous lesions 28.9%; Embolisms 60.9%	Significant differences between HIV+ and HIV-: Cardiac failure, Neurologic signs, Cutaneous Lesions									No significant association between mortality and HIV infection
Ferraris et al. (2013)	x	Embolizations 44%; Heart failure 26%; Arrhythmias 21%; Intracardiac abscess 9%; Renal failure 6%; Septic shock 4%		x	52%	16.3%	7.4%					HIV+ patients were younger, more frequent male, and had a higher frequency of right-sided IE than HIV-
Gansera et al. (2016)	None	50% had multiple emboli		100%	100%							HIV positive patients are in need of surgical treatment
Gebo et al. (2006)	Vascular phenomena 31%; Pulmonary infarcts 22%	N.A.	Significant decline in the incidence of IE between pre-HAART and current HAART	7.8%	N.A.	16%	N.A.					IE rates have decreased in the HAART era. Significant morbidity and 1-year mortality in HIV-infected patients with IE
Losa et al. (2003)				25%	N.A.							IE develops in patients with advanced HIV-1 infection

Authors	Study design; Country	Cohort	Sample characteristics											Mortality						
			Comparison group or objective	Number of patients	Age	Sex (% male)	IV drug users (%)	HIV+ (%)	Viral load	Average CD4 count/ul	ART Use	Type of ART	Duration of ART use	Overall Mortality	HIV+				HIV-	Significance
															All Pt	<200	>200	Unknown CD4 Count		
Martin-Dávila et al. (2005)	Retrospective chart review; Spain	1985-1999	Mortality and risk factors of native valve endocarditis in IVDU	220	Median 27.8	Unknown	100%	64.5%		Only available in 96 patients: 17.7% had >500; 41% had 500-200; 42% had <200	No			6.4%	7.7%	36.4%	18.2%	45.5%	3.8%	HIV not related to mortality (p=1.0 (OR 1.08 (0.25-4.7))); CD4 <200 not related to mortality (p=0.39)
Meel et al. (2014)	Retrospective case series; South Africa	Unknown	Cases of HIV-positive patients with right-sided IE due to IV nyaope use	3	Mean 26.3	100%	100%	100%		Available in one patient: 576	No			0%	0%				N.A.	
Meel et al. (2018)	Retrospective case series; South Africa	2014-2017	Characteristics of patients with IE due to IV nyaopa use	68	Mean 25.8	97.1%	100%	76.1%		Median 437; 8.8% had <200	Yes (4.4%)	Unknown	Unknown	14.7%	19.2%				0%	No higher mortality in HIV+ patients p=0.06
Mestres et al. (2003)	Retrospective chart review; Spain	1985-2002	HIV+ patients undergoing cardiac surgery	21	Mean 28.2	85.7%	95.2%	100%		Available in 13 patients: mean 353,62	No			28.6%	28.6%				N.A.	Mortality became 50% after long-term follow-up (no p given)
Nel et al. (2014)	Prospective in-hospital case study; South Africa	2004-2007	Describes echocardiographic features of IE patients and compare IE in HIV positive vs negative patients	77	Mean 31.2	54.5%	0%	22%		Mean 189	No			23.4%	23.6%	75%	25%		23.3%	Similar mortality rate between HIV+ and HIV-
Pulvirenti et al. (1996)	Retrospective chart review; USA	1987-1990	HIV+ vs HIV- IVDU patients with IE	102	Unknown	75.5%	100%	44.1%			Yes (2%)	Unknown	Unknown	10.8%	13.3%	83.3%*	16.7%*		8.8%	Higher mortality in HIV+ with CD4 <200 compared to CD4 >200 (OR 14.7 (2.64-81.9)); No difference in mortality between HIV+ and HIV- (OR 1.6 (0.46-5.61))
Ribera et al. (1998)	Prospective in-hospital cohort; Spain	1984-1995	Characteristics of HIV positive vs negative IVDU patients with IE	283	Mean 26.8	79%	100%	76.3%		36.1% had <200; 45.8% had >200; 18.1% Unknown	No			9.2%	7.9%	100%*	0%*		13.4%	Higher mortality in CD4<200 (p<0.001); No difference between HIV+ and HIV- mortality (p=0.17)
Robinson et al. (2000)	Retrospective chart review; USA	1990-1995	HIV+ vs HIV- IVDU with IE	126	Mean 34.5	56%	100%	58.7%		43.2% had <200; 29.7% had >200; 27% Unknown	No			Unknown						
Sambola et al. (2010)	Prospective 1-year cohort; Spain	2000-2008	Characteristics of IE in men vs women	271	Mean 57	67.5%	Unknown	7%			No			29%						Female gender related to higher mortality (p=0.05)
Smith et al. (2004)	Retrospective case study; USA	1999-2003	HIV+ patients with vs without IE	10	Mean 37.8	80%	20%	100%		Median 84.5	No			30%	30%	66.7%	33.3%			Small sample size limits statistical correlation

Supplementary 2. Baseline Table (continued).

Authors	Complications			Requiring surgery		Recurrent IE		Hospital stay		Rehospitalization (%)		Effect of HIV on IE
	HIV+	HIV-	Significance values	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-	
Martín-Dávila et al. (2005)				4.9%	5.1%							No significant association between mortality and HIV infection. Mortality seems to be increased in patients with CD4 <200 (no signif)
Meel et al. (2014)	66.7% pulmonary embolisms	N.A.		33.3%	N.A.							Likely to encounter more cases of IV nyoape use with HIV infection
Meel et al. (2018)	x	Septic pulmonary embolisms 61.8%; Systemic emboli 11.8% Right ventricular failure 42.6%	Clinical characteristics of IE were similar in HIV+ and HIV-	x	5.9%	x	4.4%					No significant association between mortality and HIV infection
Mestres et al. (2003)				100%	N.A.			41.1	N.A.			Increased number of HIV-1 patients referred for surgery. Cardiac surgery did not worsen HIV nor IE prognosis
Nel et al. (2014)	Congestive heart failure (29.4%); Emboli 17.5%	Congestive heart failure 46.7%; Emboli 10%	Only congestive failure was significant different between HIV+ and HIV-, any other clinical features of IE were similar	35.3%	56.6%							The clinical profile of HIV+ patients is similar to HIV- patients. Similar rate of morbidity and mortality between HIV+ and HIV- patients
Pulvirenti et al. (1996)	Embolus 73.3%; Cardiac complications 6.7%; None 8.9%; Both 11.1%	Embolus 54.4%; Cardiac complications 14%; None 15.8%; Both 15.8%	Clinical characteristics of IE were similar in HIV+ and HIV-	2.2%	10.5%							Lower CD4 count was significantly associated with higher mortality rates
Ribera et al. (1998)	Congestive left-sided heart failure 15.7%; Renal failure 19.9%; Major systemic emboli 6.9%	Congestive left-sided heart failure 32.8%; Renal failure 37.3%; Major systemic emboli 17.9%	Significant differences between HIV+ and HIV- were: Congestive heart failure, renal failure and major systemic emboli; Neurological complications increased as CD4 cell counts decreased	7.4%*	23.9%*	29.2%	31.3%					Mortality and clinical presentation between HIV+ and HIV- was similar. Among HIV+, mortality was significant increased in immunosuppressed patients
Robinson et al. (2000)			HIV infection is not associated with lower maximum temperature					28.3	30.9			HIV infection was not associated with lower maximal Temperature, but was associated with decreased WBC count
Sambola et al. (2010)												More men than women were HIV+ among IE patients
Smith et al. (2004)			No differences in clinical characteristics of HIV-positive patients with and without IE.	30%	N.A.							There were no differences in clinical presentation of HIV positive patients with and without IE

Supplementary 3. Side of cardiac involvement. Percentages are given per total number of patients in the study population, as percentage of number of HIV-positive (HIV+) or HIV-negative (HIV-), or as percentage of intravenous drug user (IVDU) or non-IVDU (Non).

Authors	Endocarditis diagnosis				HIV+/HIV- differences	Significance
	Right sided (%)	Left sided (%)	Both sides (%)	Not identified		
Studies with HIV-positive patients only						
Cicalini, S. (2001)	53.7%	34.2%	11.2%	0.9%		Higher prevalence of any right-sided heart involvement compared to any left-sided heart involvement
Gebo, K. A. (2006)	46.5%	53.5%	31.3%			
Losa, J. E. (2003)	14%	86%				
Meel, R. (2014)	100%					
Mestres, C. A. (2003)	52.4%	47.6%				
Smith, D. T. (2004)	30%	70%				
Studies comparing HIV-positive to HIV-negative patients						
Alagna, L. (2014)						
Bor, D. H. (2013)						
Bouza et al. (2001)	39.5%	60.5%			86% episodes in HIV+ was right-sided IE	
Cicalini, S. (2006)	IVDU: 61.5%; Non: 2.6%	IVDU: 31.4%; Non: 87.8%	IVDU: 5.9%; Non: 0.8%	IVDU: 1.2%; Non: 8.8%		Not mentioned on side of involvement
De Rosa et al. (2007)	HIV+: 54.6%; HIV-: 56%	HIV+: 37.4%; HIV-: 32%	HIV+: 6.7%; HIV-: 11%	HIV+: 1.2%; HIV-: 1%		IE in IVDU is more frequently localized to the right side of the heart (OR 2.24 (1.55-3.23))
Fernandez Guerrero, M. L. (2009)	HIV+: 80.3%; HIV-: 15.7%	HIV+: 19.6%; HIV-: 84.2%			Most HIV+ patients had right-sided endocarditis (most died of left-sided IE); Most HIV- patients had left-sided endocarditis	Left-sided involvement in HIV- (OR 0.05 (0.023-0.14)); Mortality and morbidity were caused by location of heart
Ferraris, L. (2013)	HIV+: 48%; HIV-: 17%	74%	5%			Higher incidence of right-sided IE in HIV+ patients compared to HIV-
Gansera, L. S. (2016)						
Martín-Dávila, P. (2005)	88.1%	8%	0.4%	3%		
Meel, R. (2018)						
Nel et al. (2014)						
Pulvirenti, J. J. (1996)	HIV+: 42.2%; HIV-: 28.1%	HIV+: 26.7%; HIV-: 47.4%	HIV+: 31.1%; HIV-: 24.6%			Cardiac side involved is significant between HIV+ and HIV-
Ribera, E. (1998)	HIV+: 75%; HIV-: 44.8%	HIV+: 10.2%; HIV-: 47.8%	HIV+: 7.4%; HIV-: 7.5%	HIV+: 7.4%; HIV-: 0%		Significant right side in HIV+, left side in HIV-, and unknown in HIV+ compared to HIV-
Robinson et al. (2000)						
Sambola, A. (2010)						