Chapter 8

Novel Prospective Treatment Options

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Additional information is available at the end of the chapter

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1. Introduction

The acquired immunodeficiency syndrome (AIDS) caused by the Human immunodeficiency virus (HIV) is by far the most feared disease by any person on the planet. The root cause of this fear stems from its relentless worldwide spread and the absence of an effective therapeutic / preventive modality. Since its discovery in the early 1980s, it has been over three decades of war between mankind and HIV. All attempts to overcome the virus have so far been unsuccessful due to the unique features of HIV such as, the abilty to undergo rapid genetic evolution and to establish latent reservoirs. The higly active anti-retroviral therapy (HAART) is currently the only available strategy to limit the disease progression. However, the principal flaw in HAART is that it fails to eliminate the infection and warrants life long therapy. Long term treatment with anti-retroviral drugs has the disadvantage of poor compliance due to adverse effects and also possesses the risk of selecting resistant mutants. These reasons justify the need to search for novel therapeutic options superior to conventional anti-viral therapy.

Success stories of the Mississippi baby and the Berlin patient have revolutionized the concepts of HIV treatment and offered hope that HIV is not invincible. The strategies that have been developed / being developed against HIV, either affect the virus directly at different phases of its natural course or enhance the immunity to clear the infection from the body. Novel natural and synthetic compounds, peptides, DNA and RNA manipulatory techniques are being explored for their ability to impede the virus. Immune based therapies to accentuate specific anti-HIV immunity have stemmed from the failures encountered with vaccines. Apart from this, various alternative therapies are also being sought by patients. This chapter elaborates the various novel options that are currently being developed for HIV treatment and discusses their potential uses and impediments.

2. Why should we look beyond the HAART?

The major problem faced with HAART is that it only controls the infection and never eliminates it. When used continually on a lifelong basis, the HAART provides an infected individual with a significant improvement of clinical condition, enhancement of the quality of life and drastic reduction of circulating viral load. Nevertheless, treatment cessation at any point results in a rebound viremia, stripping off all the benefits that the patient had enjoyed during therapy. This could be stated as the inherent flaw in HAART, which exerts its inhibitory effect only against the actively replicating viruses in circulation and has little or no effect in destroying the quiescent viruses hidden in the latent cellular and anatomical reservoirs. Due to several reasons, this proviral reservoir gets activated at a later date proceeding to active viral replication and viremia, which can occur unchecked upon discontinuation of HAART [1-4].

Another worrisome aspect of HIV is its ability to undergo rapid genetic evolution as a consequence of its voracious mutating capacity. For a virus with such a feature, lifelong therapy with anti-retroviral drugs can be deleterious by itself, as the constant drug pressure eventually selects the resistant mutant viral populations. Such mutant viruses, resistant to the currently used anti-retroviral drugs have already emerged and are being disseminated in various countries across the globe. There is a possibility that these strains may replace the drug susceptible ones and render the HAART inactive.

Lifelong HAART also faces the practical constraint of continuous patient adherence to the prescribed regimen and also its discontinuation due to adverse drug effects. Optimistic predictions of worldwide HIV control using HAART would be just a mirage if the impending failure of the HAART in the future is not foreseen from the present. In the light of these issues, any attempt to curtail the HIV pandemic warrants the need for novel anti-retroviral agents and/or strategies to supplement, if not to supplant the HAART [5].

3. Novel agents and strategies currently being tried against HIV

All of the therapeutic strategies which are either currently available or under experimental research, involve attacking HIV at any one of the different phases in its infection course. (Figure-1) All these strategies use one or more of the following principles including administration of active chemical compounds, nanotechnology, DNA manipulation, RNA based techniques and cellular transplants.

Apart from these therapeutic strategies which directly impede the viral activity, several immune based therapies are also being developed which intend to enhance the ability of the immune system to overcome HIV on its own. These strategies also act at one or more points in the natural course of HIV infection allowing the immune system to suppress and possibly eliminate HIV [6].

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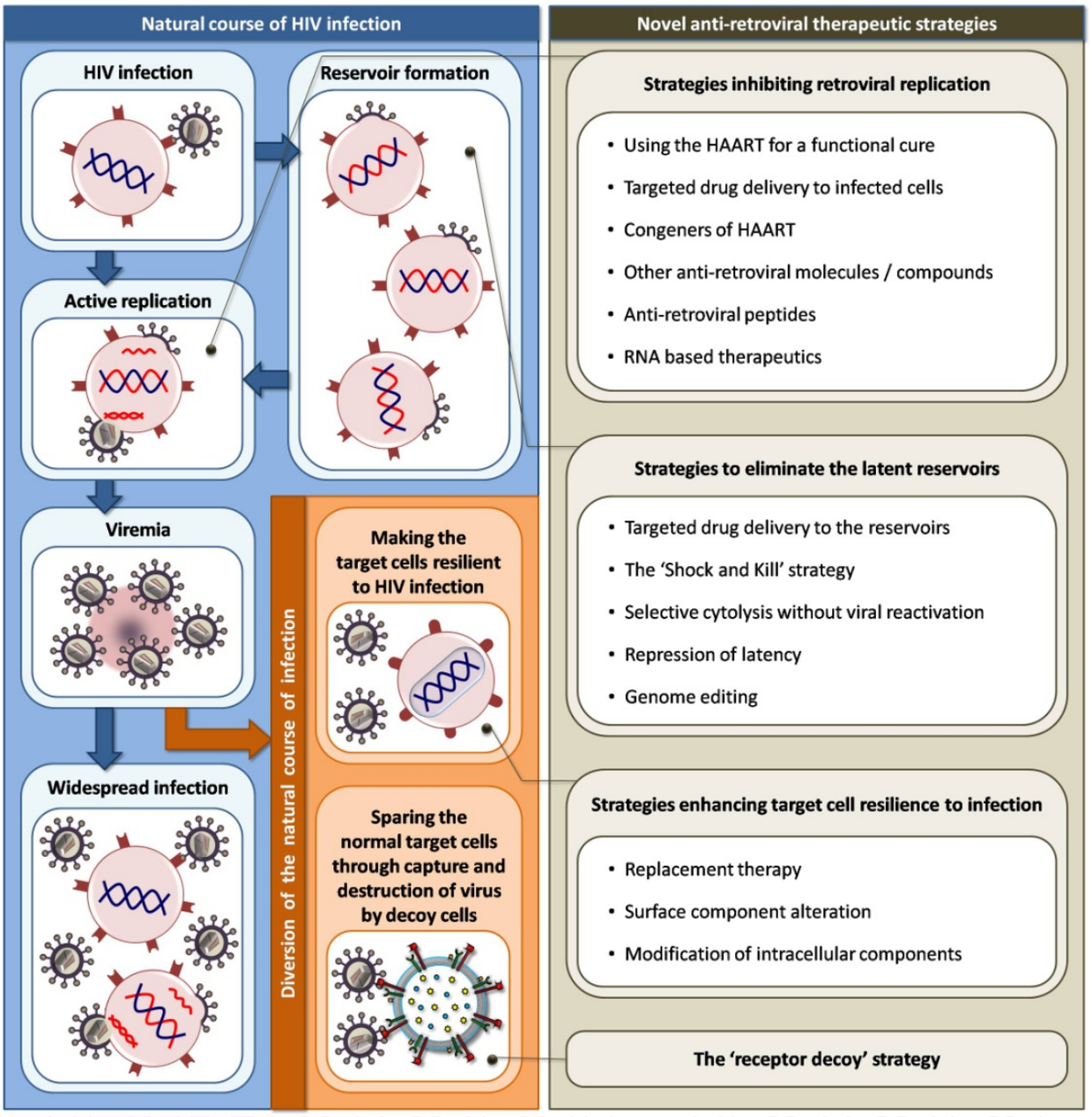

Figure 1. Natural course of HIV infection and the targeting points of therapeutic strategies

4. Agents and strategies inhibiting retroviral replication

4.1. Using the HAART for a functional cure

The HAART and the other anti-retroviral agents halt the natural course of HIV infection at the phase of active viral replication. This section elucidates the newer concepts that propose to maximize the utility of the existing anti-retroviral agents and also sheds light on novel compounds and targets which could be exploited for use against HIV.

The scope of the HAART to achieve a 'functional cure' is being studied extensively. Functional cure is said to be achieved when the infected individual treated aggressively with HAART early in the course of infection, does not develop rebound viremia on cessation of therapy for several months [7]. This concept had stemmed from the story of the Mississippi baby, where a mother seropositive for HIV-1 gave birth to an infant who was also found to be infected. Anti-retroviral therapy was initiated to the baby at 30 hours of birth and was continued till 18 months of age after which the therapy was discontinued. Surprisingly at 30 months of age, the child was tested negative for plasma HIV-1 RNA, proviral DNA in peripheral-blood mononuclear cells and serum HIV-1 antibodies [8].

In an attempt to replicate the observations of the Mississippi baby, the VISCONTI study conducted in France has effectively achieved a functional cure in a cohort of 14 adults who currently are free of viremia even after several years of interruption of anti-retroviral therapy [9]. Likewise, functional cure has been reported in an elderly German patient who had deliberately interrupted treatment after five years and has controlled rebound viremia for nine years after the cessation of treatment [10].

The reason hypothesized behind functional cure is that, early aggressive treatment prevents the build-up of large latent reservoirs and also reduces the viral load low enough that the immune system clears off the residual infection without continued use of antiretroviral drugs [11]. The drawback of this concept is that it necessitates the stoppage of the anti-retroviral drugs that have helped in the control of viremia. It is not known whether this might result in a functional cure or cause a rebound viremia. As it would be unethical to stop treatment in an individual with good viremic control without knowing the correlates of protection, well designed clinical trials must be conducted prior to implementation of this concept, to obtain answers to questions of uncertainty such as; what is the time period within which the treatment should be initiated following infection to favour functional cure? How long should the treatment be given? When is the ideal time for the interruption of treatment? What are the host factors involved in protection?

By the time the functional cure concept offered some hope, the occurrence of rebound viremia in the Mississippi baby smashed all the excitement [12]. It might only be a matter of time before we know whether the other patients claimed to be functionally cured, progress to viremia or remain "cured". Nevertheless, the Mississippi baby and the other studies have hinted that control of viremia is possible on treatment cessation. Further studies are required to find how to prolong this drug-free control of viremia.

4.2. Targeted drug delivery to infected cells

To achieve viremic control by anti-retroviral drugs, the patient should also face a plethora of untoward effects. Drug interactions, synergistic toxicity of combination, frequent dosing and pill burden are the various adverse effects of the HAART causing a poor compliance. Novel techniques based on nanotechnology are being developed to overcome the shortcomings of conventional anti-retroviral drug therapy.

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Targeted drug delivery is the technique that is extensively being applied to accentuate the beneficial effects and to simultaneously reduce the adverse effects of anti-retroviral drugs. In the course of infection, HIV gains entry into its target cells by membrane fusion, leaving behind its envelope and surface glycoproteins on the surface of the infected cells. During active replication of HIV within the target cells, the infected cells also express these viral glycoproteins synthesized de novo. Active targeting exploits these viral components on the cell surface to selectively identify the infected cells from the uninfected ones [13]. Active replication of HIV *in vivo*, from the point of infection of a CD4 T-cell to release of viral progeny takes an average of 52 hours for completion [14]. This long generation time of HIV could be effectively utilized to specifically deliver the anti-retroviral drugs to the infected cells by nanotechnology methods. This would prevent the dissemination of the viral progeny and reduce the viral load more effectively than systemically administered anti-retroviral drugs.

The nanoparticles used for targeted drug delivery contain a carrier vessel straddled with a targeting moiety. The carrier nanoparticles are tiny containers which could be loaded with the drug of interest and are capable of delivering their cargo into cells upon fusion. Various types of nanocarriers such as; liposomes, micelles, dendrimers, nanocapsules, nanoemulsions, solid lipid nanoparticles, polymeric nanoparticles, gold nanoparticles and nanocrystals have been successfully loaded with one or more known anti-retroviral agents. The active targeting moiety tagged to the surface of these carriers guides them specifically to the target cells. Recombinant CD4 molecules or Fab fragments of monoclonal anti-gp120 antibodies which possess high affinity to HIV envelope glycoproteins are used to actively target the envelope glycoproteins of HIV present on the surface of the infected CD4 cells [15].

Targeted drug delivery possesses significant advantages over conventional chemotherapy of which, the most important is the reduction of the adverse effect profile of the anti-retroviral agents. This is because, the technique concentrates the drug only to the necessary sites and hence not only it reduces the administered dose, but also avoids the accumulation of drug at unwanted sites. Besides this, some of the nanoparticles have inherent antiretroviral property and function synergistically when loaded. This promising technique however, is not devoid of pitfalls. Nanoparticles face the major problem of opsonization and phagocytic clearance which occurs almost rapidly following their administration. The process of pegylation which involves in coating of the nanoparticles with polyethylene glycol overcomes this disadvantage by making the nanoparticles invisible to immune clearance. However, the fusion kinetics of these 'stealth' nanoparticles is diminished when compared to the non-pegylated ones [16]. Nanocarriers packed with a single drug are more stable and addition of more drugs within the same nanocarrier makes it more unstable. As monotherapy can accelerate viral resistance, stable models of multidrug nanocarriers are now being developed [17]. Poor oral bioavailability, causation of target cell membrane instability and cytotoxicity, complications in renal clearance and high production cost are some of the problems that have to be solved before nanotherapeutics come into effective use [13].

4.3. Congeners of HAART

The currently available anti-retroviral drugs specifically inhibit a select few steps of HIV replication such as HIV entry and fusion, reverse transcription, protease action and integration. Apart from their beneficiary action, the anti-retroviral drugs possess significant adverse reactions which form the principal reason behind poor compliance and drug withdrawal. With the advent of pharamacological techniques, congeners of anti-retroviral drugs are being developed with the aim of minimizing the side effects.

The congeners are molecules of any particular drug class which are engineered to overcome the pitfalls faced by the existing members of the same drug class. They possess minor structural modifications that confer one or more favourable properties such as increased biovailability, increased target site binding affinity or longer half life. By virtue of these properties, the congeners can be effectively administered at reduced dose and interval and hence possess a low adverse effect profile. The congeners are subject to clinical trials and would probably fail or get pass the trial stages to get approved by the United Staes Food and Drug Administration (FDA) and relevant international bodies. Table-1 summarizes the FDA approved anti-retroviral dugs for use in the USA and their congeners in the pipeline (updated to October 2014) [18-21].

Stage of HIV replication inhibited	Anti-retroviral drug class	FDA approved agents	Congeners
Attachment to CCR5 co-receptor	Entry inhibitors	Maraviroc	Cenicriviroc Vicriviroc Adaptavir INCB-9471 PRO-140
Fusion of envelope with cell membrane	Fusion inhibitors	Enfuvirtide	Albuvirtide
Reverse transcription	Nucleoside / Nucleotide Reverse Transcriptase Inhibitors (NRTIs)	Zidovudine Lamivudine Stavudine Didanosine Emtricitabine Abacavir Tenofovir disoproxil fumarate	Tenofovir alafenamide Hexadecycloxy propyl tenofovir Amdoxovir Racivir Festinavir Elvucitabine Dexelvucitabine
	Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)	Nevirapine Efavirenz Delaviridine Etravirine Rilpivirine	Doravirine

Stage of HIV replication inhibited	Anti-retroviral drug class	FDA approved agents	Congeners
Proteolytic cleavage of precursor polyprotein into functional proteins	Protease inhibitors	Ritonavir Nelfinavir Indinavir Saquinavir Atazanavir Foamprinavir Tipranavir Darunavir	-
Integration of viral DNA to the host cell genome	Integrase inhibitors	Raltegravir Dolutegravir	Elvitegravir Cabotegravir

Table 1. Anti-retroviral drugs approved by the FDA and their congeners under consideration

4.4. Reducing the pill burden

One of the practical difficulties faced by the multi-drug HAART regimen is the pill burden. It is glaringly obvious and even scientifically proven that, therapeutic regimens with lesser number of pills have a better patient adherence [22]. In this context, pills with a fixed dose combination of more than one anti-retroviral agent are being developed and few have also been approved by the FDA. Table-2 summarizes the list of combinations approved by the FDA as of October 2014 [18].

Name of the pill	Drugs contained
Epzicom	Abacavir and lamivudine
Combivir	Zidovudine and lamivudine
Truvada	Emtricitabine and tenofovir disoproxil fumarate
Triumec	Abacavir, dolutegravir and lamivudine
Trizivir	Abacavir, zidovudine and lamivudine
Atripla	Efavirenz, emtricitabine and tenofovir disoproxil fumarate
Complera	Emtricitabine, rilpivirine and tenofovir disoproxil fumarate
Striblid	Elvitegravir, cobicistat, emtricitabine and tenofovir disoproxil fumarate

Table 2. Fixed dose combinations of anti-retroviral drugs approved by the FDA

Designing a single drug molecule containing the active moieties of two different anti-retroviral compounds is yet another strategy to reduce the pill burden. These molecules termed 'port-manteau inhibitors' can exert their anti-retroviral action at two different steps of HIV replica-

tion and could thereby reduce an extra pill. Caffeoyl-anilide compounds are evaluated for their dual action in inhibiting HIV integrase and blocking the CCR5 receptor mediated entry [23].

4.5. Synergistic enhancers of anti-retroviral drug action

Certain compounds such as pharmacokinetic boosters and virostatics, are being evaluated for their role in potentiating the action of anti-retroviral drugs. The property of pharmacokinetic boosting is exclusively exploited for protease inhibitors which are metabolized and cleared from the body by the cytochrome P-450 CYP3A4 enzyme. The effect of protease inhibitors can be boosted if they are co-administered with compounds that inhibit this enzyme. The protease inhibitor ritonavir with the propensity to inhibit the cytochrome P-450 CYP3A4 enzyme, when co-administered with another protease inhibitor not only enhances its bioavailability, but also acts synergistically to minimize the dosage of both agents [24]. The combination of lopinavir boosted by ritonavir has been identified to provide maximum benefits with minimal adverse effects and hence is approved by the FDA and marketed as a fixed dose combination pill under the name, kaletra. Cobicistat is another unrelated compound which is a potent inhibitor of the cytochrome P-450 CYP3A4 enzyme without having any inherent anti-retroviral activity. As it is also capable of boosting the beneficial effects of integrase inhibitors, it is approved for use along with the fixed combination of elvitegravir, emtricitabine and tenofovir under the trade name, striblid [18, 25].

Virostatics is an abbreviation for the combination of antiviral (viro) and cytostatic drugs (static). This combination intends to reduce viremia by inhibiting HIV replication with anti-retroviral drug and simultaneously reducing the number of the viral target cells (normal CD4 T-cells), using the cytostatic drug. It has been observed that the antiviral drug didanosine functions synergistically with hydroxyurea, an inhibitor of cellular proliferation to achieve viral load reduction. As this strategy depends on hydroxyurea induced immunosuppression for reduction of viremia, more knowledge has to be gained on its practicality, with respect to didanosine resistant HIV mutants and flare-up of opportunistic infections [26, 27]. The anti-parasitic / anti-inflammatory drug leflunomide is also found to have similar synergistic effect to inhibit HIV replication when used along with nucleoside reverse transcriptase inhibitors [28, 29].

4.6. Novel targets and synthetic molecules to inhibit active retroviral replication

From binding to release, the replication of HIV involves several steps of which, only a few steps are targeted by the currently available anti-retroviral drugs and their congeners. As the various sub-cellular and molecular mechanisms of HIV replication are gradually being unraveled, novel compounds are also being identified to target and inhibit one or more steps of HIV replication. Most of these are small synthetic molecules identified based on computational analysis of their structure and ability to dock at the required site and further screened for functionality by *in vitro* bioassays. As the discovery of newer anti-retroviral compounds is an ongoing process, table-3 summarizes a non-exhaustive list of novel synthetic anti-retroviral compounds and the phase of HIV replication they inhibit.

Phase of HIV replication inhibited	Novel mechanism of action	Compound	Reference
Entry and fusion	Binding to HIV gp 120 and prevention of its attachment to CD4 receptor	Temsavir and fostemsavir	[30]
		BMS-488043	[31]
		NBD-556	[32]
		DCM-205	[33]
		Curdlan sulfate	[34]
	Attachment to the CXCR4 co-receptor and prevention of HIV envelope fusion to cell membrane	AMD-11070	[35]
		T-22	[36]
		KRH-1636	[37]
Uncoating	Binding to HIV capsid / cyclophilin-A and prevention of uncoating by stabilizing the capsid	Pyrrolopyrazolone derivatives	[38]
		Quinoxaline derivatives	[39]
	Destabilisation and dissolution of the HIV capsid after entry	PF-3450074 (PF74)	[40, 41]
		I-XW-053	[42]
Reverse transcription and DNA synthesis	Inhibition of reverse transcription by novel nucleoside analogues	Adenosine analogs	[43]
	Inhibition of HIV DNA synthesis and introduction of lethal mutations	Ribonucleoside analogs	[44]
		KP-1461	[45]
	Reversal of susceptibility in NRTI resistant mutants	Foscarnet	[46]
Transcription	Inhibition of viral protein (tat) or inhibition of the leader sequence of HIV mRNA (TAR) or inhibition of tat-TAR interaction to prevent the initiation of transcription	Quinolone derivatives (HM13N, NM13)	[47]
		Acetylpromazine derivatives	[48]
		N1-aryl-propane-1,3-diamine	[49]
	Inhibition of cellular factors responsible for the elongation of HIV mRNA	Flavopiridol derivatives	[50]
		Seliciclib	[51]
		Roscovitine	[52]
		Iron chelators	[53]
		Garcinol derivatives (LTK-14)	[54]
		Ribofuranoside and carbonitrile derivatives	[55]
		Miltefosine	[56]
	Inhibition of post transcriptional processing of HIV mRNA	Mycophenolic acid	[57]

Phase of HIV replication inhibited	Novel mechanism of action	Compound	Reference
Cytoplasmic export of HIV mRNA	Inhibition of host proteins involved in nuclear export of HIV mRNA	PKF050-638	[58]
Translation	Inhibition of cellular ATPases involved in the initiation of HIV mRNA translation	Rhodanine and triazine compounds	[59, 60]
Assembly and release	Binding to capsid protein and Inhibition of assembly	benzodiazepines and benzimidazoles	[61]
	Blocking the ion channel function of the HIV protein vpu	BIT225	[62]
Maturation of progeny after release	Preventing the release of capsid protein from its precursor protein in the progeny virions and thereby rendering them non-infectious	Bevirimat	[63]
Viral DNA integration	Inhibition of lens epithelium derived growth factor protein p75 (LEDGF/p75) which helps in viral DNA integration	2-(quinolin-3-yl) acetic acid derivatives	[21]
		Chicoric acid derivatives	[64]
Enhancing the anti-viral restriction factors of the host cell	Inhibition of HIV virion infectivity factor (vif) to indirectly improve the viral restriction by APOBEC proteins	RN-18	[65, 66]
		VEC-5	[67]
	Direct enhancement of APOBEC proteins	SN-2	[68]
Multiple steps	Inhibition of uncoating and assembly	Thiourea compounds	[69]
		Acylhydrazone derivatives	[70]
	Inhibition of viral entry and transcription	Aminoglcosides (Neomycin, netilmicin)	[47]
Direct destruction of free HIV virions	Cation mediated destruction of HIV envelope	Ceragenins (CSA-54)	[71]
Selective cytolysis of HIV infected cells	Inhibition of acute viral infection and activation of apoptotic pathways specifically in cells infected with HIV	Ciclopirox and deferiprone	[72]

Table 3. Novel synthetic anti-retroviral compounds and their mechanism of action

Although most of these compounds have shown potent *in vitro* anti-retroviral activity, their *in vivo* efficacy needs to be evaluated. A few of the listed drug candidates are undergoing clinical trials and are expected to be available for use in the near future. The concept of using antiviral compounds for treatment of HIV poses a significant drawback. Unlike other viruses,

HIV possesses an immense potential to undergo genetic variation and the anti-retroviral agents rapidly select out resistant mutants. This could be well explained with the experiences faced with the experimental uncoating inhibitor PF74 and the maturation inhibitor bevirimat. The action of both these drugs can be nullified by HIV with simple mutations causing amino acid substitutions and these resistant mutants are expected to emerge rapidly if these drugs get approved for widespread use [41, 73]. While comparing the length of time and amount of work required to approve new anti-retroviral molecules acting on viral components, it is almost an instantaneous process for HIV to develop resistance against the drugs. In this context, compounds that inhibit the genetically more stable host components to produce anti-retroviral effects such as the CCR5 / CXCR4 inhibitors hold promise, until the virus finds an alternative to evade their action.

4.7. Natural derivatives with anti-retroviral property

Apart from the multitude of synthetic anti-retroviral compounds that are being generated every day, many naturally occurring compounds have also been identified to inhibit HIV [74, 75]. Plants, marine organisms, arthropod venoms and bacteria form the principal sources of these compounds. Extracts from natural sources are tested *in vitro* for anti-retroviral activity and the most active component identified, purified and studied for possible mechanism of action. Interestingly, some of the natural compounds have been found to perform better than their synthetic counterparts [73]. Recently, virtual methods are being developed for screening natural compounds for their anti-retroviral property [76]. If the *in vitro* effects could be effectively achieved *in vivo*, it would not be surprising when some of these compounds soon make their way across clinical trials and be available for use in the near future. Table-4 summarizes some recent compounds of renewed interest among various naturally occurring anti-retroviral compounds already identified.

Source	Source organism	Compound	Mechanism of anti-retroviral action	Reference
Plants	*Camellia sinensis* (Green tea)	Epigallocatechin-3-gallate	Inhibits HIV transcription. Also degrades the semen-derived enhancer of virus infection and prevents mucosal transmission	[77, 78]
	Eucalyptus globoidea (Australian Eucalyptus tree)	Globoidnan A	Inhibits HIV integrase	[79]
	Betula pubescens (and several other plants)	Betulinic acid	Prevents the release of capsid protein from its precursor protein in the progeny virions and thereby renders them non-infectious	[73]
	Isatis indigotica (Chinese medicinal plant)	Indirubin-3'-monoxime	Inhibits host cellular machinery responsible for HIV transcription	[80]

Source	Source organism	Compound	Mechanism of anti-retroviral action	Reference
	Salvia miltiorrhiza (Chinese medicinal plant)	Tanshinone II A	Inhibits host cellular machinery responsible for HIV transcription	[81]
	Pelargonium sidoides (German medicinal plant)	Unidentified compounds	Inhibits HIV entry	[82]
	Grapes and berries	Resveratrol	Inhibits tat-induced HIV transactivation. Also possess cystostatic effects on CD4 T-cells similar to hydroxyl urea	[83]
	Grapefruit juice	Unidentified compounds	Neutralizes the CYP3A4 in the GI tract and increases the bioavailabity of anti-retrovirals	[84]
Marine species	*Griffithsia* species (Red algae)	Griffithsin / Grifonin-1	Binds to various HIV surface components including gp41 and gp120 and inhibits entry	[85, 86]
	Corticium simplex (Sponge)	didehydro-cortistatin A	Most potent inhibitor of tat-dependent HIV transcription identified till date	[47, 87]
	Fascaplysinopsis reticulata (Sponge)	Fascaplysin	Inhibits host cellular machinery responsible for HIV transcription. Also inhibits HIV reverse transcriptase	[88]
	Cassiopia *Andromeda* (Jelly fish)	Cnidarian proteins	Various anti-HIV activities including inhibition of HIV protease, inhibition of HIV entry and cytotoxicity of infected cells	[89, 90]
	Galaxura filamentosa (Red algae)			
	Litophyton arboreum (Soft coral)			
Bacteria	*Nostoc ellipsosporum* (Cyanobacterium)	Cyanovirin-N	Binds to various HIV surface components including gp41 and gp120 and inhibits entry	[91]
	Scytonema varium (Cyanobacterium)	Scytovirin		[92]
	Sorangium cellulosum (Myxobacterium)	Ratjadone-A	Inhibits host proteins involved in nuclear export of HIV mRNA	[93]
	Streptomyces species (Actinomycete)	Leptomycin-B	Inhibits the viral protein rev which helps in nuclear export of HIV mRNA	[94]
Arthropods	Bee venom	Melittin	Binds to various HIV surface components and disintegrates the envelope	[95]
	Scorpion venom	Kn2-7	Interacts with HIV envelope and inhibits entry	[96]

Table 4. Anti-retroviral compounds isolated from natural sources

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4.8. Anti-retroviral peptides

Eukaryotic organisms are known to secrete antimicrobial peptides as a part of their innate immunity, which have a broad spectrum of activity against various pathogenic microbes [97]. These peptides are usually short sequences of 12 - 20 amino acids, but some of them may also be around 40 amino acids long. Apart from the inherent anti-retroviral peptides produced by the human body, numerous studies have identified several peptides from natural and synthetic sources that are capable of inhibiting HIV replication [98]. With the FDA approval of the fusion inhibitor peptide enfuvirtide, coupled with the growing problem of resistance to anti-retroviral drugs, much interest is being shown in the development of novel anti-retroviral peptides. Currently, there are nearly a thousand HIV inhibiting peptides identified and new members are being added on a daily basis [99].

The anti-retroviral peptides identified till date are predominantly from natural sources ranging from bacteria to plants to primates. Also a significant number of these peptides are derivatives of HIV itself, which structurally mimic the viral substrates and competitively inhibit the various replicatory processes. On the other hand, synthetic production using techniques such as phage display, offer the scope of combination of related or unrelated peptides to achieve maximal anti-retroviral effect. Most of the anti-retroviral peptides inhibit one or more of the phases of HIV replication. Majority of the peptides act extracellularly to inhibit HIV attachment or its fusion while others inhibit the intracellular phases of replication such as reverse transcription, transcription and integration. Apart from the direct administration of peptides for therapy, these agents could also be administered in their complementary DNA form which on recombination with the host cell genome and subsequent expression, makes the target cell resilient to HIV infection (subsequently discussed). Certain peptides possess the unique property of cell penetration and hence being evaluated for their possible role in targeted nanotechnological and cellular delivery techniques [99, 100].

The advantages of therapeutic peptides include their high specificity for the site of action, rapid break down into harmless amino acids which are eliminated easily and hence possess less toxicity and adverse effect profile [98]. However, this rapid breakdown of peptides by the peptidases could also be a drawback since they could be cleared off before they exert their action. Currently, synthetic peptides incorporated with D-isomers of amino acids (instead of the naturally occurring L-isomers) or with added non-peptide moieties, overcome this drawback and possess extended half life [101]. Peptides are highly antigenic and elicit the production of antibodies. Hence the extracellular acting peptides face the problem of antibody mediated clearance. Although the intracellular acting peptides are devoid of the antibody problem, availability of efficient delivery systems into the cell and degradation by the intracellular peptidases are the challenges they face. Poor oral bioavailability, inability to cross biological barriers and high production cost are further problems to be addressed prior to approval for therapeutic use [100, 102]. The FDA however, has approved enfuvirtide, this approval has offered hope for the success of other anti-retroviral peptides currently undergoing clinical trials [103]. With advancements in nanotechnology and cellular delivery systems, peptide therapy might become a reality for HIV infection in due course of time.

4.9. RNA based therapeutics

RNA based therapeutic strategies exert their action between the phases of transcription and translation. Numerous RNA based techniques have been developed which are classified by their mechanism of action. They include; inhibitors of messenger RNA (mRNA) translation (antisense oligonucleotides), the agents of RNA interference (RNAi), catalytically active RNA molecules (ribozymes) and RNAs that bind proteins and other molecular ligands (aptamers) [104, 105] (Table-5). These techniques can be utilized for the treatment of any viral disease by engineering specific, complementary inhibitory RNA particles to the viral transcription components. Among these techniques, RNAi and to a lesser extent antisense oligonucleotides, have been tried out to inhibit retroviral replication. As these techniques can also target the host cellular processes, they are being exploited in strategies to increase the resilience of target cells to HIV infection, which is discussed later [106].

RNAi is an endogenous mechanism which involves the down regulation of mRNA activity during transcription and post transcription phases using short double stranded RNA (dsRNA) called micro RNA (miRNA), which are about 20-30bp long. The identification of this regulatory process has provoked the interest of controlling unwanted viral replication using exogenously administered specific sequences of short dsRNA. The exogenously administered agents of RNAi therapy include small interfering RNA (siRNA) and short hairpin RNA (shRNA). These agents act on the post transcript mRNA and either cause direct sequence specific cleavage when there is a perfect sequence complementarity match, or lead to translational repression and degradation of mRNA when the interfering RNA sequence is of limited complimentarity to the targeted mRNA. As the siRNA get cleared off after their action, their effects are only transient and need repeated administration similar to agents of chemotherapy. On the other hand the action of shRNA is similar to gene therapy, as they get expressed on promoters and cause long term effects. Various viral and non-viral delivery mechanisms and active targeting strategies have been developed to deliver these active agents into the target cells [107, 108].

Systems employing siRNA and shRNA to target the gene products of tat, rev, nef, env, vif and pol have been designed and evaluated for efficacy [47, 106]. However, the use of this technology against viral replicatory processess is threatened by the genetic variation exhibited by HIV. Very simple mutations allow HIV to escape from the action of both siRNA and shRNA [109, 110]. Four possible solutions are being tried to tackle the problem of these escape mutants. The first attempt is to expand the RNAi technique to simulataneously inhibit multiple HIV mRNA targets similar to the concept of multidrug use. Recent studies have demonstrated that concurrent inhibition of HIV mRNA with three different shRNAs can prevent viral escape *in vitro* [111, 112]. In the second possible solution, inhibitory RNAs with a complete match to the most commonly encountered viral escape sequences are being designed. When used along with the inhibitory RNA of the wild type virus, these could prevent a majority of the mutants from escape [113]. The third solution involves identifying novel, genetically conserved sequences of HIV which do not usually undergo mutation. Targeting these stable sites would favour the success of RNAi [114]. Finally, RNAi techniques are also being designed to restrict the 'genetically more stable' host factors that help in HIV replication (discussed later under strategies to enhance target cell resilience to HIV infection).

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Apart from the disadvantages posed by the virus per se, RNAi technology has numerous setbacks. The short lived action of siRNA warrants its repetitive administration thereby compounding the treatment cost. The overexpression of shRNA can result in cell death and hence needs strict dose titration. RNA is immunogenic and is rapidly neutralized by antibodies in circulation, hence effective vectors are needed for the *in vivo* administration of RNA. Even after successful intracellular delivery, the administered RNA moieties are easy targets for degradation by cytoplasmic ribonucleases. Engineered RNA with modifications in sugar moieties, nucleotides or their backbone are found to possess improved cytoplasmic stability and are being evaluated for their superiority and efficacy [115].

Due to the numerous setbacks, almost all RNAi techniques that exclusively inhibit the viral targets have stagnated at the level of pre-clinical testing and none have entered into clinical trials. A recent technique which involves the simultaneous use of three shRNAs to specifically inhibit three corresponding targets of HIV, has been found to be safe and effective and is hopeful to enter phase I trials in the near future [111].

5. Strategies to eliminate latent reservoirs

The ability to integrate its nucleic acids with host cell genome and co-exist in a genetically quiescent proviral state is one of the distinct features of HIV, which makes eradication of infection next to impossible. The latent reservoirs of HIV are categorized into two broad groups namely the cellular and the anatomical reservoir. The cellular reservoir comprises the long lived resting CD4 T-cells. With an extended lifespan averaging 400 days, these cells bear the provirus and release the progeny upon activation at a later time [116]. Infection of the cells of the monocyte-macrophage lineage and subsequent compartmentalization of these infected cells into various organs / tissues such as the reticulo-endothelial system, lymph nodes, gastro-intestinal tract, brain and lungs, lead to the formation of anatomical reservoirs. Within the macrophages of these anatomical reservoirs, the HIV either remains quiescent inside the chronically infected cells or maintains a continuous low level replication [117]. Thus, the reservoirs act as Trojan horses spilling the progeny virions into circulation at periodic intervals. Although the mechanisms involved in proviral repression are gradually being unraveled, specific factors / agents reactivating the viral replication are yet to be clearly identified. Although early aggressive treatment helps in achieving a functional cure by limiting the reservoir formation, it does not offer a solution to the already established reservoirs. The new strategies and techniques that are being developed for eradicating the established viral reservoirs are subsequently discussed.

5.1. Targeted drug delivery to the reservoirs

Apart from attempts to limit viral replication in actively infected CD4 T-cells, nanotechnology methods are widely studied for the elimination of the latent viral reservoirs. Strategies include passive and active targeting and the use of surface moieties which enhance the penetration of biological barriers by the nanoparticles.

Upon systemic administration, most of the nanoparticles are instantaneously opsonized with plasma proteins and rapidly cleared off from circulation by the phagocytosis. The macrophages of the reticulo-endothelial system are the principal cells which are involved in the degradation and clearance of the nanoparticles [118]. This forms the basis of passive targeting which aims at achieving high concentrations of anti-retroviral agents in the reticulo-endothelial reservoir as a consequence of phagocytosis of the drug loaded nanoparticles. Studies have demonstrated the achievement of higher drug concentrations in the reticulo-endothelial cells following the administration of liposomes, nanocapsules or polymeric nanoparticles loaded with anti-retroviral drugs [119]. Likewise, passive targeting of the lymphatic reservoir can be achieved by incorporation of the nanoparticles with lipids such as phosphatidylcholine and cholesterol or by surface coating of the nanocarriers with polyethylene glycol [120, 121].

Apart from passive targeting, specific drug delivery to the anatomical reservoirs can be accomplished by active targeting strategies. Nanocarriers tagged on their surface with galactose or mannose residues or anti-HLA-DR monoclonal antibodies, effectively localize in the reticulo-endothelial system which have abundant receptors for these ligands such as the galactose and lectin receptors and the HLA-DR determinant of MHC-II respectively. Active targeting of the cellular reservoirs is based on the aforementioned principle of employing nanocarriers with homing ligands to viral components on infected cell surface. The infected resting CD4 T-cells containing HIV envelope glycoproteins on their surface attract the drug loaded nanoparticles coated with recombinant CD4 molecules or Fab fragments of monoclonal anti-gp120 antibodies [13].

Nanotechnology also offers a solution for the problem of poor drug penetration in certain anatomical sites. One such anatomical reservoir is the brain, where the infected microglial cells rest safely with the protection conferred by the blood brain barrier against the systemically administered anti-retroviral agents. The blood brain barrier functions not only by preventing the permeation of the circulating anti-retroviral compounds into the brain tissue, but also by efflux of a considerable portion of the compounds that have managed to cross through. With the ability to increase the crossing and reduce the efflux of drugs, nanotechnology methods help in reservoir elimination by overcoming the privilege offered by the blood brain barrier [122].

Nanotechnology methods that increase the drug transport across the blood brain barrier function by mimicry of natural substrates, utilization of cell penetrating peptides or by active targeting of molecules of abundance in neuronal vasculature with suitable surface ligands. The non-ionic surfactant 'polysorbate-80' has found to be an effective enhancer of drug delivery to the brain using the principle of substrate mimicry. Upon systemic administration, the drug loaded nanoparticles coated with polysorbate-80 adsorb various apolipoproteins in circulation to form a complex that mimics lipoproteins. Presuming them as natural substrates, the blood brain barrier permits the entry of these complexes by receptor mediated transcytosis resulting in delivery of the drug to the brain tissue [123]. Nanoparticles coated with cell penetrating peptides such as the HIV-1 tat peptide has shown enhanced efficacy in crossing the blood brain barrier. As the microvasculature of the brain is rich in receptors such as transferring receptor,

low-density lipoprotein receptor and β2 receptors, their respective ligands such as transferrin, apolipoprotein-E and β2 agonists are considered possible candidates for active targeting.

The use of nanotechnology for reservoir elimination poses some peculiar problems in addition to other drawbacks mentioned earlier. The effectiveness of nanotechniques that rely on antiretroviral drugs to eliminate the reservoir is questionable as these agents are lethal to the virus only during active replication and do not affect the inactive provirus. The final clearance of the nanoparticles from the anatomical sites, especially from the brain is not clearly known and accumulation overtime with repeated doses can possibly lead to neurotoxicity. Acquisition of more knowledge through carefully designed clinical trials is needed so that the potential of this technology could be put into adequate use [122].

5.2. The 'shock and kill' strategy

Considered as the 'holy grail' of HIV eradication research, this strategy aims at depletion of the latent reservoirs by widespread activation of the provirus using 'shocking agents' and subsequent killing of the actively replicating virions. Reactivation of the quiescent viral genes can be achieved either by inactivating the repressing factors and/or by activating the transcription factors [13].

Deacetylation and methylation of histones and DNA methylation are the few processes that have been identified to favour the proviral repression and hence the inhibitors of these processes are considered as attractive candidates for shocking agents. Inhibitors of the histone deacetylase group of enzymes; valproate and vorinostat have been tried out for viral reactivation with limited success [124]. Congeners of vorinostat such as givinostat, belinostat, panobinostat and droxinostat and other novel histone deacetylase inhibiting compounds such as oxamflatin, romidepsin are being evaluated for their efficacy in reactivating viral replication. Histone methyltransferase inhibitor chaetocin and DNA methyltransferase inhibitors azacytidine and decitabine are also being assessed for their efficacy as shocking agents [125].

Provirus could also be drawn into active replication by making the intracellular milleu conducive for transcription [126, 127]. This could be achieved by activating the transcription factors such as the NF-κB and transcription elongation factor- b. Initial studies that employed NF-κB activators such as interleukin-2 and monoclonal anti-CD3 antibodies failed to relieve the provirus from repression. This led to the identification of a gatekeeper kinase which has to be concurrently activated along with NF-κB to facilitate viral replication. Other compounds such as prostratin and bryostatin-1 are also being evaluated for efficacy in the activation of NF-κB pathways [128]. Activators of elongation factor-b such as hexamethylene bisacetamide, disulfiram and certain quinolones are being evaluated as shocking agents. Apart from these, interleukin-7, agonists of Toll like receptor-9 and novel syenthetic molecules have been identified to activate viral replication in the latent reservoirs [125, 129]. All of these transcription activators are being assessed for stand-alone use or for use in combination with inhibitors of proviral repression.

None of the agents of this attractive strategy has provided satisfactory results so far. The dosing and combinations have to be worked out to extract maximum effects out of these shocking

agents. As most of the shocking agents are related to anti-cancer drugs, they are likely to have a significant adverse effect profile. The activators of the NF-κB pathway are notorious for triggering a plethora of cytokines and can cause a fatal cytokine storm if administered at higher concentrations. Another important drawback is that, even if an efficient shocking agent is identified, killing of the actively replicating virions depends on anti-retroviral drugs which are expected to lose their efficacy in the future due to the evolution and dissemination of resistant viral population. Nevertheless, this strategy has immense potential in eradicating viral reservoirs if the various setbacks are addressed over time [130].

Apart from the trial of different compounds, techniques of gene therapy have also been tried out to activate viral reservoirs with limited success. Herpes virus and lentiviral vectors loaded with genes coding for key viral proteins involved in replication were found to induce replication and release of the provirus from the latent CD4 T-cells [131]. Problems with *in vivo* administration, lack of specificity in targeting the cellular reservoir, low frequency of recombination are the various setbacks which have withheld the progress of this technology in reservoir elimination [132].

5.3. Selective cytolysis without viral reactivation

Another interesting finding pertaining to reservoir eradication is the property of the gold complex drug 'auranofin' to selectively destroy the retroviral cellular reservoir. Although developed and used as an anti-rheumatic agent, the unique 'anti-memory T-cell effect' of auranofin has kindled interest in its possible role against HIV. Auranofin exerts its cytocidal action by inducing intracellular oxidative stress. The memory T-cells with low antioxidant defenses are highly vulnerable to the oxidative stress induced by auranofin and perish along with the integrated provirus. Combination of auranofin and buthionine sulfoximine, an inhibitor of glutathione synthesis is found to act synergistically by causing further imbalance in the redox pathways [133].

Auranofin has shown promising results in studies utilizing the simian AIDS model. Simian immunodeficiency virus (SIV) infected macaques, treated with a combination regimen of auranofin, buthionine and HAART have not only shown a prolonged post treatment drug free control of viremia but also developed enhanced cell mediated immunity with SIV specific cytotoxic CD8 T-cells following treatment suspension [134, 135]. However, well designed human clinical trials are required to know more about this anti-HIV reservoir compound before its flamboyance could be translated to practicality.

5.4. Repression of latency

With the mechanism exactly opposite to the shock and kill strategy, this strategy aims at achieving viremic control by keeping the viral reservoirs continually in the inactive state. As mentioned under the shock and kill strategy, concurrent activation of the gatekeeper kinase and NF-κB favours reactivation of the provirus. The compound Jun N-terminal protein kinase inhibitor-5 which is a potent inhibitor of the gatekeeper kinase strongly prevents viral reactivation even upon strong stimulation of the NF-κB pathway under *in vitro* conditions. Inhibitors

of the NF-κB pathway such as aloisine A and roscovitine also inhibit HIV reactivation to a lesser extent [128]. Various other molecules such as C-terminal truncated STAT5, Staf 50, prothymosin α, thioredoxin reductase, glucosamine and OKT-18 zinc finger protein have been identified to prolong the proviral repression. As the HIV protein 'Tat' activates proviral transcription after getting itself activated by acetylation, molecules that could specifically inhibit host cell acetylases which, eventually subdues the activity of the Tat peptide, can serve as attractive candidates for continuing viral repression [136]. Also, molecules that enhance the activity of the histone deacetylase if identified, could serve as suitable agents for this strategy [137].

5.5. Genome editing

Genome editing techniques confer the possibility of excising out specific genetic sequences from the whole genome. This novel concept is currently being used for research involving in eukaryotic genome manipulation to produce gene knockout animals. The ability of this technology to specifically excise the integrated provirus from the genome of the latent reservoir cells is being evaluated.

Three different genome editing systems are currently available such as the zinc finger nuclease (ZFN) system, the transcription activator like-effector nucleases (TALEN) system and the clustered regularly interspaced short palindromic repeat (CRISPR) with CRISPR-associated protein-9 (Cas9) known as the CRISPR/Cas9 system (Table-5). Of the three systems, the ZFN and the CRISPR/Cas9 system are being tried in the genome editing of the latent reservoir cells. *In vitro* studies employing the CRISPR/Cas9 system have demonstrated the ability of the technique to remove the HIV internal genes and suppress proviral reactivation in T cells [138]. Alternatively, studies employing the ZFN system have demonstrated the ability of the technique to excise the full length HIV proviral DNA from the infected human T cell genome [139].

This advanced technology also has its own drawbacks. The efficacy of genome editing strategies observed with different *in vitro* studies is about 30% though research work is still ongoing, addressing the challenges and improving on the efficacy. Suitable transport systems are yet to be developed for *in vivo* delivery of the editing machinery to the viral reservoir cells. All genetic editing systems have a certain proportion of 'off-target activity' where they excise out genes unrelated to the targeted site. As this can result in undesired gene modification events, the genome editing systems must be made more specific before its utilization for anti-HIV treatment [140].

6. Strategies enhancing target cell resilience to HIV infection

6.1. Replacement therapy

The discovery of HIV cure by replacement therapy happened by chance from the observations made on Timothy Brown, widely referred as the 'Berlin patient'. The patient was seropositive for HIV and had undergone an allogenic bone marrow transplant on developing acute myelogenous leukemia. Incidentally, the transplanted donor stem cells carried a homozygous

CCR5Δ32 deletion and produced T-cells with a truncated chemokine receptor CCR5 conferring resistance to infection with CCR5 utilizing virus. The patient had discontinued HAART after the transplant yet has had no detectable viremia for over five years [141]. The experiences with the Berlin patient, introduced the concept of 'sterilizing cure' which essentially comprises of eradicating all replication competent viruses from the body including the ones inside the latent reservoirs.

Replacement with resilient target cells alone does not suffice to achieve a sterilizing cure. As seen with the Berlin patient, the replacement therapy must be preceded by eradication of latent reservoirs. This has been effectively achieved in the Berlin patient by the myelo-ablative procedures prior to bone marrow transplant and continued by the graft versus host disease which occurred following the transplant [142].

Attempts to replicate the cure of the Berlin patient have not been successful so far. A similar treatment provided for two adults in Boston resulted in a brief period of aviremic state, subsequently followed by rebound viremia in both the patients. A few reasons have been postulated for the failure of sterilizing cure in the Boston patients. The first reason being, the initial myelo-ablative procedure was milder than that which was given to the Berlin patient and hence would have not effectively destroyed the viral reservoirs. Also, the donor cells transplanted to the Boston patients carried a heterozygous CCRΔ32 deletion which might allow HIV infection, when compared to the more resilient homozygous mutant cells transplanted to the Berlin patient [143].

Although well substantiated, the concept of sterilizing cure still has many lacunae. The exact correlates of protection involved in pre-transplant reservoir ablation, establishment of a graft versus host disease and homozygous versus heterozygous CCR5Δ32 deletion are yet to be identified. It has been observed that CCR5Δ32 deletion is associated with increased susceptibility to infections with West Nile virus. There may be other serious adverse effects which are to be identified before using defective CCR5 as replacement therapy for HIV infection. Another thought provoking issue is the ability of defective CCR5 in protecting infections caused by CXCR4 tropic viruses [144].

Apart from the above mentioned hurdles, the major setback which could limit the reality of this strategy is the availability of matched donors with the required mutation. Autologous bone marrow transplant has been considered to overcome these stringent requirements of allogenic transplants. Transplants of uninfected haematopoietic stem cells harvested earlier from the same person and made resilient to HIV infection by *in vitro* genetic modification (discussed below) do not confer as much protection as their allogenic counterparts. The poor performance of autologous transplants is attributed to the lack of the 'allo-effect' in clearing the infected reservoirs by establishing a graft versus host disease [145]. Hence it has to be borne in mind that replacement with resilient target cells is not a stand-alone strategy and must be compulsorily preceded by reservoir eradication procedures, in order to achieve an effective sterilizing cure.

6.2. DNA manipulation

Techniques of DNA manipulation have immense potential and offer a wide scope in tackling HIV. Apart from their use in peptide delivery and in the shock and kill and the genome editing strategies for reservoir elimination, the different techniques of genetic manipulation can be exploited to confer resilience to the natural target cells against HIV infection. DNA manipulatory techniques can confer CD4 T-cells with resilience to HIV infection by either modification of the natural cellular components which are utilized by HIV or by the administration of engineered genetic material which get expressed to produce HIV inhibitory peptides. The techniques utilized for this purpose include gene therapy and genome editing.

Although the term 'gene therapy' is interchangeably used to denote all the DNA and RNA based techniques, this much earlier developed technique actually involves the delivery of specific genetic elements to the target cells by a suitable vector. This is followed by homologous recombination of the transferred genetic element with the recipient cell genome and its eventual participation in transcription and translation to express the desired phenotype. However attractive it may be, the success of this technique depends on the effective recombination of the foreign gene with the target cell genome. Low frequency of recombination is the principal drawback faced with this technology [146].

The more recent gene editing techniques as mentioned earlier, comprises excising specific portions of genetic material from the target cell genome. The principle of this technology makes it a good strategy for reservoir elimination as it can cut off the unwanted proviral genes without any subsequent untoward effect. Although when used for host cell genetic manipulation, the success of these techniques relies on the identification of suitable sub-cellular targets, which on manipulation cause significant impairment of HIV replication without affecting the normal cellular function. These novel techniques are found to have a higher success rate than other dated techniques of gene therapy [140].

Though the functional mechanism differ in the techniques, they still have several processes in common. Firstly, both techniques require an effective carrier-delivery system called vectors which can deposit the genetic elements / editing machinery specifically to the cells that are to be modified. Various viral vectors utilizing adenovirus, baculovirus, canary pox virus or lentivrus have been developed for this purpose. *In vivo* use of viral vectors face the problem of antibody mediated clearance. To overcome this challenge, non viral vectors based on nanoparticles like dendrimers are being developed. Cell electroporation is yet another technique that has been developed for the *in vitro* delivery of nucleic acids into target cells [147]. The second common feature is that, both the techniques can be developed for either *in vitro* or *in vivo* use. The *in vitro* methods involve in harvesting of the cells of interest, genetic modification in laboratory conditions followed by transfusion of the modified cells to the recipient, while the *in vivo* methods rely on vector mediated delivery by active targeting of the cells of interest. Another feature of the genetic manipulation procedures is that they could be performed on either mature cells or stem cells. Modification of mature CD4 T-cells confers protection only during their life span and warrant the need for repetitive transfusions over time. On the other hand, genetically modified stem cells such as the CD34+ haematopoietic

stem cells can be effective with a single transplantation as exemplified with replacement therapy [148].

Both techniques attempt to make the target cells resilient to HIV infection by either modifying the cell surface components required for HIV entry or by altering the intracellular contents which are utilized by the virus during replication, or a combination of both. Strategies involved in limiting viral entry by surface component modification possess certain remarkable advantages. It has been detected that among all HIV related cell death events, over 95% are caused by apoptosis initiated by the cells immediately after viral entry [149]. Hence, the target cells can be saved from committing 'suicide' if they are made impervious to viral entry.

With the serendipitous discovery of its curative effects in the Berlin patient, CCR5 which acts as the co-receptor for HIV entry is the most sought-after target for genetic modification. A homozygous deletion of a specific 32 base pair sequence from the CCR5 gene confers complete protection, while a heterozygous deletion of the same nucleic acids confers partial protection from HIV entry, without causing any glaring change in the CD4 T-cell function. All the three systems of gene editing namely the ZFN, the TALEN and the CRISPR/Cas9 system are being evaluated for this purpose and are found to have nearly 50% efficacy in disrupting CCR5 in mature CD4 T-cells and around 25% efficacy in adult haematopoietic stem cells. In this regard, it is intriguing to use induced pluripotent stem (iPS) cells to introduce delta32-like mutation and test their viral resistance. *In vitro* studies of CXCR4 disruption has also shown promising results but this might not serve the purpose *in vivo*, as the deletion is expected to cause functional derangement.[140]. Yet another instance of gene therapy under human trials is the SB-728-T, a zinc finger DNA-binding transcription factor. It binds to the DNA of target cells and disrupts the gene responsible for CCR5 co-receptor production [150]. Apart from the ones mentioned here, there are numerous other techniques of DNA manipulation, RNA based and peptide based techniques being tried to harness the potential of CCR5 alteration in curtailing HIV infection (Table 5).

The target cells can also be made resistant to HIV infection by increasing the expression of intracellular factors that restrict the viral replication process. TRIM5α, APOBEC3G and tetherin are the well known restriction factors of HIV infection. TRIM5α is a cytoplasmic protein which inhibits HIV by binding with the incoming capsid and preventing further the process of replication. The APOBEC3 family of mRNA editing proteins, especially the APOBEC3G inhibits HIV replication by introducing lethal mutations during reverse transcription. Tetherin is another host cellular protein involved in HIV restriction by preventing the release of viral progeny from the infected cell.[151] As the vif and vpu proteins of the virus neutralize the effect of APOBEC3G and tetherin respectively, they can be made resistant to their respective viral proteins by introducing point mutations. Single amino acid substitution, D128K in APOBEC3G and T45I in tetherin makes them overcome the viral factors vif and vpu respectively. Gene therapy techniques to increase the expression of TRIM5α and mutated APOBEC3G and tetherin in CD4 T-cells are known to enhance their resilience to HIV infection. Mov 10 and CPSF6 are the newer restriction factors that are being considered for development in this strategy [106, 152]. Recently, the interferon inducible family of proteins called the myxovirus resistance proteins (Mxs) have been identified as potent restriction factors which

act by inhibiting HIV uncoating [153]. Also, TSG-101 the intracellular protein derivative of tumor susceptibility gene has been identified to inhibit the HIV protein p6 thereby blocking viral budding and release [154]. These proteins could possibly serve as candidates for anti-retroviral gene therapy.

Administration and expression of extraneous genetic material can also confer resistance to the target cells against HIV. Much of recent interest is towards the surface modification using the synthetic peptide 'C46'. When expressed on the CD4 T-cell surface, this peptide binds with gp41 of the approaching virions and prevents envelope fusion. Stable expression of C46 can be achieved on CD4 T-cells following delivery of the corresponding gene using retroviral vectors. As the cells made resilient by CCR5 alteration alone remain vulnerable to CXCR4 tropic viruses and the reverse also holds good, C46 can be effectively used to inhibit infection with both of the viral strains [152]. Extraneously administered genetic material coding for dominant negative inhibitory proteins of HIV replication such as the M-10 and those coding for intrabodies and intrakines can also cause favourable intracellular modifications in the T-cells making them resistant to HIV infection [148].

Newer studies advocate the combination of both surface and intracellular modifications of the target cells to obtain improved resilience against HIV [147]. Apart from their role in enhancing resilience to HIV infection, techniques of genetic manipulation are also useful in conferring resistance to viral integration and thereby restricting the reservoir formation [155]. Although the techniques of genetic manipulation has numerous setbacks; poor frequency of recombination faced with gene therapy, unwanted off-target effects and double strand break induced apoptosis occurring with gene editing are the principal challenges that have to be overcome. Nevertheless, few of these techniques have entered into clinical trials and give hope for a promising future [152]. Apart from their use in therapeutic strategies, techniques of gene therapy are being evaluated in DNA vaccines for prophylactic use and also in some of the strategies of 'immune therapy' [156].

6.3. RNA based mechanisms

Studies employing RNA based therapeutics such as antisense oligonucleotides, RNAi, ribozymes and aptamers have shown to be effective in down-regulation of CCR5 and subsequent inhibition of HIV replication in humanized mice [106, 157]. Of all the above mentioned technqiues, silencing of CCR5 using RNAi is the most widely studied. RNAi techniques have also been studied for the downregulation of other cellular factors of HIV replication such as CXCR4, CD4, NF-κB, LEDGF/p75 and DDX-3 [158].

RNAi techniques for host cell modulation are devoid of the problem of viral escape mutants that is faced, when the same technique is employed for inhibiting viral targets. However, the adverse effects faced with these techniqiues are due to loss of other essential functions of the target cell as a consequence of cellular factor silencing. Increased susceptibility to West Nile virus infections has been observed with the loss of CCR5 function. CXCR4 downregulation can disrupt the homeostasis of the lymphopoietic pathway as these receptors are essential for the homing of stem cells into bone marrow and their subsequent differentiation into T-cells [159].

Hence, the success of this strategy depends on the identification of suitable cellular co-factors of HIV infection which can be down regulated without altering the normal cellular physiology. In the search for such targets, genome wide profiling studies have revealed numerous previously unknown cellular factors that participate in HIV infection. Subsequent knock down studies have validated the effectiveness of silencing these factors for limiting HIV replication [160-162]. Further studies are needed to reveal the adverse effects following the knock down of these novel cellular factors.

The technique related pitfalls are the same as the ones mentioned earlier, which include the problems faced with delivery of inhibitory RNAs into specific target cells, off-target effects of the inhibitory RNAs and cytotoxicity to the administered cell. As of date, only one RNAi technique has entered clinical trial. The technique was designed to inhibit both viral (tat protein and TAR RNA) and cellular factors (CCR5). However, the trial was terminated in phase-0 and the results were not disclosed [163]. Table-5 broadly summarizes all the nucleic acid based therapeutics that are currently being tried against HIV.

Type	Cells targeted	Phase acted on	Mechanism		Technique employed
DNA based techniques	Infected reservoirs (In vivo)	Elimination of latent reservoirs	Direct excision of integrated proviral DNA		Genome editing systems - CRISPR/Cas9, ZFN and TALEN
			Activation of viral replication (Shock and kill)		Gene therapy - Vectors based delivery of genes coding for viral proteins of replication
	Uninfected susceptible cells (In vivo / In vitro)	Diversion of natural course of infection to make the target cells resilient	Modification of normal cellular components	Cell surface modifications	Genome editing systems - CRISPR/Cas9, ZFN and TALEN to knock out cellular co-factors that support HIV replication
				Intracellular modifications	
			Expression of novel protective proteins	Cell surface expression	Gene therapy - Vector based delivery of cDNA of novel peptides followed by their recombination and expression
				Intracellular expression	
RNA based techniques	Actively infected cells (In vivo)	Inhibition of active viral replication	Inhibition of viral mRNA		Agents of RNA interference (siRNA and shRNA) and to a lesser extent other RNA based techniques (Antisense oligonucleotides, ribozymes and aptamers) delivered in viral / non-viral vectors to the desired target cells by active targeting
			Inhibition of host cellular factors involved in viral replication		
	Uninfected susceptible cells (*In vivo* / *In vitro*)	Diversion of natural course of infection to make the target cells resilient	Modification of normal cellular components	Cell surface modifications	
				Intracellular modifications	

Table 5. Nucleic acid based therapeutics for HIV infection

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7. The 'receptor decoy' strategy

The receptor decoy strategy is a novel concept which helps to salvage the natural target cells of HIV by diverting the virus to infect decoy particles, thereby altering the natural course of HIV infection. This involves the usage of decoy cells termed 'cancellers', which possess HIV entry receptors on their surface and do not contain the machinery required for retro-viral replication. The cancellers (Figure-2) intend to function as decoys and get infected by the free HIV virions thereby preventing the infection of the natural target cells. Apart from protection of the natural target cells, the cancellers also serve to limit viremia as the trapped virions cannot replicate inside the cancellers due to the absence of replication machinery. Optionally, the trapped virions could be destroyed by packing anti-viral agents within the cancellers [164].

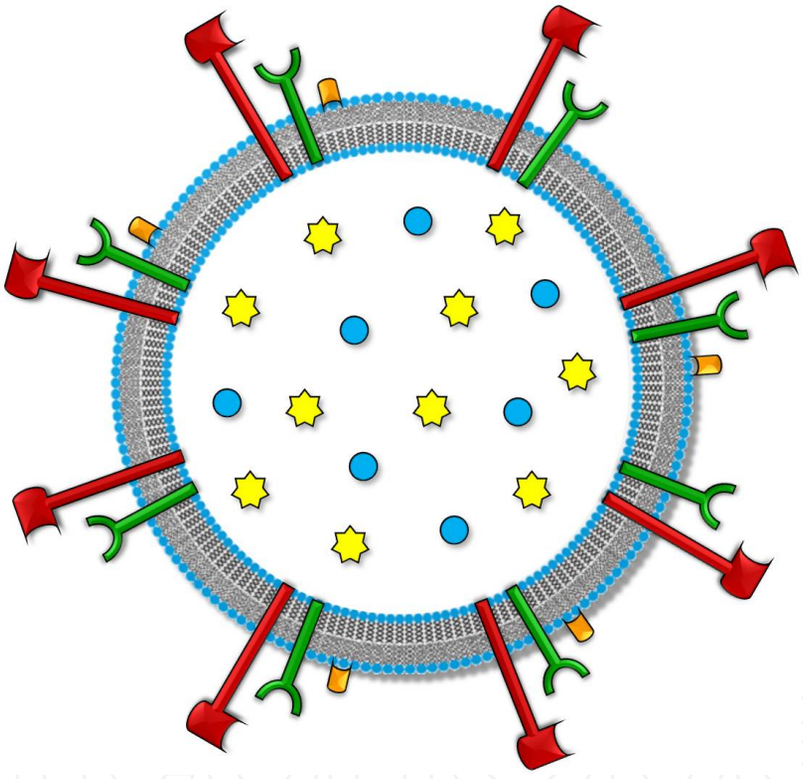

Figure 2. Schematic representation of a canceller particle. RBC particle reconstituted with receptors on the membrane surface and loaded with various molecules. CD4 (red) and CCR5 (green) receptors are involved in the active targeting of HIV virions. Accessory surface molecules (orange) may be added to enhance the fusion process and/or to prevent elimination of the canceller by reticulo-endothelial system. Molecules capable of viral destruction (blue spheres and yellow stars) such as protein and RNA damaging agents are packed within the canceller.

The superiority of the receptor decoy strategy over the conventional anti-retroviral drugs is that it mimics natural conditions and is thereby devoid of the 'adverse factor' effect in selecting out resistance. This attractive strategy is in the concept phase and more discussions and comments could be made only after its implementation. Similar strategies employing modified erythrocytes or nanoparticles as viral traps are being studied for their use against HIV [164].

8. Immune based therapies

Since the discovery of HIV repeated attempts to achieve effective protection using conventional methods of immunoprophylaxis have resulted in outright failure. The failure of the preventive strategies has lead to the concept of 'immunotherapy' or 'immune based therapy'. This strategy involves in the manipulation of the immune system in a therapeutic motive to eliminate the already acquired infection, rather than preventing new infection. The immune based therapies involve in enhancing the potency of the immune system to counteract HIV by reducing inflammation, preventing immune activation by HIV-1 or promoting effective immune responses against HIV. The different modalities used to achieve this effect can be broadly classified into non-HIV antigen specific therapies and those which use the HIV antigens (Table-6)[6, 165].

None of the immune based therapies have provided satisfactory results so far. Among the available methods of immunotherapy, HIV specific CTL induction using the dendritic cells is of recent interest and appears to be a promising strategy. Recent studies reveal that a therapeutic vaccine using autologous monocyte-derived dendritic cells pulsed with heat-inactivated whole HIV, stimulated anti-HIV immune response and shifted the virus/host balance in favor of the host when administered to patients on HAART with CD4+ T-cell count >450 cells/mm. At week 12 after ART interruption, 55% of people in the vaccinated group represented decrease of their viral load by at least 10-fold or 90%, compared with just 9% in the control group receiving non-pulsed DCs. These proportions dropped to 35% and 0%, respectively at week 24. This significant decrease in plasma viral load observed in vaccinated recipients was associated with a consistent increase in HIV-1-specific T cell responses. These data indicate that HIV-1-specific immune responses are elicited by the therapeutic DC vaccination. This could significantly reduce plasma viremia after ART interruption in HIV patients chronically infected but controlled with sufficiently high CD4 numbers. Thus, this study warrants further investigation with new candidates and/or new optimized strategies of vaccination toward the final goal to achieve a functional cure of HIV infection. Although heat-inactivated whole HIV was used as the antigen in this strategy, direct expression of the mRNA derived from patient's cells can also be considered using DCs in the immune therapy which expects highly precise antiviral efficacy targeting not only HIV but also non-viral antigens [166].

9. Complementary and alternative therapies

It is a common feature that people living with HIV or AIDS across the world resort to products and practices that are not presently considered to be part of conventional anti-retroviral medicine. When these are used together with conventional anti-retroviral medications, they are referred to as complementary therapies and if used as a stand-alone modality instead of conventional medications, they are referred to as alternative therapies [167]. A plethora of complementary and alternative strategies are available from all over the world which predominantly involves the use of natural products and/or mind and body practices. Herbal

Types	Components	Examples	Mechanism
Non-antigen specific therapies	Cytokines	Interleukin-2 (Proleukin)	Augmentation of CD4 T-cell proliferation and cytolytic function of CD8 T-cells
		Interleukin-7	Improvement of T-cell homeostasis
		Interleukin-12	Enhancement of Th1 response and increasing the cytotoxic activity of T-cells and NK cells against virally infected cells
		Interleukin-15	Expansion of effector and memory subsets of CD* T-cells
		Interleukin-21	Proliferation and enhancement of the cytolytic potential of effector CD8 T-cells
	Drugs	Chloroquine	Reduction of IFN-α in order to reduce the immune activation to prevent depletion of CD4 T-cells and progression to AIDS
		Hydroxy chloroquine	
		Aspirin	Reduction of T-cell activation due to its broad anti-inflammatory property
		Celecoxib	Inhibition of cyclo-oxygenase type-2 enzyme to reduce T-cell activation
	Antibodies	Anti-CD4 (Ibalizumab)	Competitive inhibition of viral entry receptors
		Anti-CCR5	Competitive inhibition of viral entry receptors
		Anti-PD-1	Blockage of the negative co-stimulatory molecules PD-1 and CLTA4 on the surface of T-cells to prevent CD8 T-cell dysfunction and enhancement of CD4 T-cell proliferation
		Anti-CLTA4	
	Prebiotics and probiotics		Modulation of the gut microbiome and amelioration of HIV induced mucosal damage
Antigen specific therapies	Vaccines	Inactivated whole virus	gp120 depleted, inactivated HIV strain with incomplete Freund's adjuvant
		Antigenic sub-units of HIV	Recombinant immunodominant proteins of HIV with suitable adjuvants
		Viral vectors expressing HIV antigens	Various HIV specific antigens expressed on different viral vectors
		DNA	Plasmids containing one or more genetic determinants coding for HIV proteins
		Dendritic cell	Delivery of viral antigens to dendritic cells to ensure activation of both CD4 and CD8 T-cells

Table 6. Immune based therapies being developed against HIV

remedies derived from the ancient medical science forms of different countries are the most widely sought after modality of complementary or alternative therapies. The various mind and body techniques include spirituality, meditation, yoga and other body manipulatory procedures, acupuncture and energy therapies [168].

Observations across the globe reveal that 30 - 90% of the HIV infected patients seek for complementary or alternative therapies of which, majority are females and educated individuals [169, 170]. The usefulness of these therapies is controversial. Spiritual methods such as prayer, faith healing and meditation are found to improve the psychological state by helping to overcome anxiety, depression and stress thereby providing a feeling of well being [171]. The compound IGM-1, obtained from herbs used in traditional medicine was observed to alleviate the symptoms of HIV infection but did not have any effect in reduction of viremia or improving the immune status. Many of the other Chinese herbal medicines tested were found to be unsatisfactory in altering the viral and immune parameters [172]. To much despair, several studies have highlighted the deleterious effects of complementary and alternative medicines. Recent reports indicate that patients on concurrent complementary therapy have reduced adherence rate to conventional anti-retroviral therapeutic regimens [173]. Homeopathy, a traditional health system has been proven ineffective for the treatment of HIV infection and has been disregarded by the WHO [174]. Herbal preparations containing St. John's Wort and those containing garlic extracts reduce the therapeutic levels of conventional anti-retrovirals. Apart from reducing the efficacy, many other herbal preparations have also been observed to increase the HAART related side effects [169].

Despite these assumptions, complementary and alternative therapies cannot be totally overlooked. This is due to the fact that natural compounds with antiretroviral property such as indirubin monoxime and tanshinone II A have been isolated from herbal medications [80, 81]. Meta analyses on the efficacy of herbal preparations have yielded only inconclusive results but not ineffective [172, 175]. Hence rigorous clinical trials including large study population are required to refute or accept the potential benefits of these therapeutic modalities. National Centre for Complementary and Alternative medicine, a division of the National Institute of Health, USA is an organization dedicated for research in alternative and complementary medicines and thus provides funding to various studies in this field [167].

10. Conclusions

The HAART has played its crucial role in curtailing the HIV pandemic so far. However, its inability to eliminate the infection and the rise of resistant mutants is a fundamental challenge. This has initiated the frantic search for novel compounds and strategies to counteract HIV. Desperate measures have resulted in the discovery of a plethora of anti-retroviral compounds and strategies, but none could be unanimously agreed upon as completely reliable. The much hyped functional cure concept has taken a big blow with the occurrence of rebound viremia in the Mississippi baby. Moreso, studies to replicate the sterilizing cure of the Berlin patient have provided disappointing results. Despite the development of various novel antiviral

compounds, HIV is well set before-hand to readily evolve and overcome all anti-viral actions. Novel techniques such as gene therapy, gene editing, RNA interference, anti-retroviral peptides may have sound concepts and appear attractive but in reality they face numerous roadblocks. By virtue of the ability to undergo rapid genetic evolution and the tendency to establish latent reservoirs, HIV has thwarted all the overcoming attempts. Currently, efforts are being stepped-up by scientist globally to overcome the challenges discussed herein, though the strategy(ies) may face limitations today but they still have the potential to achieve the required success of sustained functional cure.

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