From convention to innovation, promising strategies in HIV-1 treatment

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Abstract

As a causative agent of acquired immune deficiency syndrome (AIDS), human immunodeficiency virus (HIV) attacks the immune system and without interruptions the immune system ultimately is destined to be paralyzed. Although cellular immunity can be partially preserved if antiretroviral therapy (ART) is administrated in the early stage of HIV infection, efficient approaches to cure HIV are still in the study and all existing treatments adopted have their own limitations, for which more novel tactics are being exploited. In this paper, an extensive repertoire of tactics is being introduced to provide a general review of the frontiers of research and development in HIV treatment, spanning conventional treatments in clinical use and emergent strategies. Despite all merits demonstrated respectively, their security should be the first priority in practice and we are trying to strike a balance between risk and return all the time.

Keywords

Convention to innovation, HIV-1 .

1. Introduction

In 2016, an estimated 36.7 million people were living with HIV (including 1.8 million children) – with a global HIV prevalence of 0.8% among adults [1] [2]. Without efficient treatment, most of patients would gradually develop to the stage of AIDS and die of varied kinds of HIV-associated complications with poor prognosis, especially malignant tumors and opportunistic infections. Nowadays, with the widespread administration of conventional ART, HIV-infected individuals are expected to be older as times goes on. More patients can reach a lifespan similar to normal individuals, but considering increased vulnerability related to aging, more challenges are met in the clinical care. Hence, HIV remains to be a serious concern of public health. To fight against HIV, like other infectious diseases, great efforts have been focused on prophylaxis and treatments. Although efficacious vaccine appears to be the best alternative to stop the global epidemic of AIDS, many trials culminated in unexpected failure. The most effective vaccine is RV 144, which demonstrates an approximately 30% reduction in infection rates [3], but the others show no efficacy. Therefore, more attention is being paid to treatments. Despite progression made by ART, there also exist many challenges including delayed efficacy of ART [4], undesirable side effects of therapeutic agents, lack of compliance, endemic resistance and persistent dormant latency. Following that, more specific and potent therapies emerge, involving burgeoning genetic therapeutic technology, broadly neutralizing antibodies(bNAbs) and chimeric antigen receptor T-Cell immunotherapy (CAR-T). In this review, we introduce those potential and promising approaches and provide the outlook in HIV-1 treatment.

2. Antiretroviral therapy

The life cycle of HIV-1 includes: entry and fusion, reverse transcription and integration, assembly and release. HIV-1 may also be latent in infected cells and would start replication again upon activation. Continuous and uninterrupted viral budding causes the exhaustion of the membrane of the

host cell, leading to cell lysis. When process from entry to release was interfered, not only can the function of the immune system be maintained, other uninfected cells can be protected. Thus, many therapeutic agents work on different targets in that process. Those agents include chemokine coreceptor antagonists, fusion inhibitors, reverse transcriptase (RT) inhibitor, integrase inhibitor, protease inhibitor and pharmacokinetic enhancers.

RT inhibitor, as the first drugs for HIV treatment, has limited efficacy and many administered patients still progress to AIDS in the end. Nonetheless, compared with respective drug, combination of two and more different kinds of drugs demonstrates more potent and sustained suppression of HIV replication [5]. Since then, combination has become the essence of the drug administration. Besides treatment, it also decreases the possibility of transmission. If the HIV-infected partner was treated with antiretroviral therapy, heterosexual transmission was reduced by 92% [6]. To improve the compliance and avoid resistance induced by poor adherence, fixed dose combination (FDC) drugs is accessible, containing multiple drugs in a single pill or tablet. The recent efforts of ART focus on extending effective duration, but the critical challenges in ART is the state of HIV latency and the side effects of drugs, for which researchers turn to other novel approaches in order to cure HIV.

3. Promising strategies

Not like the closely arranged visceral cells, such as hepatocyte and epithelia, the target cell in gene therapy of HIV, namely the lymphocyte, has its specialty and advantage contributed to its widespread travel routes and handily accessible. Therefore, HIV gene therapy, may evolve with the progress in techniques, gain the first access to the fruits of scientific frontiers and on the frontiers of clinical translation. At the time of December 2017, human immunodeficiency virus (HIV) infection is the major target in the gene therapy of infectious diseases (69.2%) [7]. Gene therapy has become the indispensable part of HIV treatment, although the history of gene therapy is just beginning.

There has been nearly thirty years since the first human gene therapy, which saved 4-year-old girl with adenosine deaminase (ADA) deficiency by an infusion of autologous T cells into which a normal ADA gene had been inserted [8]. When gene editing had not begun to emerge, they chose processed and relatively safe virus as vectors carrying the new gene. Nowadays, as vectors in gene therapy, viruses are still very useful and efficient, such as the adeno-associated virus (AVV), which has been introduced in clinical trials for gene therapy of Spinal Muscular Atrophy and Severe Hemophilia A [9] [10]. With the development of technology, instead of being integrated randomly into the genome, techniques with more accurate orientation and insertion into target gene comes to the focus of public and gradually applied to clinical use with novel sequence specificities. Zinc-finger nucleases (ZFN), transcription activator-like effectors nuclease (TALEN), and clustered regularly interspaced short palindromic repeats-associated Cas9 (CRISPR/Cas9) systems are the most concerned gene editing techniques utilized by scientists and doctors [12] [15] [20] [21] [29]. In addition, direct product of translation, or RNA, also provides an alternative target for disruption. Both antisense oligonucleotide (AON)-induced exon skipping, via synthesized nucleic acid which inactivates the gene by binding to the mRNA produced by the gene, and RNA interference (RNAi), via microRNA (miRNA) and small interfering RNA (siRNA), which play a single guide RNA (sgRNA)-like role inducing cleavage by Argonaute 2 (Ago2) once they pair with complementary sequence in mRNA, interfere with the normal translation of RNA, paving another road for novel therapy of HIV [11] [12]. (See Table 1.) Besides these genetic therapeutic technologies, immunotherapy also provides alternative strategy. Intensifying the adaptive immunity, namely the humoral and cellular immunity, can enrich more possible combinations to optimize current therapy to a more effective, continuous, economical and accessible one. Here, we focus on transfer of bNAbs [13] and CAR-T [14].

Based on these advancements of science, numerous kinds of treatment programs develop and we are more closer to the final goal—cure and eradicate HIV. To date, these progressions have been broadly utilized to relentlessly pursue better alternative approach by inhibition of normal HIV life cycle and its interaction with host cell. Here, according to the difference of targets, the therapy strategy is

divided into two categories, inhibition of HIV life cycle and intensification of adaptive immune response.

3.1 Inhibition of HIV life cycle

In the regular ART treatment mentioned above, there are two main challenges on the way to eradicate HIV in infected individual, persistence of latent viral reservoir and anatomic compartment where effective drug concentration cannot be reached. Both of them pose a major threat of rapid relapse of viremia when the regular ART stops. Local administration solves the problem of medicine differential distribution, and to eliminate latent viral reservoir, there are many theoretical models, such as genome editing towards relevant genes (genetic treatment) [20] [21]. Globally activating the virus with or without concomitant ART (non-genetic treatment) are the focus of recent efforts [16]. When latency is reversed in non-genetic treatment, another question being noticed recently is that appropriate activation of HIV-1-specific immune response, especially directing cytotoxic lymphocyte (CTL) responses to unmutated viral epitopes, may be required [17] [18]. In genetic treatment, to modify the CD4+ T cell becomes the main goal to clear HIV-1 latency because of the great majority of latently-infected cell pool in those T cells [19]. Targeting gene should contain both 5' and 3' long terminal repeat (LTR) to prevent potential transcription of toxic proteins in the case of modification in other sites. Moreover, the LTRs of HIV are highly conserved and critical for viral replication, which avoids the frequent mutations and resistance in some degree. Compared with the costly and time-consuming engineering of ZFN and TALEN, the facile and versatile Cas9/gRNA technology platform is the first priority [20] [12] and there has been demonstrated the possibility of Cas9/gRNA technology in reducing HIV expression via mutating provirus LTR [21].

Other than disruption of latency of provirus, there are other working sites towards translation of virus pre-mRNA and inhibitors impeding the entry of virus. Alternative splicing of virus pre-mRNA can be directly inhibited by blocking the specific viral splice sites with antisense sequences and interfering with translation of the key regulatory protein of HIV-1, Tat and Rev [22] [23]. Disrupts the expression of Tat and Rev can also be achieved by RNAi which have been utilized to interfere with the expression and decreasing concentration of relevant positive regulator—SR protein, a conserved family of proteins related to RNA splicing [11].

The interaction between virus and host is another focus of genetic treatment. Entry inhibitors, either peptides or molecules, hinder the interaction of viral glycoproteins gp41 and gp120 with the main receptor CD4 and the coreceptor C-X-C chemokine receptor type 4 (CXCR4) and C-C chemokine receptor type 5 (CCR5). In the host side, in fact, only the CCR5 is the practical choice because the role of CD4 in T cell receptor (TCR)-induced activation are indispensable, without which immunodeficiency may become worse, and CXCR4 also has an important function especially for retention of hematopoietic stem cells (HSCs) [24]. In contrast, removal of CCR5 is safer and more reliable, because: 1) spontaneous homozygous CCR5(Δ32) mutation occurs in approximately 1% of Caucasians and rather than being affected with severe immune disorders, they are highly resistant to HIV-1 infection [25]; 2) "Berlin patient", who fully recovered from HIV after a successful transplantation of allogeneic stem cells homozygous for the CCR5 Δ32 allele, stopped ART for more than 20 months, suggesting the feasibility and practicality of CCR5 disruption [26]. To disrupt the expression of CCR5, there are two targets, mRNA and gene coding that. In gene editing, binding of respective engineered nuclease of different gene editing to specific genomic site, via designed DNA binding domain, triggers those "scissors" and DNA double stranded break (DSB) forms through which non-homologous end joining (NHEJ), one of major DSB repair pathways in mammalian cells, starts. The error-prone repair pathway is NHEJ-mediated inactivation, blocking the expression of CCR5 [15]. In contrast to gene editing, RNAi utilizes si/shRNA to hinder the translation of related mRNA and can be used to silence host factors without undesirable levels of off-target effects and toxicity [12].

Table 1. Different blocking sites in inhibition of HIV life cycle

Extrinsic inhibition of HIV life cycle	Normal life cycle of HIV	ART (mentioned above)	non-genetic interventions to clear HIV latency	Genetic therapeutic technology			
				Antisense therapy	RNAi therapy		Gene editing
				virus	virus	host cell	ZFN TALEN Cas9
The HIV-1 Entry and Fusion Inhibitors	gp120 binds with CD4						
	gp41 binds with CCR5 or CXCR4 HIV fuses with membrane of target cell	chemokine coreceptor antagonists; fusion inhibitors	inhibition of new transmission [16] [17] [18]			DRa [12]	DRa [14] [29]
The HIV-1 Integration Inhibitors	Reverse transcriptase trigger reverse transcription of ssRNA, and new RNA-DNA hybrids form	RT inhibitor					
	HIV dsDNA form followed by the RNA degradation by ribonuclease H						
	cDNA inserted into the host genome by integrase enzyme	integrase inhibitor					
Elimination of latent viral reservoir	Provirus in a latent state		reversal of latency. [16] [17] [18]				genetic interventions to clear HIV latency: provirus excision [20] [21]
The HIV-1 Assembly Inhibitors	Transcription of proviral DNA into mRNA or new copy of the RNA genome			ASRb [22] [23]	ASRb [11]		
	Translation of mRNA into precursors						
	Viral protease cleaves precursors into viral proteins	protease inhibitor and pharmacokinetic enhancers					
	Viral envelop forms and newborn virion releases						

[a] Downregulation of CCR5 expression

[b] Alternative splicing regulation

3.2 Intensification of adaptive immune response

Humoral immunity

There have been many vaccine trials failing to effectively protect naïve individual from the risk of HIV infection since the last century. Turning to passive immunization becomes another choice. Passive transfer of neutralizing antibody gains prominent achievements in macaques in the recent trispecific broadly neutralizing HIV antibodies, implying a promising future for passive immune protection [13]. The trispecific Abs superior characteristics of a combination of breadth and potency, compared with all possible combination of bnAbs, and obvious consequence of prophylaxis of non-human primates (NHP) in vivo protection, help eradicate and purge the HIV in the early stage. Its excellent efficacy also ameliorates the life-long ART and provides durable protective immunity in the state of drug-free remission. In addition, strategy of vector-mediated gene transfer, like AAV vectors for delivery of HIV bNAbs and antibody-like proteins, to generate long-term production of bNAbs in the absence of immunization is under study [27].

Cellular immunity

As mentioned above, CTL responses play important role to clear the latency of HIV and therefore, it's also critical in the progress of HIV. Herein, how to intensify the cellular immunity pave another road for us to treat HIV. In CAR-T therapy, alternative receptors, as chimeric antigen receptors in modified T cell, contain single-chain variable fragments(scFvs) derived from known broadly neutralizing antibody, which circumvents MHC recognition, increases the targeting efficiency, accelerates the process of activation and promotes more powerful cellular immunity. There has been confirmed that engineered T cell has the ability to recognize HIV-1-infected cells, suppress and kill viral replication [28]. The combination with CCR5 knock-out through insertion of CAR gene into a CCR5 locus shows more valuable and promising results in the inhibition of virus replication [14].

4. Conclusion

It's the first time ever that more than half of the HIV-infected population in the world, about 19.5 million people, are under antiretroviral treatment [30]. As the most therapeutic regimen, ART benefits millions of infected people and protect more susceptible individuals from HIV. Nonetheless, there is an urgent need for more potent treatments which will replace the position of ART and cure HIV in the end. To date, any regimen in the study or in trials has its inevitable disadvantages, leading to the restriction of clinical application. (See Table 2.) Inherent barrier of respective regimen, recombination between two RNA genomes of HIV and lack of repair during reverse transcription make single strategy nearly impossible to eradicate HIV in vivo. Consequently, like combinations in ART, combinations of targets in different strategies is vital to prevent viral escape and resistance [12], such as introduction of a designed CAR gene into a CCR5 locus [14] and reversal of latency combined with ART [16] or appropriate CTL response [17] [18]. Accordingly, rational designs, in which different strategies are administered in different orders, would make promising candidates for HIV eradication in the near future.

Table 2. Features of different targeted objects and technologies

Features / Objects/ Technology			Advantages	Disadvantages	Clinical application	
					HIV	Others
objects	host cell	hematopoietic stem cell (HSC)	Long-term efficacy in a degree of functional or even complete cure of HIV.	Impairment contributed to common pathway or unexpected disruption.	"Berlin patient" [26]	SCID-X1 [31] [32] [33]
		mature immune cell	Relative safety and easily controllable.	Finite effective period and repetitive treatment required.	N/Aa	ADA deficiency [8] Lung cancer [34]
		virus	Better specificity and less side effects.	Resistance contributed to frequent mutation.	ART [5]	Interferons
ART			Widespread use and reliable safety.	Side effects due to long-term administration.	-	-
Interventions to clear HIV latency			Longer drug-free remission; depletion and eradication of infection.	Possibility of exacerbation of host cell function and further HIV spread to uninfected CD4+ T cells; technically challenging to identify a cell harboring HIV persistently.	N/Aa	N/Aa
Genetic therapeutic technology	Antisense therapy		Specificity in targeted mRNA.	Structural delicacy and difficult delivery.	N/Aa	Familial hypercholesterolaemia(mipomersen) [38]
	RNAi therapy		Non-toxic in protection of uninfected cells.	Difficult delivery and continuous presence of siRNA is necessary.	N/Aa	Familial neurodegenerative and cardiac

					syndromes caused by mutations in transthyretin (TTR) [39]
	Genetic editing: ZFN TALEN Cas9	Permanent disruption of targeted genes in a single treatment; combination with other strategy through insertion of designed sequences.	Complex sequence design and vectors construction; high inclination for off-target effects and undesirable toxicity.	N/Aa	Lung cancer [34]
Immunotherapy	Trispecific Abs	Unparalleled characteristics in breadth and potency; decline in potential escape mutations.	Safety and efficacy are N/Aa in vivo [13].	N/Aa	Multiple Sclerosis [35]
	CAR-T	MHC independent and better targeting efficiency; rapid proliferation of modified T cells and elimination of targeted cells upon antigen contact.	Severe anaphylaxis reactions [36] and increases in infected cell numbers.	N/Aa	B-cell malignancies, such as acute lymphoblastic leukemia (ALL) [37]

a Not available

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