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Antiretroviral Drugs Development; Past, Present and Future

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Authors' contributions

This work was carried out in collaboration among all authors. All authors were equally contributed to design, write and revise the manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Thirty years after the discovery of human immunodeficiency virus (HIV), more than 29 antiretroviral have been introduced. HIV at present can be managed though; it comes with consequences such as toxicity due to long term use of antiretroviral, development of resistance by HIV-1 strains and other viral or bacterial infections associated with it. Issues such as latency, socio-economic problem in the developing world has been of considerable concern. The benefits of highly active antiretroviral therapy (HAART) in the developed countries far outweighed those in the underdeveloped nations. HIV belongs to the genus Lentivirus and family *Retroviridae*, possess a diploid RNA and a cone shaped capsid core particles. The virus consists of major and minor structural and nonstructural proteins that perform different roles in the virus life cycle. In this review we seek to give a comprehensive account of the past, present and future directions in the development of antiretroviral drug. There are five classes of antiretroviral inhibitors which target HIV-1's reverse transcriptase, protease, integrase, envelope fusion and co-receptor binding thereby disrupting virus replicative cycle. Strategies have emerged on how to better manage HIV

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patients such as simplification of drugs, complete HAART withdrawal, use of microbicides, targeted PrEP (pre-exposure prophylaxis), and vaccine development. There are several host (example; CRIM-1) proteins and virus (example; Rev and Tat) proteins that remain unexplored and could serve as potential druggable targets, thus the need for further research in this direction.

Keywords: HIV; antiretrovirals; present and future.

1. INTRODUCTION

Antiretroviral drug development for the treatment of human immunodeficiency virus/acquired immunodeficiency syndrome infections has witnessed significant progress in the past two decades. The collective efforts by major players in antiretroviral development research coupled with a speedy regulatory approval of new drugs has enhanced the expansion from two antiretroviral drug that targeted two mechanisms in the viral life cycle and had been introduced before 1996, to drugs that have the potential to target seven different mechanisms in the viral life cycle [47-49].

In 2010, an estimated 34 million (between 32 million and 35 million) people have been infected with HIV. An estimated newly infected people in the same year stand at 2.7millions, which is much significantly less (21%) when compared to the yearly incidence cases for new infection at the zenith of the epidemic in 1997. The number of people dying as a result of AIDS or AIDS related causes has dropped from 2.2 millions in the mid-2000s to 1.8 millions in 2010 (www.worldbank.org). Two and a half decade after the discovery of the first antiretroviral drug, AZT (zidovudine) which was first described in 1985 as an antiviral agent inhibiting the infectivity and cytopathic effect of HTLV-III [(human T-lymphotropic virus type III)/LAV (lymphadenopathy-associated virus)] which were original names for HIV] *in vitro* [1-4]. More than 25 single antiretroviral drugs have been introduced, and mechanism of action classified into seven (7) mechanistic classes and can be used alone or in multiple combinations towards suppressing viral load and eventually eradicating the virus. These mechanistic classes are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) [7] non-nucleoside reverse transcriptase inhibitors (NNRTIs) [8-10], protease inhibitors (PIs) [11-14], entry/fusion inhibitors (FIs), CCR5 antagonists [17-20] integrase inhibitors [21-23] and maturation inhibitors (although drug development investigation had been dropped) [24].

The presently available treatment options for HIV/AIDS have immensly benefited people living with AIDS in the developing/developed world. Given the challenge, the eradication of the infection remains a long term prospect. The issue of socio-economic factors, especially in underdeveloped countries, needs to be addressed, and complications associated with the disease, long term toxicity effects, more so, there must be a shift in focus in developing strategies for combating these challenges. There is a need for intense efforts in implementing the extensive use of preventive measures as these will drastically reduce the rate of transmission in the population. In the area of research, focus must be on exploring new targets either in the human host or virus to abate the resistance experienced currently with existing drugs and ultimately coming up with drugs that will be capable to cure HIV/AIDS infections.

The increasing use of therapeutics drugs, emergence of resistance to some existing antiretroviral along with the potential long term toxicity of these agents has raised question on the cure for HIV, and this has led to a major paradigm shift on how treatment in the future should be handled and ways of tackling present limitations along with strategies on future direction.

In this review, we seek to detail, the past, present events in antiretroviral drug development and also what are expected to be/new directions of exploration in the future that will revolve around the ultimate goal of finding a cure for HIV/AIDS infection.

1.1 Overview of HIV Genome Organization and Structural Biology

The HIV is a member of the Lentivirus genus in the family *Retroviridae* that possess diploid RNA and exhibit a cone-shaped capsid core particle. Other Simian immunodeficiency virus (SIV), Feline immunodeficiency virus (FIV), HIV-type 2 which are all closely related to human immunodeficiency virus type 1(HIV-1). Dealing with the menace of HIV will require strategies that will yield desired results as per the

prevention of the spread of the infections. This calls for a broad and extensive understanding of the mechanisms involved in virus evasion of host immune system, virus genomic organization and its structural biology. The anticipation of antiretroviral drug development in the future lies on this tenet.

1.1.1 Genome organization of HIV

The HIV genome is a homodimer of linear, positive sense, single-stranded RNA, the genomic organization is depicted in Fig. 1. The HIV-1 virion is functionally diploid. The interaction between the two 5' ends of RNA in a self-complementary region known as dimer linkage structure (DLS) (see Fig. 2) maintains this dimer. The RNA is capped at the 5' end, using the common m7G5'ppp5'GMP structure (see Fig. 3); and contains a poly (A) sequence (see Fig. 2) which is about 200 nucleotide long, at the 3' end. The HIV genome codes for *gag*, *pol* and *env* structural genes which are required for production of infectious virions [23] (see Fig. 1). There are several additional and overlapping open reading frames (ORF) of unknown functions at the present, and non-structural Vpu [24-26] and Nef.

During replicative life cycle, three primary HIV-1 translation products are initially synthesized as polyprotein precursors, which encode structural proteins or enzymes. These synthesized polyprotein precursors are subsequently processed within the pre-assembled virus particles. The Gag precursor $Pr55^{Gag}$ (see Fig. 1) is cleaved into the matrix (MA), capsid (CA), nucleocapsid (NC) and p6 proteins (see Fig. 4) during release of progeny virions [25]. Synthesis of protease (PR) results from autocatalytic cleavage of 160kd Gag-Pol polyprotein, $Pr160^{Gag\text{-}Pol}$ (see Fig. 1), also synthesis of the following protein, heterodimeric RT and integrase (IN) result from autocatalytic cleavage by PR of $Pr160^{Gag\text{-}Pol}$. Proteolysis by cellular enzyme(s) converts the glycosylated *env* precursor gp 160, into the gp120 surface (SU) and g41 transmembrane (TM) proteins (see Fig. 4) The six HIV-1-encoded non-structural proteins (Vif, Vpr, Tat, Rev, Vpu and Nef) (see Fig. 1) are the primary translation products of spliced mRNA [27-34].

HIV-1 has multiple sequence elements within its genomic RNA that direct the balanced and coordinated production of progeny virus. Although many of these cis-acting RNA elements (see Fig. 3) are present in other retroviral genomes, few of them are unique to the primate Lentiviruses such as Simian Immunodeficiency virus (SIV) [35]. The 5' termini untranslated region of HIV-1 genomic RNA is highly structured and contains multiple elements that mediate transcriptional elongation, splicing, genomic RNA dimerization, and packaging of full length viral RNA and reverse transcription. The primer binding site (Pbs) stem (see Fig. 3) in HIV-1 genome is encompassed by three regions which participate in the placement and stabilization of the transfer RNA $(tRNA)^{Lys3}$ primer. The tRNA is incorporated into virus particles and is required for reverse transcription initiation. The nucleocapsid domain of the Gag polyprotein, acting as a molecular chaperone, helps in the unwinding of both the tRNA primer and Pbs stem and assists in the incorporation of primer into nascent virions [36]. This domain also helps in directing the packaging of HIV-1 genomic RNA (gRNA) into the virion. This role depends on its interaction with four RNA stem-loops (Ψ-region, Fig. 2) [38,39].

The genome dimerization initiation sequence (DIS) is located at the crown of SL1 and contains a complementary and exposed sequence (GCGCGC) (see Fig. 2) [40-42], and the process is initiated via a kissing loop mechanism involving base-pairing between the palindromic GCGCGC sequences on each HIV-1 genome [43]. The stabilization of the RNA dimer in a more extended duplex structure maintained by other regions of SL1 [43,44]. The Ψ packaging domain also contains the major splice donor (MSD) (see Fig. 2) that have been demonstrated to suppressed polyadenylation activities [45-47] and the AUG Gag start codon (see Fig. 2).

The AAUAAA binding site for the cleavage and polyadenylation factor (CPSF) and the GU/U-rich binding sites for the 3' terminal cleavage stimulation factor (CSF) of HIV-1 are situated within the repeat (R) (see Fig. 3) region at each end of the viral genomic RNA [48]. It has been reported to switch conformation from a multiple hairpin to a rod like structure which in turns promotes either packaging or translation of the viral RNA [49], respectively. This switch occurs during RNA synthesis, but after RNA synthesis, the entire HIV-1 sequence converts to a stable structural rod form that contains an expose AUG Gag start codon and the occluded DIS motif, which promote the translation of virus mRNA into viral-encoded proteins. Subsequent switch to an alternative form usually exposes the DIS and Ψ elements, favouring dimerization, packaging and amplification of viral RNA [50-52].

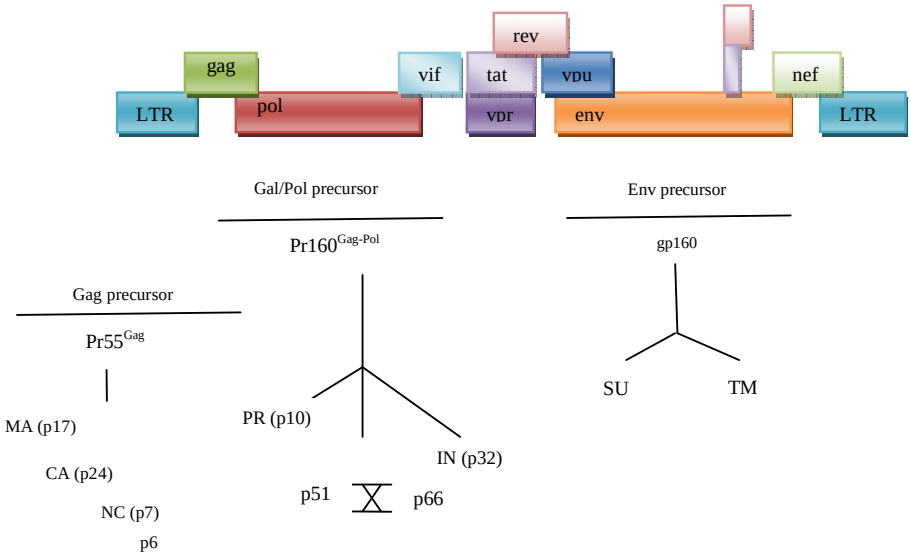

Fig. 1. Diagrammatic presentation of HIV-1 genomic organization with their encoded proteins and relative position of genes. The resulting polyprotein are shown with protease processing into mature virus proteins. Adapted from [21]

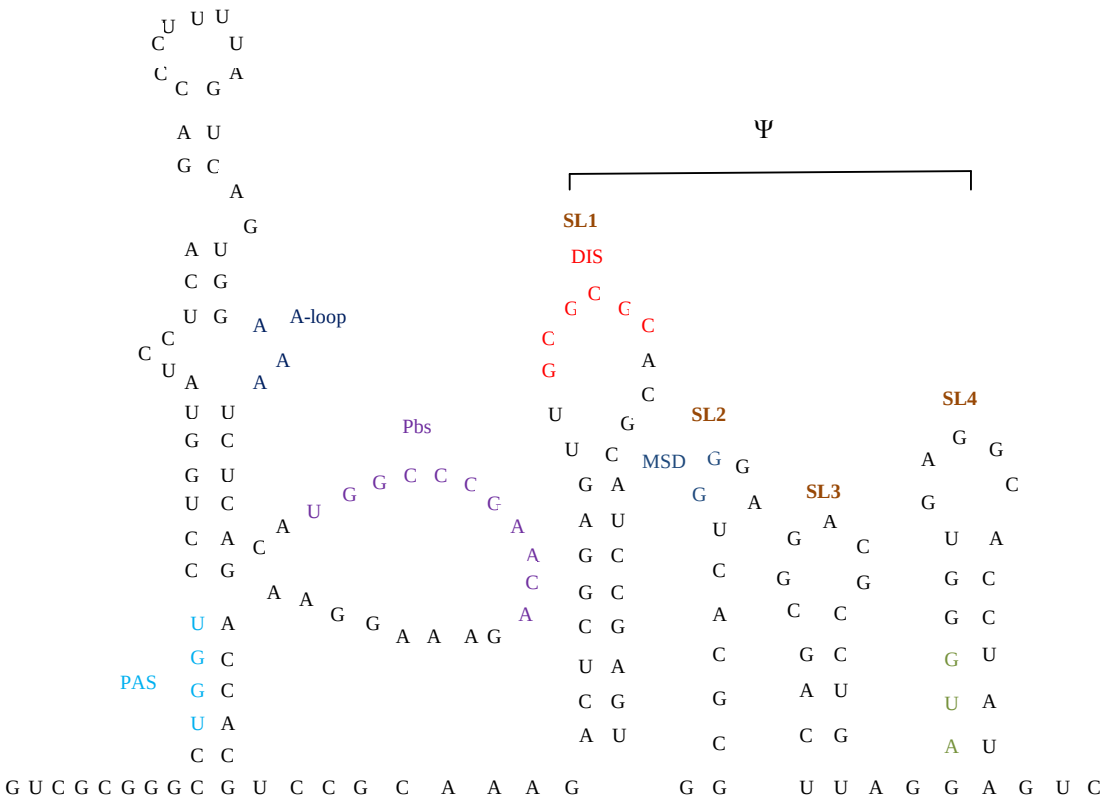

Fig. 2. Diagrammatic presentation of the secondary structure of HIV's mRNA at 5' end showing the RNA packaging site(Ψ), major splice donor(MSD), SL1;2;3;4, A-loop, PAS, DIS and Pbs. Modified from [28]

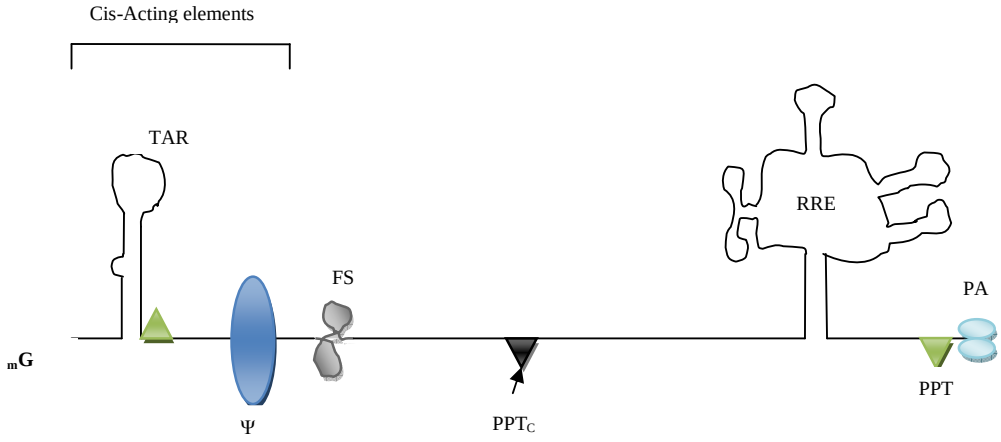

Fig. 3. Schematic presentation of the Cis-acting element of HIV-1 genome showing the Tat-responsive stem-bulge-loop structure (TAR), frame shifting motif (FS), central polypurine tract (PPT_C), Rev-responsive element (RRE), canonical 3' polypurine tract (PPT), polyadenylation signal (PA) and the methyl-capped terminal G residue at the 5' end($_mG$). Adapted from [31]

The synthesis of (+)-strand viral DNA is associated with a unique central polypurine tract (PPT_C) (a distinctive feature for the Lentivirus family that distinguish them from other retroviruses) along with canonical U3-proximal PPT (see Fig. 3). Viral gRNA possesses also heptameric UUUUUUA slippery sequence within the *gag* gene were ribosomal frame shifting (FS) (see Fig. 3) occur. Two virus RNA motifs also fold into the complex structures; transactivation response region (TAR) (see Fig. 3) and Rev-responsive element (RRE) (see Fig. 3) which are involved in RNA synthesis and transport, respectively.

There are multiple inhibiting sequences scattered throughout the *gag*, *pol* and *env* genes which are associated with the instability or nuclear retention of virus transcripts [53].

1.1.2 Structural biology of HIV

HIV, when assembled in infected cells is released in the form of immature particles that contain unprocessed Gag and Gag-Pol precursor polyproteins which eventually make up the mature virus. In the immature state, virion is spherical, with a characteristics electron-lucent centre. Once the virion is mature, the precursor polyproteins are cleaved, this changes the structure of the virion nucleocapsid (NC) into a conical, electron dense shape. The event that precedes the processing of Gag precursor during maturation is the collapse of capsid (CA) protein resulting in the formation of a more ordered lentil-like (hence Lentivirus) crystalline core [53-56]. The general features of the mature HIV and a schematic depiction of the major and minor structurally characterized viral proteins are shown in Fig. 4. The mature virus is spherical with a diameter of 100-120nm. At the exterior, the virus is enveloped by a lipid bilayer that is derived from the membrane of the host cell. Exposed on the virus envelope membrane are spikes known as surface glycoproteins (SU, gp120) which are anchored to the virus through interactions via transmembrane protein (TM, gp41). Lying beneath the inner part of the lipid bilayer is a matrix shell comprised of the matrix protein (MA, p17), and a conical capsid core particle comprising of capsid protein (CA, p24), located at the center of the virus. These nucleocapsids encapsidate copies of unspliced viral genome, which is stabilized as a ribonucleoprotein complex with copies of nucleocapsid (NC, p7) and also contain essential virally encoded enzymes: protease (PR; black), reverse transcriptase (RT) and integrase (IN). Selected virus proteins which are relevant to drug design/target will be discussed in brief.

1.1.3 The HIV Reverse Transcriptase (RT)

Is a heterodimer with a large subunit (p66) (see Fig. 5) containing the DNA polymerase (able to incorporate deoxyribonucleotides using either an RNA or a DNA as a template) and RNase H (whose activity degrades RNA in the heterodimer duplex form) domains, and a small subunit (p51) lacking RNase H. These subunits are folded

differently resulting in an overall asymmetric structure. p66 is similar to the structure of a right hand (see Fig. 5), where the nucleic acids lie in the grip of the hand. YXDD motif at the active site for DNA polymerase lies at the base of the palm, while RNase H domain is attached to the wrist position of the hand. p51 subunit lies under the hand, not making direct contact to nucleic acid [55], and it has been suggested that the p51 does not participate in the RT processes.

1.1.4 Integrase

Integrase is encoded at the 3'-end of the HIV *pol* gene [56] of which the polyprotein precursor is cleaved by protease during maturation, resulting in the formation of the IN polypeptide that is packaged along with the newly formed virus particle. The HIV-1 IN is a 32,000 Da polypeptide that composed of 288 amino acids residues consisting of three functional domains [56-58] which are the amino-terminal domain (amino acids 1–50) that is made of the conserved zinc-binding motif HHCC (His12 and 16, Cys 40 and 43). This amino-terminal domain plays a key role in protein multimerization. The catalytic core domain (amino acids 50–212) contains the catalytic DDE motif, known to be conserved among all retroviral integrases and composed of the active site residues D64, D116, and E152 in HIV-1 IN (see Fig. 6) that are crucial for binding viral DNA ends and also metal ions such as Zn^{2+} or Mg^{2+}. The last one which is the carboxyl-terminal domain (amino acids 213–288) is important for nonspecific DNA binding of sub terminal viral DNA and of the host cell's DNA [59-63]. It's also contains an SH2-like motif 3 [64]. One unique feature of the IN domains is that they form dimmers and functions as tetramer [65].

HIV-1 IN recognizes the specific sequence 5'-GCAGT-3' at the ends of each viral long terminal repeat (LTR) and binds tightly to those LTR ends [72] see Figs. 6 and 7 [73-75]. The IN reaction takes place in a two steps manner following reverse transcription and the binding of IN to the LTR ends within the PIC [76], resulting in the assembly of the PIC onto the 5'-GCAGT-3' LTR sequence of the viral DNA ends generating a complex that will be subsequently used for integration. The reaction starts with a 3'-processing, that results in the removal of two nucleotides from the 3'-ends of each viral DNA LTR. The phosphodiester bond between the deoxyadenosine and deoxyguanosine is hydrolyzed by water in a nucleophilic attack in the presence of Mg^{2+} or Mn^{2+} [77,78], from which the terminal 3'-end of the viral DNA is released beyond the conserved CA dinucleotide [79,80], resulting in the formation of a recessed 3'-hydroxyl ends at each terminus. With subsequence translocation of the PIC, the second transesterification reaction is been catalyzed by IN, where the 3'-hydroxyl ends of the viral DNA act as a nucleophiles attacking the DNA phosphodiester backbone of a host chromosome resulting in a staggered insertion that is sealed by host DNA repair enzymes [80].

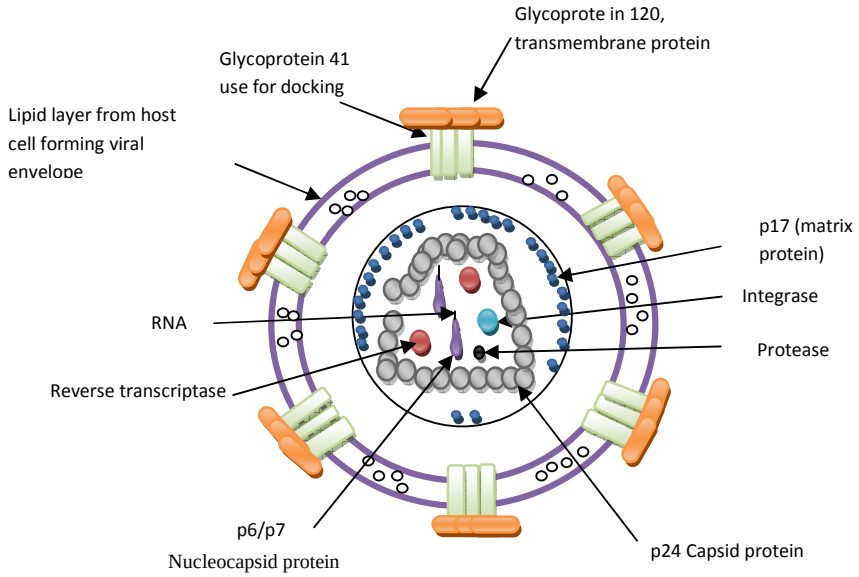

Fig. 4. Schematic representation of the structure of human immunodeficiency virus showing position of some important proteins involves in virus life cycle

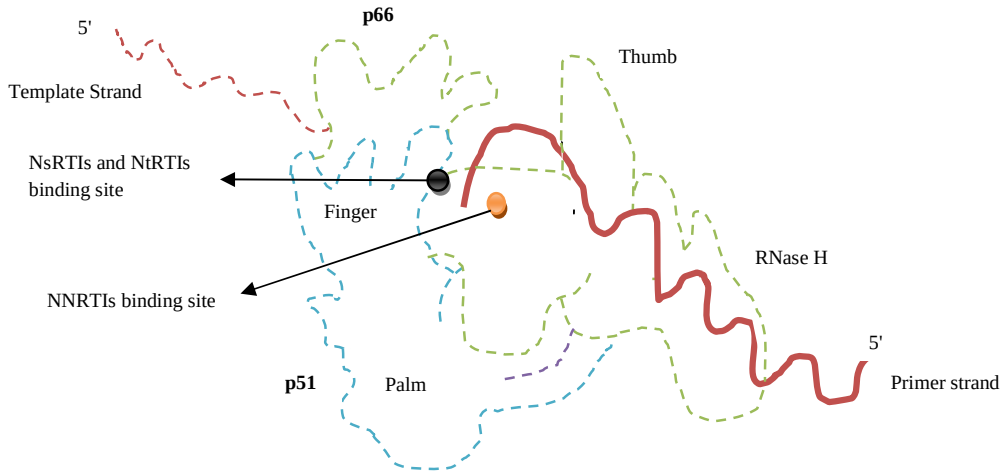

Fig. 5. Schematic of heterodimer reverse transcriptase of HIV-1 in an arrangement similar to the human right hand with the palm pointing up depicting p51 and p66 subunits and RNase position. Modified from [66-69]

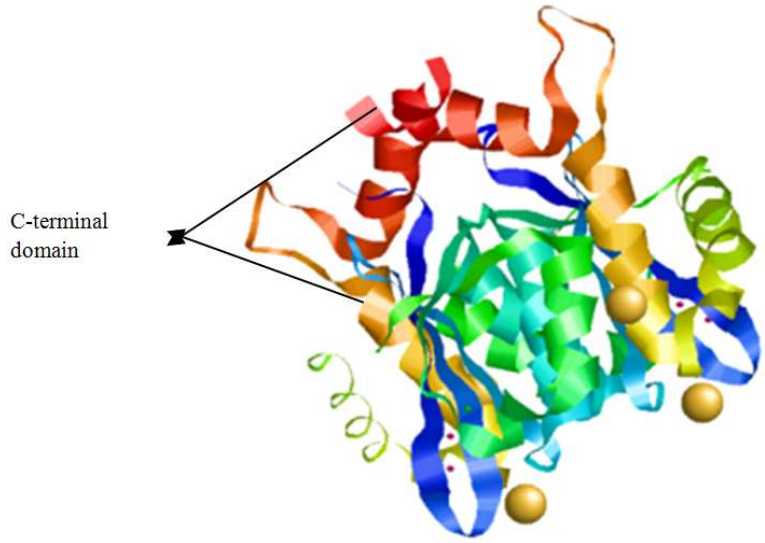

Fig. 6. Ribbon presentation of HIV-1 integrase catalytic core domain. Adapted from [70-71]

1.1.5 Protease

The protease is aspartyl protease with sequence similarity to members of the cellular family of aspartyl proteases [81]. This enzyme is small, contains about 100 amino acids, and is a functional homodimer. Each subunit makes a contribution of a single aspartate residue to the common active site, and also has a flap consisting of an antiparallel β-sheet with a β-turn that covers the active site cleft, and moves away to allow for binding of substrate. The PR homodimer flap comprise of residues Lys45-Met-Ile-Gly-Gly-Ile-Gly-Gly-Phe-Ile-Lys55, which fold into two antiparallel β-strands. These flaps are involved in the binding of a substrate in the active site cavity of PR [82,83] see Fig. 8. The importance of the flap residues for PR activity has been reported [84]. It has also been demonstrated that residues Met46, Phe53 and Lys55 tolerate most, a great number of substitutions, while residues Ile47, Ile50, Ile54 and Val56 are conservative in their tolerance to substitution [85]. So therefore, an alteration in the flap residues could result in a change to PR's flap enzyme activity, conformation and flexibility [86].

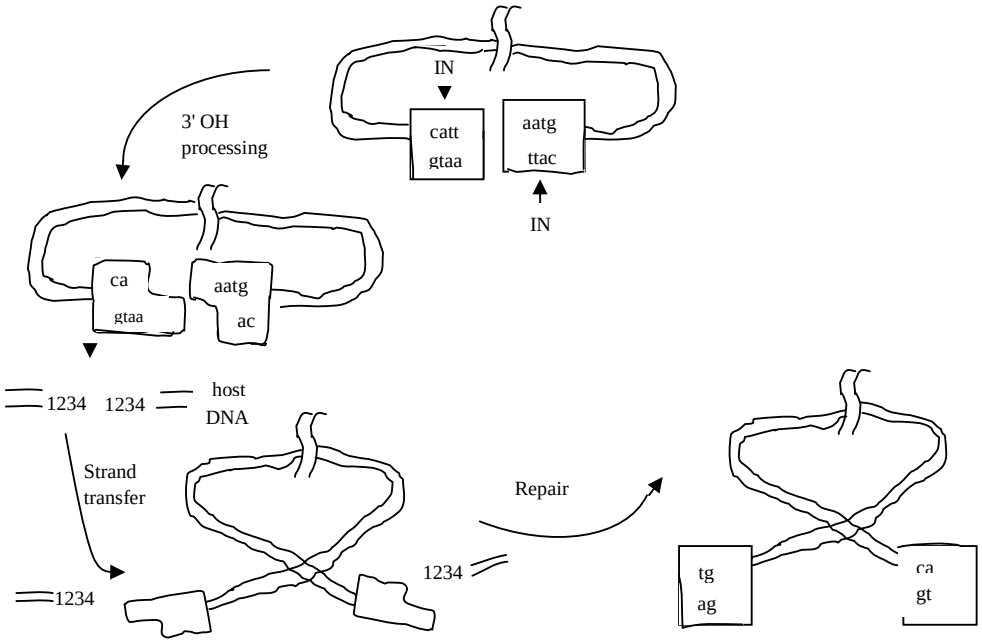

Fig. 7. Schematic presentation of HIV integration mechanism showing three crucial steps in the process; 3' processing, strand transfer and repair that leads to integration of virus genome into the host cell chromosome. Adapted from [78]

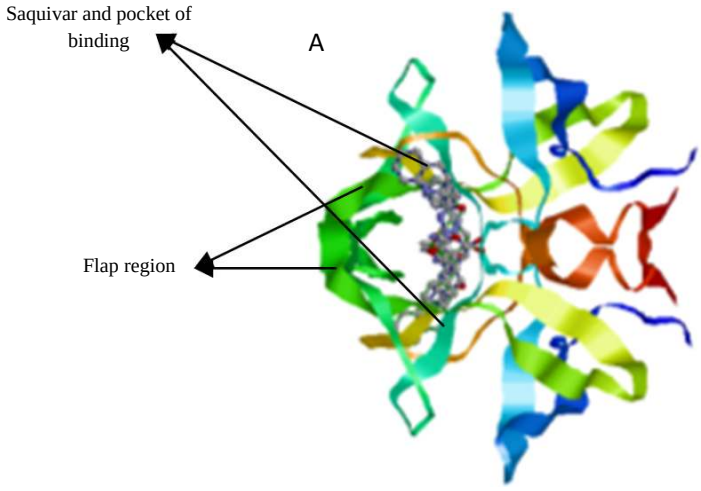

Fig. 8. Ribbon presentation of HIV-1 Protease enzyme chain with saquivar [79]

So therefore, an alteration in the flap residues could result in a change to PR's flap enzyme activity, conformation and flexibility [86].

1.2 Historical Milestones in the Development of Antiretroviral Drugs

Almost three decades elapsed, since the discovery of HIV as the etiology agent for AIDS [87-90]. The development of antiretroviral drug within these years has witnessed unprecedented series of breakthroughs (see Table 2) that have resulted in the successful management of the chronic infections. The first compound shown to inhibit HIV replication both *in vitro* and *in vivo* was suramin [91] but never saw the light of the day as its benefits were far less than its shortcomings. However, this led to the

development of the first anti-HIV agent approved for clinical use, zidovudine in 1987 (see Table 2). This agent was first described in 1985 as an antiretroviral agent inhibiting the infectivity and cytopathic effect of HTLV-III/LAV *in vitro* [92]. As time went on, there was a need to evaluate the use of fixed dose combination (FDCs) as it will give a greater efficiency compare to a single drug with just one mechanism of action. The first of its kind was a two-drug combination; Combivir (AZT+3TC) approved by FDA for clinical use in 1997, after this subsequent combination were approved including Keletra (LPV+RTV; 2000), Epzicom/Kivexa (ABC+3TC; 2004), Truvada (TDF+FTC; 2011), also in the arsenal was the first three-drug combination Trizivir (ABC+3TC+AZT; 2000), Atripa (TDF+FTC+EFV; 2006), Quid in 2012, Dolutegravir; 2014, With the advancement in antiretroviral drug development, new agents have been developed in the last 12 years, some of which inhibit entirely new targets that have not been explored previously, including integrase inhibitors (Raltegravir) approved in 2007 (see Table 2), (Maraviroc) entry inhibitor, approved in 2007(see Table 2), Quid (complete single tablet regime; one of its component Cobicistat has the ability to inhibit cytochrome P4503A) was approved in 2012 (see Table 2) by Food and Drug Administration (FDA), also approved on May 11, 2012, by FDA was the use of Truvada as a pre-exposure prophylaxis for the prevention of HIV/AIDS in high risks individuals and Dolutegravir, 2014 (Table 2).

2. CLASSES OF ANTIRETROVIRAL DRUGS

In the last 30 years of research into antiretroviral drug development, a considerable advancement has been made and there is an increasing thirst for the development of new agents that will ultimately lead to the cure of HIV/AIDS. At the present, over 26 agents have been approved by FDA for the management/treatment of HIV infections. These compounds fall within different categories depending on their target in the HIV replicative cycle. Here, the mechanisms of action of these agents will be discussed in brief in chronological order along with examples. The targets that had been of immense interest in the drive for the development of antiretroviral agent are reverse transcription and viral protease. Also of note are the recently recognized therapeutic targets, viral entry (virus-cell fusion and interaction with host cell's co-receptor), and proviral DNA integration.

2.1 Reverse Transcription (Reverse Transcriptase) Inhibitors

The process of reverse transcription (catalyzed by reverse transcriptase) is one of the major targets for antiretroviral drug during virus life cycle. Three forms of inhibitors were developed including nucleotide RT inhibitors (NtRTIs), nucleoside RT inhibitors (NsRTIs) and non-nucleoside RT inhibitors (NNRTIs). The NsRTIs and NtRTIs interact with the catalytic site (substrate-binding site) of the reverse transcriptase but the result for phosphorylation of each of them differs as NsRTIs give triphosphate form and NtRTIs gives diphosphate forms, whereas the NNRTIs interact with a noncatalytic site [87]. Currently, there are eight NsRTIs that have been approved by FDA for use in the treatment of HIV/AIDS including Zidovudine (AZT), didanosine (ddi), Zalcitabine (ddc), Stavudine (d4T), Lamivudine (3TC), Abacavir (ABC), Emtricitabine (FTC), Quid (complete single tablet regimen; for composition see Table 1). We will discuss these inhibitors with respect to the site of binding to RT in the following section.

2.1.1 Nucleoside Reverse Transcriptase Inhibitors (NsRTIs)

These drugs are nucleoside analogues, which upon intake by host cell, phosphorylate to a (pass through three phosphorylation steps to be converted to an active form) 5'-monophosphate, 5'-diphosphate and 5'-triphosphate following this pathway; ddNMP—ddNDP—ddNTP) of which the 5'-triphosphate form will now act as a competitive inhibitor/alternate of the normal deoxynucleoside triphosphate (dNTP) substrate. As a competitive inhibitor it acts by thwarting the incorporation of the substrate into the growing DNA chain, where as an alternate substrate it is incorporated into the chain as ddNMP, thus serving as a terminator since ddNMP lacks the 3'-hydroxyl group needed for further chain elongation. During DNA polymerization, Mg^{2+} cations co-ordinated by the catalytic residues Asp110, Asp185 and Asp186 from the palm subdomain (see Fig. 5) activate the DNA primer 3'-hydroxyl group, resulting in the stabilization of the pentavalent α-phosphorus intermediate state within the substrate 2'-deoxyribonucleoside 5'-triphosphate (dNTP) [92-94]. The evolutions of resistance by the virus to NRTIs in the past decade have been of serious concern as NRTIs are principal component of highly active antiretroviral therapy (HAART). Studies have

shown that mutant RT that is resistant to the first commercially approved NRTIs; AZT achieved this feat by effectively incorporating AZT monophosphate into the viral DNA as suppose the prevention of its incorporation. The mutant enzyme is able to do this because it has developed the capacity to excise the incorporated molecules. The process is achieved through the effective use of ATP as a pyrophosphate donor to excise the molecules as an AZT-adenosine tetraphosphate adduct, resulting in the generation of 3'-hydroxyl primer terminus following the mechanism which is akin to polymerization step reversion [89-91]. It has been reported that resistant mutants (Lys 70 Arg, Thr 215 Tyr & Lys 219 Gln) to AZT create an optimal ATP-binding site between fingers and palm subdomains of RT that promote the excision reaction [92].

2.1.2 Nucleotide Reverse Transcriptase Inhibitors (NtRTIs)

These are nucleotide analogues that require a two-step phosphorylation to be converted to an active state. One of its unique properties is that it contain a phosphonate group that cannot be cleaved by hydrolases, thus posing a serious deal of challenge to cleave-off, once incorporated at the 3'-terminal end (Kohlstaedt et al. [102]). The reverse is the case for their regular nucleotide counterpart including AZTMP, ddAMP, ddC [95-97]. An example of NtRTIs is tenofovir disoproxil fumarate (TDF) frequently prescribed for use in the treatment of HIV/AIDS.

2.1.3 Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

They are allosteric inhibitors in the sense that they bind to a noncatalytic site of RT and induce the formation of a flexible binding pocket through large conformational changes involving Tyr 181, Tyr 188 and the primer grip, all of which lie within the palm subdomain [98-100]. The mechanism of inhibition is due to the displacement of the primer grip [101-103] or the three-stranded β-sheet that contains the catalytic triad [104-105]. The first compounds that were classified as NNRTIs were derived from 1-(2-2-hydroxyethoxymethyl)-6-(phenylthio) thymine (HEPT) [106] and tetrahydroimidazole(4, 5, 1)(1, 4) benzodiazapin-2 (1H)-one and-thione (TIBO) [107] whose prototype were emivirine and tivirapine which never saw the light of the day in drug market, reasons being attributed to their complicated synthesis (tivirapine) or whose activity was considered not to be very high (potent). This led to the development of the first generation NNRTIs such as nevirapine, delavirdine, efavirenz and etravirine. The interaction between the aromatic side chains of Tyr 181 and Tyr 188 with this first generation drugs in an aromatic manner have resulted in a potent drug affinity [98]. On the other hand, a mutation of just one aromatic side chain confers resistance [94].

The evolution of NNRTIs resistant HIV strains against first generation drugs led to the development of etravirine and rilpivirine approved for clinical use in 2007 and 2009, respectively, whose potency is retained against first generation NNRTIs resistant strains as they possess inherent flexibility that considerably enhances their binding affinity to NNRTIs resistant strains [108]. Certain host cellular factor such as APOBEC3G inhibits HIV-1's RT by converting nascent cytidines in the viral cDNA to uracil [109-101] thereby preventing viral DNA elongation [102]. HIV-1 countermeasure to this host defense process is Vif protein which antagonizes the incorporation of APOBEC3G by binding to it, resulting in its degradation in virus infected cells [103]. This process of defense-antagonism by viral and host cells proteins serves as a druggable target and as such, small molecules that could interfere were been investigated (Vif degradation of APOBEC3G), see detailed discussed in the reference provided [106].

2.2 Protease Inhibitors (PIs)

Over the past decade more than 10 protease inhibitors have been approved for clinical use (see Table 1). The mechanistic functions of all the PIs are based on the 'peptidomimetic' principle, as they contain a hydroxyethylene scaffold that is being cleaved by HIV-1 protease, but itself cannot be cleaved with the exception of tipranavir whose mechanistic action is based on a coumarin scaffold [107]. Primary sequence homologies are displayed by the nine different peptides sequences within Gag and Ga-Pol which are cleaved by PR (protease). It has been demonstrated that substrate shape is more relevant rather than the primary sequences using cocrystallized six peptide substrates with PR which defined the substrate envelope [109].

Table 1. Viral proteins/enzymes needed for proper functioning (infectivity) of HIV-1 and exploration as drug target

Proteins	Functions	Explored as drug target
Major structural proteins Reverse Transcriptase (RT)	Synthesis of DNA copy of the viral RNA genome which is integrated into the host catalyses.	Yes
Integrase(IN)	It integrate/insert DNA copy of viral genome into infected cell genome. This process elicits latency of virus.	Yes
Protease(PR)	Cleaves long polyproteins into proper functional subunits.	Yes
Viral protein u(Vpu)	Play a role of letting newly formed virus particle to escape from host cell during budding by weakening the interaction of cell receptors with the new envelope protein. Also functions in forming ion channel in host membrane endoplasmic recticulum.	No
Viral infectivity factor(Vif)	It counters cellular defense system.	Under investigation
Viral protein r(Vpr)	Helps in guiding viral genome into nucleus after infection of host cell.	No
P6	Helps in the incorporation of Vpr into newly formed virus.	No
Negative regulatory factor(Nef)	Involves in the progression of HIV infection as it compels host (infected) cells to stop making several proteins that are importance in its defense. That is in defense with host cell protein synthesis.	No
Regulator of virion(Rev)	Helps regulate the splicing and transport of viral RNA by binding to a hairpin in the viral RNA.	Under Investigation
Transactivation of transcription(Tat)	Binds to hairpin of viral RNA, which influences the translation of protein.	Under Investigation
Major structural Proteins Matrix protein (MA)	Critical during the budding of new virus. formed coat assembles on the inner surface of virus membrane.	No
Capsid protein(CA)	Delivers viral RNA into the cell during infection. it forms a cone-shaped coat around the viral RNA.	No
Envelope protein(SU & TM)	Binds to host cells surface receptor leading to fusion. Also enable virus to evade host cell immune system as it is highly decorated with carbohydrate.	Yes
Nucleocapsid(NC)	Protects viral RNA by forming a stable complex with RNA.	No

Commercially available PIs are competitive inhibitors as they occupy the inhibitor envelope when they bind in the enzyme active site. Evidence has it that the inhibitor envelope protrudes beyond the substrate envelope thereby making contact with amino acid residues of PR which do not contact substrate residues, this confer resistance if changed [110]. It has been hypothesized that if PIs bind precisely within residues that are only essential for PR function, then the resistance mutation would not come by as their interaction would distort substrate-binding capacity of PR [111]. With this in mind, there is a need for further research to see if substrate envelope-based PIs will defy HIV-1 PIs resistance strains, though it has been reported that amprenavir-based compounds show improved binding to drug resistant PR strains than the non-amprenavir-based compounds in vitro [112].

2.3 Fusion Inhibitors (FIs)

There is at present one fusion inhibitor (FIs) approved for clinical treatment of HIV/AIDS, Enfuvirtide (see Table 1). It is a 36 amino acids polypeptide that is involved in a coil-coil interaction with the heptad repeat (HR) regions of the viral glycoprotein gp41 [113]. It is derived from the C-terminal sequence and is highly potent. This interaction blocks the fusion of virus particle with the host cell membrane. Also, the compound is polymeric in nature and as such is not orally biovailable and must be injected subcutaneously twice daily. This challenge, coupled with frequent resistant mutations

observed in gp41 against the drug, makes it unfavourable as an effective antiretroviral. Peptides derived from gp41 N-terminal or C-terminal [114] sequences have also been reported and help to disrupt the formation of the six helical bundles, resulting in a membrane fusion blockage which exhibit potent antiviral efficacy. D-peptides compound that target a pocket at the base of the gp41 N-terminal helical structure has also been reported as potent drugs that could overcome the shortcomings of enfuvirtide [115].

Table 2. Historical milestone in antiretroviral drugs development

Generic name	Marketed name	Producer	Mechanism of action	FDA approved date
Zidovudine	Retrovir	Glaxosmith Kline	Inhibit RT	19/03/1987
Didanosine	Videx (tablet)	Bristol-Myer Squibb	Inhibit RT	9/10/1991
	VidexEC (Capsules)	Bristol-Myer Squibb	Inhibit RT	31/10/2000
Zalcitabine	Hivid	Hoffman-LaRoche	Inhibit RT	19/06/1992
Stavudine	Zerit	Bristol-Myer Squibb	Inhibit RT	24/06/1994
Lamivudine	Epivir	GlaxoSmith Kline	Inhibit RT	17/11/1995
Squinavir	Invirase (Hard capsule)	Hoffman-LaRoche	Protease Inhibitor	6/12/1995
	Fortovase (Soft capsule)	Hoffman-LaRoche	Protease Inhibitor	7/11/1997
Ritonavir	Norvis	Abbot Laboratories	Protease Inhibitor	1/03/1996
Indinavir	Crixivan	Merck	Protease Inhibitor	13/03/1996
Nevirapine	Viramune	BoehringerIngeiheim	NNRTI	21/06/1996
Nelfinavir	Viracept	Agouron Pharmaceuticals	Protease Inhibitor	14/03/1997
Delavirdine	Rescriptor	Pfizer	NNRTI	4/04/1997
Efavirenz	Sustiva (USA)	Bristol-Myer Squibb	NNRTI	17/09/1998
	Stocrin (Europe)	Merck	NNRTI	17/08/1998
Abacavir	Ziagen	GlaxoSmith Kline	NNRTI	17/12/1998
Amprenavir	Agenerase	GlaxoSmith Kline	Protease Inhibitor	15/04/1999
Lopinavir+Ritonavir	Kaletra (high-income countries)	Abbott Laboratory	Protease Inhibitor	15/09/2000
	Aluvia (low-income countries)	Abbott Laboratory	Protease Inhibitor	15/09/2000
TDF(TenofovirDisoproxil Fumarate)	Viread	Gilead Sciences	Unknown	26/10/2000
Enfuvirtide	Fuzeon	Hoffman-LaRoche&Trimeris	Fusion inhibitor	13/03/2003
Atazanavir	Reyataz	Bristol-Myer Squibb	Protease Inhibitor	20/06/2003
Emtricitabine	Emtriva	Gilead Sciences	NRTI	2/07/2003
Fosamprenavir	Lexiva (USA)	GlaxoSmith Kline	Protease Inhibitor	20/102003
	Telzir(Europe)	GlaxoSmith Kline	Protease Inhibitor	20/10/2003
Tipranavir	Aptivus	Boehringeringelheim	Protease Inhibitor	22/06/2005
Darunavir	Prezista	Tibotec Inc.	Protease Inhibitor	23/06/2006
Maraviroc	Celsentri	Pfizer	Entry	18/09/2007

	(Europe)		Inhibitor	
	Selzentry(USA)	Pfizer	Entry Inhibitor	18/09/2007
Raltegravir	Isentress	Merck & Co., Inc.	Integrase Inhibitor	12/10/2007
Etravirine	Intelence	Tibotec Therapeutics	NRTI	18/01/2008
FDC(Fixed dose combination)				
Lamivudie&Zidovudie	Combivir	GlaxoSmith Kline	RT inhibitor	27/09/1997
Abacavir, Zidovudie&Lamivudie	Trizivir	GlaxoSmith Kline	NNRTI & RT inhibitor	14/11/2000
Abacavir&Lamivudie	Epzicom (USA), Kivera (Europe)	GlaxoSmith Kline	NNRTI & RT inhibitor	02/08/2008
TDF&Emtricitaine	Truvada	Gilead Sciences	NRTI Prophylaxis	02/08/2008 2/07/2012
Efavirenz, emtricitabines&TDF	Atripla	Bristol-Myer Squibb& Gilead Sciences	NNRTI & NRTI	12/07/2006
Rilpivirine /tenofovir/emtricitabine	Rilpivirine	Bristol-Myers Squibb	NNRTI	08/2011
Tenofovir/FTC and rilpivirine	Complera	Gilead Sciences	NRTI& NNRTI	10/08/2011
Complete single tablet regimen				
Elvitegravir, Cobicistat&Emtriatabine	Quid		Cytochrom e P450 3A (CYP3A) inhibitor, integrase inhibitor & NRTI	11/05/2012
	Stribild		Integrase inhibitor	2014
	Dolutegravir		Integrase inhibitor	2014

Modified from [43]

2.4 Co-receptors Inhibitors (CRIs)

These inhibitors interacts with the CCR5 or CXCR4 co-receptors used by R5 or X4 strains of HIV-1, respectively, to access target cell. In these events, the interaction of viral gp120 with co-receptor falls between that of viral glycoprotein gp120 with the CD4 receptor on T-cells and subsequent fusion of the viral glycoprotein gp41 with the host cell outer membrane [116]. Over the last seven years only one CRIs has been approved for clinical use, the maraviroc (see Table 1), a CCR5 antagonist [117]. Its major flaw is that it is active against R5 HIV strains and will select only these strains in a mixed population of X4/R5 strains. A more potent and specific CXCR4 antagonist AMD 3100, has been discussed in detail [118-126], though it is not orally bioavailable, research is on-going at the present to see how it could be deliver to HIV/AIDS patients. In 2009, there was a report about the safety of using CCR5 as a drug target [127].

2.5 Integrase Inhibitors (INIs)

The HIV intergrase performs two imperative catalytic functions: 3'-processing and strand transfer. Commercially available INIs including raltegravir(see Table 1) and similar small molecules that are in development inhibits DNA transfer activity which give them a highly effective potency against HIV-1 [128]. These compounds interact with instasome and not with the free integrase. The intasome consists of a dimerofdimers of IN, with only one subunit of

each dimer binding a viral DNA end [129]. In a similar manner as RT, functional IN active sites are delegated to a subset of protein molecules within the multimeric complex of which the intasome accommodates the target DNA within a cleft between the functional active sites, in a severely bent conformation. This bend out of the normal shape in the target DNA allows the intasome active sites separated from one another by a distance of 26.5 Å to access their target split phosphodiester bonds [130]. The Asp and Glu residues of the catalytic motif D,DX_{35}E coordinate two divalent metal ions, which activate the 3′-hydroxyl nucleophile and destabilize the target phosphodiester bond during strand transfer [131-134]. Reversal of the reaction appears to be restricted by a conformational change that causes a 2.3 Å displacement of the newly formed viral DNA–target DNA phosphodiester bond from the IN active site following transesterification [135]. The currently available INIs, raltegravir, and similar small molecules that are in development preferentially inhibit DNA strand transfer activity.

The clinically approved HIV1 IN inhibitor, The integrase strand transfer inhibitors (INSTIs) consist of co-planar heteroatoms that chelate the active site metal ions [136] and halogenated benzyl group moieties, resulting in a slight change in its positions within the IN active site. The latter moiety interacts with the pen ultimate viral DNA G-C base pair and a 3_{10} helix in Pro 154-Gln 146 of HIV-1IN as it takes the position of the terminal adenine ring, resulting in the ejection of viral 3'-dA along with 3'-hydroxyl nucleophile from the active site [137,138]. There has been addition to this cohort Stribild [139] and Dolutegravir [140] and Elvitegravir, [141].

3. THE PRESENT STATE OF ANTIRE-TROVIRAL THERAPY

In the last 30 years, over 28 antiretroviral drugs including both single and multiple fixed dose combinations (FDCs) agents have been approved by FDA for clinical use. These classes of antiretroviral were discussed in detail in the preceding chapter. This arsenal of antiretroviral agents provides medical doctors managing HIV/AIDS patients with options of constructing highly effective combination therapies, nonetheless, they also present challenges as per the complexity of specific drug profiles. Also of note are conditions that could complicate treatment, which include the patient's health status, age, treatment history, drug resistance and co-medications etc.

The ultimate aim of administering antiretroviral is to achieve a long lasting suppression of viral load below the levels of approved standard detection system along with immune system boost, assessed by the measurement of CD4+ cell count with the drugs having minimal side effects treated individual. Speedy facilitation of treatment decisions has contributed immensely to the management of HIV/AIDS. The US department of Health and Human Service (DHHS) do update guidelines for the treatment of adults, adolescents, pregnant women and pediatric patients, its latest update can be found here (http://aidsinfo.nih.gov/guidelines). Also organization such as the US Centre for Disease control and prevention (CDC) and the National institute of Health (NIH) make available recommendations for treatment and are updated annually, taking into consideration newly generated clinical data [142].

Various determinants in the cause of therapy could lead to a change in treatment regimen, including drug resistance, inadequate drug exposure, toxicity or incompatibility with other essential medications. This necessitates treatment decisions to be taken on a case-by-case basis. At present, there is no defined treatment for experienced patients (patient who are on antiretroviral therapy), with the exception of drug combinations that should be avoided. At this stage of treatment, genotypic or phenotypic resistance screening becomes necessary for choosing optimal regimen [143].

3.1 Administration of Antiretroviral and Side Effects

The issue of side effects that arise from the administration of antiretroviral is crucial during HIV/AIDS management. Despite the advances in drug tolerability, the challenge (adverse effects) is still eminent and can be tackled through the following: drug discontinuation, switching or dose adjustment and supportive treatment. Such an example was the demonstration that statin reduces HAART (highly active antiretroviral therapy)-related hypercholesterolemia and metformin and other anti-diabetic medications can be used to manage HAART-associated insulin resistance [144,145]. During antiretroviral administration, certain requirements are necessary for the treatment of special individuals such as pregnant women, which require that

changes in the pharmacokinetics of some antiretroviral should be put into consideration before treatment. Also, the pros and cons of HAART as per preventing mother to child transmission must be weighed against available options. In the case of pediatric patients, conscious effort must be made in distinguishing metabolism and/or toxicity profiles of the various drugs [146]. Complication in the treatment of an individual who are co-infected with either virus such as HCV [147,148] or bacteria such TB [149-151] should be taken into account as drug-drug interaction, changes in drug toxicity profiles, and overlapping activities of antiretroviral and TB drug(such as rifadin) could worsen the situation.

In rare cases, individual's genetic make-up affects treatment strategies and as such, drug pharmacokinetics, efficacy, and/or side effect of specific antiretroviral can be influenced by gene polymorphism. One such example is the role of HLAB 5701 allelic polymorphism in the hypersensitivity to abacavir, as the researchers seek to find the association between MHC (major histocompatibility complex) alleles and abacavirhypersentivity [152]. They observed that in a cohort of participants in whom they studied, withholding abacavir in them reduces the prevalence of hypersensitivity from 9% to 2.5% on exposure to abacavir. Details on other examples of the relationship between antiviral hypersensitivity and individual's genetic make-up, pharmacokinetics during HAART treatment have been reported [153]. Although, numerous treatment options and diagnostic tools are available at the present to meet the requirement of diverse patient population, its long term success requires an in-depth knowledge, experience and strategic planning for various treatment outcomes.

3.1.1 The challenges of strict adherence to prescription

For the goal of antiretroviral therapy to be achieved, that is the suppression of viral load to an undetectable level in the plasma and to avoid the emergence of drug resistance, there must be a near 100% adherence to the antiretroviral daily dosage. On initiation of HAART, it must be taken for life, the challenge here lies in the difficulty to maintain this treatment regimen [154,155]. Nowadays the adherence by individuals is inversely proportional to the number of pills and doses assigned to be taken per day. Also of note are medication side effects and daily life interferences [156-159]. Among the various strategies that could help improve the adherence are: pharmacist-led individualized interventions, cognitive-behavioural educational interventions based on self-efficacy theory and cue-dose training [160,161] with monetary reinforcement [162-164]. Above all, simplification of drug treatment revolutionalized antiretroviral therapy. Fixed drug combinations (FDCs) are becoming an important part of antiretroviral regimens with the introduction of such first drug combination in 1997(Combivir). About 7 such antiretroviral are currently available on the market, which represent a complete FDCs regimen taken once daily. Currently, FDRs (fixed drug regimens) are being explored only in treatment-naive patients, underlining the need for FDRs development for a patient who experienced drug resistance as this is not available currently, thus the need for a search in this direction with the view of developing FDRs for treatment experienced patient as it could reduce complication that may arise from long term treatment.

3.1.2 The challenges of drug toxicity

With the administration of antiretroviral, there still exist acute drug toxicities; however are no longer life-threatening, but can affect the quality of life and patients' willingness to adhere to their treatment regimen. The effect of drug toxicity could lead to a change in initial HAART for some individual and could require new alternatives with better tolerance and more convenient dosing schemes. It should be noted that long term toxicity could lead to drug administration discontinuation or regimen switching as a result of unforeseen side effects. There are emerging strategies that could help alleviate some of these effects.

3.1.3 The development of resistance by HIV strains

Drug resistance during antiretroviral therapy could arise from suboptimal treatment efficacy and inadequate adherence [165-170]. There is therefore a need to use potent antiretroviral regimen, available new drugs classes(such as integrase and fusion inhibitors) along with the latest new generation drugs that inhibit old targets(NNRTIs and PIs) which are active against resistant threaten strains may be used to reduce to the barest minimum the emergence of drug resistant strains. These available options could be employed also to constructs regimen that would be active against multiple drug-resistant viruses. New agents with improved

capabilities of countering resistant pose by HIV-1 virus should be used in formulating novel FDCs for treatment-experienced patients, as the availability of such will help reduce resistance to the barest minimum.

3.1.4 Socio-economic challenge in the underdeveloped world

It was reported that, the number of individuals on antiretroviral therapy in the developing world had increased by 10-fold during the year 2001-2007 with an estimate of 3 million at the end of 2007 [165,166]. Currently, there has not been any significant increase in the number of an individual who accessed HAART as the health care sector for these countries is plagued by poor administration, inadequate facilities, high cost of antiretroviral (as majority of the population of these developing countries wallow in poverty). The worldwide goal of achieving general access to therapies and care requires dealing with the high cost of antiretroviral in these countries. One of the ways to achieve this goal is through the building of laboratory, clinical infrastructure and generating adapted guidelines for treatment that reflects the Nation's regional, economic and cultural status. Other factors that could serve as an impediment for implementation of this lofty goal are nutritional deficiencies, co-infections with endemic pathogens which could affect HAART response [167]. There is a need for sustained provision of antiretroviral and at a non-suboptimal regimen, as these are necessary for a future success of HAART.

3.1.5 Antiretroviral therapy impacts on HIV/AIDS pandemic

The discovery of the first antiretroviral agent 29 years ago, led to a sudden drop in the annual age-adjusted death rate in the United States as a result of HIV/AIDS [168]. Clinical challenges faced in the early eighties often involved both severe immunodeficiency and serious malignancy [168]. The morbidity and mortality as a result of HIV/AIDS created a high burden or an unbearable burden on relatively young people, and this still happens nowadays especially in the developing world.

In the United States the death toll among the age group 24-44 years in 1995 was reported at 32, 000 [168]. In recent time, there have been a decrease in death rate as reported in the year 2005 to be at 6000, reason for this reduction was attributed to the available effective antiretroviral therapy, that is enhanced by FDA approval of viral load kits and other molecular diagnostics [169].

A different scenario is the encounter in the developing countries especially in Sub-Saharan Africa region which has the world highest HIV/AIDS rate. Its high mortality impact is being felt in this region according to statistics (www.unaids.org/en). Also access to drug in recent time have helped to improve the outcomes of the treatment process in some countries of the region [169], as reported in the case of Malawi and South Africa. In both countries mortality rate have reduced due to their Government committed scale up of antiretroviral therapy. The area of mother-child transmission in this region is of interest as Governments of some of these nations have intensified efforts of intervention in recent time as with a promised outcome as witnessed in the reduction in transmission rate via breast milk. Breast feeding which is an indispensable thing to do in the developing world cannot be undermined and at the same time serves as a portal entry for virus transmission to the child. In such situation absence of therapy force the woman to face inaccessible alternatives such formula feeding.

3.1.6 The issue of latency

For a decade now, the issue of latency in HIV infection has been one of the greatest banes in tackling the infection as some of the cells which are in latency state cannot be access by the present antiretroviral. These re-emerge after withdrawal of treatment posing as a threat to patient. Most recently, a promising study to counter such threat was carried out by a group in the United States, they concluded that, the administration of vorinostat (a cancer drug) on patient on ART leds to the release of latent state virus [170]. Also it has been reported that cannabinoid receptors 2-Mediate the attenuation of CXCR4-tropic HIV infections in primary CD4+ T-cells [172]. It was reported that one way for purging HIV in a latent state from its reservoirs involves reactivating HIV gene expression, with their premise based on the fact that reactivating latent HIV proviral DNA within all resting CD4 T cells would eliminate these virus factories when the newly made viruses burst forth, killing the host T cells. Although this comes with a consequence as it could triggers off potentially deadly immune response. A more promising way has been strategized which involves the

activation of HIV gene expression without inducing host cell activation. Based on this path several small molecules have been identified that can accomplish this task e.g. valproic acid, cannabinoid [171-175]. Recently it was demonstrated that two classes [class I (5-chloroquinolin-8-ol) and class II (quinolin-8-yl carbamates)] of quinolines reactivate latent HIV-1 with ranges from 25% to 70% for anti-CD3+ anti-CD28 co-stimulation and that a group of quinolin-8-ol derivatives induces reactivation latent HIV-1 in a primary cell model without causing T cell activation [176].

3.2 Associated HIV Disease Complications and Antiretroviral Therapy

In HIV disease, other viral and bacterial infections such as hepatitis C (HCV), tuberculosis (TB) pose a considerable challenge in the management of a patient who are co-infected, as complication could arise and could leads to a non-response to antiretroviral and ultimately a speedy dead of the patient. HCV has been associated with increased hepatoxicity in co-infected patient who are on antiretroviral therapy (ART), although with the current ART a decline in hepatoxicity has been witnessed in the last seven (7) years. A study carried out showed that the risk of developing earlier hepatoxicity in co-infected patient who are on ART is high compare to only HCV-infected patient. HIV patients do have low bone mineral density (BMD). Recently the interaction between antiretroviral and Hepatitis C drugs along with side effect has been discussed indepth [177] and also interaction of hepatitis C direct acting antiretrovirals (DAA) [178]. This relationship along with specific ART drug is being explored. It was reported by Ofotokun and colleagues that early bone loss in HIV patient is associated with immune reconstitution on starting ART. They went further to investigate the contribution of ART to bone loss by examining markers of bone resorption and formation in patients with suppressed HIV-1 RNA who was switched from NRTI-based ART to lopinavir and raltegravir. There are antiretroviral agents associated with renal (kidney) toxicity such as tenovir. Also it has been suggested that the association of PI with renal dysfunction may be in part mediated by increased tenovir levels. HIV-infected individuals have high risks of developing malignancies (cancerous cell) such as infectious hepatocellular carcinoma (liver cancer), non-infectious cancer including, colon and lung cancer.

Tuberculosis (TB) is one of the bacterial infections that are associated with HIV. Over the years guidelines have been made for recommendation in HIV-TB co-infected individuals. This recommends that ART should start in TB patient at two weeks after the initiation of TB treatment. Compatibility of new ART agents and rifampin (which induces hepatic enzymes that lower levels of many ART drugs) is a key issue for optimal management of this disease.

4. THE EMERGING STRATEGIES USED IN ANTIRETROVIRAL THERAPY

Individuals with HIV but still healthy with a full virus suppression serves as an example of the clinical impact of HAART. The present state of success experience by an individual who are on antiretroviral therapy remains universal, but could be marred by certain factors such as individual genetic make-up, increasing age of treated population, robust ability of HIV to evolve, stringent adherence, cost of managing chronic treatment, and effect of life-long therapy have triggers the soughting for suitable strategies with a view of long term HIV management.

4.1 Simplification of Antiretroviral Treatment

The aim of simplification of antiretroviral has to do with the exploration of new combinations with the view of attaining a high response in patient's population who had limited response to established treatment. The presently available combinations contain NRTIs as a key component, of which they have their own limitations that distinguish them from other agents [179-182]. Over the years two-and three-drug NRTI combinations have been investigated of which, some were highly effective in suppressing viral load in treatment-naive patients, these were observed to evolved common resistance and adverse metabolic effects [183]. Research focuses have shifted to the exploration of more NRTI-sparing regimens as they represent an attractive option, especially for patients with drug resistance [184-188]. Among the newest strategies that have been tested is maintenance monotherapy. In years past, simplified maintenance treatment has been initiated if HIV-individuals achieved a stable, undetectable viral load on a combination therapy. Also, several drugs such as NNRTI rilpivirine, INI elvitegravir have passed their clinical trial test and have been approved by FDA (see Table 2) for treatment-experienced patients (patient who

have been on antiretroviral therapy). The need to incorporate pharmaco-enhancers [they served as 'booster', boosting the plasma levels of antiretroviral, thereby improving the efficacy and convenience of HAART. The performed this function by interfering with the action of the cytochrome P450 3A (CYP34) enzyme; a drug metabolizing enzyme, making the concentration of the drug to be high in the blood stream for a longer period] in combination therapy is necessary as it would allow for further expansion of treatment choices, an excellent example is ritonavir which is an enhancer. There has been a shift in paradigm from focusing on developing antiretroviral from existing classes to exploring novel viral and host antiretroviral targets including Vif-APOBEC3G, integrase-LEDGE and factors involved in HIV assembly and maturation [189]. While still in its embryonic state we hope for the development of new classes of antiretroviral from these potential targets.

4.2 The Prospect of Complete HAART Withdrawal

Patients on HAART for a long period of time with fully suppressed viral load do experience a reoccurrence and increase in plasma viral load upon the withdrawal of ART, as a result of the spread of virus that was in a latent state. At present, it is uncertain, the cells type for which the latent state virus are present and where the cells are located in the body, although there have been evidence suggesting that they are quiescent latently infected cells harbouring transcriptionally inactive provirus [190]. It was reported that analysis of these cells in a patient who have been on ART for a prolonged period of time showed a remarkably slow decay indicating its unlikeness to be cured by lifelong therapy [191,192].

Strategies are emerging for the reduction or elimination of this reservoir to a level that would allow for ART withdrawal, without resurgence [193]. The mechanism of HIV latency have been proposed to include transcriptional and post-transcriptional block in viral gene expression [194], as a result, various chemotherapeutic approaches have been put forward for the transcriptional activation of latent provirus through the inhibition of histone deacetylases [194] or DNA methylases. There is also a strategy of the activation of the NF-kB signaling pathway by nontumorigenicphorbol ester analogues [which could help to activate the provirus thereby being subject to antiretroviral therapy. Another strategy that has been of interest in recent time is the transplantation of HLA-matched, homozygous CCR5-Δ32 stem cells following immunosuppressive therapy in individuals with HIV-related hematological malignancies [194]. This procedure tends to eliminate viral RNA and DNA from the peripheral blood, bone marrow and rectal mucosa and results in a long-term control of HIV without HAART. This observation is particularly beneficial as clearing of reservoir cells may be sufficient to prevent virus to rebound [194]. Stem cells therapy could enable this strategy as an available option for patient infected with CCR5-tropic virus meaning it is not effective for individual infected with CXR4-strains thus the need for further study on how to develop means for tackling infection caused by CXR4-strain or a combination of both.

A trial conducted by Sangamo Sciences showed that gene therapy successfully reduced viral loads in patients who already naturally had one copy of the HIV-resistant CCR5 gene form in a phase I trial. The phase II trial is currently ongoing with the view of confirming and further investigation of results obtained from phase I. Here the investigators will explore further the gene therapy's effect in individuals who have inherent copy of HIV-resistant CCR5 gene. In the second objective, the investigators will test the effect of cyclophophanide (used in cancer patient to improve the outcome of stem cell transplant by killing patient's existing T-cells) prior to gene therapy treatment.

4.3 The Use of Microbicides

In recent time strategies have been outlined for the prevention of HIV through sexual intercourse (which is a particularly common portal entry for HIV transmission) as the method is used in protecting the reproductive or gastrointestinal tract of uninfected individual. These strategies may involved the use of immune boosting agent (such as a vaccine for preventive purpose) or chemical as provided by topical microbicides [82]. Previous investigation on the use of topical microbicides on HIV prevention was evaluated in advanced clinical trial and results showed that some of the chemical namely; cellulosesulphate gel, 1% C3IG, canraguard and buffer gel products could not prevent HIV transmission. It was reported that placebo gel achieved 30% effectiveness in reducing HIV incidence [94-98]. In 2010, a study carried out in the Centre for the AIDS programme of Research in South Africa

(CAPRISA) code named CAPRISA 004 in which tenofovir was used to formulate a vaginal gel, and was administered to volunteers. It was observed to show 39% HIV infections compared with placebo, although in 2011, NIH called for the discontinuation of the use of tenofovir vaginal gel in VOICE (vaginal and oral intervention to control the epidemic) study as it was no more effective than placebo gel (www.niaids.nih.gov). A good number of microbicides trials with topically applied antiretroviral are currently ongoing.

4.4 The Use of Targeted Pre-exposure Prophylaxis

Thequest for developing strategies for the prevention of HIV among a high risk individual has led to intensive search for a pre-exposure prophylaxis (PrEP) with antiretroviral that could potentially be used to control HIV transmission. Studies carried out on primate models to investigate rectal transmission of HIV showed that NRTIs substantially reduced infections over the course of multiple exposures [98]. These observations were also investigated in the clinic in a multiple phase's placebo-controlled trial to assess PrEP effectiveness among several subjects [99-102]. It has been reported through epidemiological assessment that HIV transmission under PrEP could substantially reduce the incidence of new infections among high risk individual in the United States [182-187]. In 2011 two clinical trials using partner PrEP and TDF2 PrEP observed that they were effective in preventing infections among heterosexuals (www.fhi360.org). Also in the same year report has made available on the study of TDF2 as PrEP among heterosexual men and women in Botswana and observed that daily oral dose of TDF/FTC could reduce HIV transmission among these individuals (www.cdc.gov). Also, it was reported the oral FTC-TDF (an antiretroviral) reduced the risk of acquiring HIV infection by 43.8% among homosexual as their detectable blood level strongly correlated with the prophylactic effect [102]. A study code named HPTN 052 that begun in April, 2005 and involved 1,763 couples with 97% of them being heterosexual, conducted in 13 cities in Botswana, Brazil, India, Kenya, Malawi, South Africa, Thailand, USA and Zimbabwe showed that treating HIV infected individuals who have relatively healthy immune system on ART protects their partners from getting infected.

4.5 The Prospect of Vaccine Development

In the last eight years effort on the discovery of HIV vaccine for the prevention or cure of HIV infection has been ongoing with little or no success, although candidate vaccines have been developed along the line to elicit neutralizing response with low efficacy [154]. One of the greatest challenges in developing an HIV vaccine is that infective HIV inoculums consists of genetically varied population, and subsequent variation is continuously generated over the course of infection allowing for immune evasion using the following mechanisms including shielding the majority of the surface envelope glycoprotein with glycan, rapidly mutating exposed epitopes without significantly affecting its replication, hiding conserved epitopes and producing immunodominant decoys [158]. A vaccine must elicit both mucosal and systemic immunity to protect against sexual transmission and virus transmitted directly into the blood stream. An attempt was also made to study the development of vaccine for HIV-1 using B-cell-lineage immunogens [189]. Over the years several different monoclonal antibodies (mAbs) have been isolated which have the capacity to recognize a range of epitopes on the HIV-1 viral spike, of which some of these broadly neutralizing antibodies (BNAbs) are directed against the membrane proximal external region of gp41 [189-194], but a large number of them recognizes gp120. Examples of such antibodies are the quaternary structure–preferring antibodies PG9, PG16, and CH01-04 [; the glycan reactive antibodies 2G12 and PGT121-137 [185]; and antibodies b12, HJ16, and VRC01-03, of which they antagonize the region of HIV-1 gp120 involved in initial contact with the CD4 receptor [160-167]. One exceptional feature of these gp120-reactive broadly neutralizing antibodies is that they easily undergo somatic mutations. Normally, antibodies do have the capacity to build up 5 to 15% changes in variable domain–amino acid sequence during the affinity maturation process [167-169], but the unusual characteristic attributed to the gp120 reactive neutralizing antibodies, is that, the degree of their somatic mutation in heavy chain ranges from 19%-46% [170-172]. Investigation have demonstrated that some genetic determinants of protection including human leukocytes antigen (HLA) genotype, cellular cofactors could be used to prevent HIV replication as alleles of HLA are involved in the suppression of HIV replication and could serve as a potential antigen regimen that should be included in a novel HIV vaccine

for virus suppression [78]. Also, genome wide association [57,58] and functional screens of HIV replication and disease progression have demystified the complex trait and virus-host interaction that could serve as potential targets to prevent HIV disease progression [67-68]. In 2010, a team led by NIAIDS (National Institute of Allergy and Infectious Diseases) scientists discovered two human antibodies (Abs) which can block more than 90% of the world known HIV strains from infecting human cells and their mechanism of antagonism, such proteins could be helpful in the design of vaccine that could elicit such Abs production in healthy individuals against HIV infection. Detail structural study has been carried out to determined the HIV binding site which would give insight on novel vaccine development [67] and a generalize picture of the preventive mechanism of HIV-1 from binding to CD4+ T cells receptor.

4.6 Future Direction

During the early years of the discovery of HIV as the causative agent for AIDS, the cardinal goal as of then was to focus on mortality and morbidity associated with the infections. With its chronic effect and being incurable, the scientific community was forced to come up with strategies on how it could be managed. Options on ART have been improved significantly in the last 4 years with new drugs and strategies for using them been advanced. Nonetheless, with these strides, the issue of toxicity, latency, morbidity and mortality are still a serious concern as no definite strategies has been put to place to contain this threat, thus the need to project into the future of possible research path and strategies that should be worked towards in order to achieved the ultimate goal of finding a cure to HIV/AIDS.

4.6.1 New therapeutic targets

The development of HAART was witnessed as a major stride in the fight against HIV/AIDS as it gave hope to millions infected by the dreaded virus. This strategy was helpful in that it reduces plasma viral load to an undetectable level, improve CD4+ cells counts, delay disease progression and ultimately increase life span of patients. Despite this advancement, there are shortcomings as it requires a life-long daily treatment and cannot eliminate virus in the latent state as there is always a rebound upon withdrawal of treatment. This has raised a question about its efficacy in the future and coupled with the toxicity due to prolong treatment, thus a need for the development of new antiretroviral drugs or innovative therapies to reduce undesired effects and new target either in the host or virus.

4.6.1.1 Targets on human host and virus

About two decades now after the discovery of CD4 as a primary receptor of HIV, with coreceptors, CCR5 and CXCR4 as being vital for HIV entry [23]. Events that followed shortly were the demonstration that homozygous deletion in CCR5 allele gives protection against HIV-1 infection [34-38] although heterozygous deletion in CCR5 were associated with delayed disease progression [101-107], enabling it to serve as a suitable target. This led to an intense research that resulted in the development of maraviroc [49], see Table 2 approved by FDA. However, several agents such as anti-CCR5 antibodies evaluation are ongoing [135-137].

Gene therapy is another strategy used for CCR5 inhibition, nonetheless, it has its shortcoming as CCR5 are crucial in protecting the body against some pathogens [45].

Thestrategy that could be used to target the HIV cofactors such as Tat and Rev, which are proteins essential for viral replication [134-136] should be worked upon. The elongation of viral transcripts is elicited by Tat protein through binding to the transactivation response element (TAR) (see Fig. 3) that is located in the HIV long terminal repeat (LTR) and acts as an adaptor for the recruitment of the positive transcription elongation factor b (P-TEFb). The recruitment of P-TEFb to the TAR is necessary for HIV-1 transcription [145], thus making it a potential target. A number of small compounds that disrupt the Tat/TAR/P-TEFb interaction have been demonstrated [123-124], although difficult in the sense that P-TEFb is necessary for the transcription of many cellular genes. So if, interfered with, would results in the disruption in its capability to transcribe other cellular genes, thereby affecting the cells [138].

Rev mediate nuclear export of unspliced viral RNA through interaction with the cis-acting Rev response element (RRE) located in the HIV env gene [132] (see Fig. 3). The Tat and Rev represent useful targets for antiretroviral drug development, no specific agents inhibiting them have yet been developed, and thus there is a need for further work in this direction.

The recent addition of an HIV-1 IN inhibitor (raltegravir) to the available HAART drugs indicates that targeting proviral integration is a useful approach. HIV-1 IN interacts with a cellular factor that may have potential as a therapeutic target: the cellular lens epithelium-derived growth factor (LEDGF)/p75 [149], a chromatin-associated protein that is essential for HIV-1 integration [146]. It has been demonstrated that disruption of the interaction between HIV-1 IN and LEDGF/p75 leads to impaired viral replication [145]. The development of a small inhibitor to prevent LEDGF/p75–IN binding remains challenging [134].

Also, post integration, the inhibition of particle budding from the plasma membrane and/or cell-to-cell transfer of the virus is a valid path to prevent viral spread. HIV-1 usurps the cellular Endosomal Sorting Complex Required for Transport I (ESCRT-I) for its release from infected cells [134-137] this pathway need to be explored as it could be a druggable target. In particular, the PTAP-type late domain of the HIV-1 Gag precursor polyprotein interacts with TSG101 (tumor susceptibility gene 101), a cellular protein normally involved in endosomal protein sorting and inhibition of this interaction or depletion of TSG101 by RNA interference suppresses HIV-1 particle release [150]. One therapeutic strategy would consider the development of molecules, which would imitate the viral PTAP motif, such as cyclic peptides [154-155]. Another potentially intriguing interaction has also been identified between HIV-1 Gag and the endosomal sorting protein Alix [167].

5. CONCLUSION AND RECOMMENDATION

The marked effect of antiretroviral therapy has been immense since the introduction of the first antiretroviral drug AZT [15,126] of which the sole aim was to reduce the mortality and morbidity rate. The subsequent introduction of HAART in the management of HIV has witnessed a tremendous improvement in the life span of an infected individual. The currently available antiretroviral that forms the core of HAART has become a threat due to their toxicity, vulnerability to resistance by HIV-1 strains and their targets such as integrase, protease, and RT are prone to mutation mediated drug resistance, thus raising the question on the reliability of the current cohort of antiretroviral used in HAART. With these challenges at heart, we therefore, put forward the following recommendation.

i) Triple drug combination regimen have been helpful as it is taken in a single once-daily tablet, nonetheless it comes with its shortcoming as the combination of non-equal potency inhibitors helps increase the number of virus mutation (genetic barrier) in a high rate that enables the virus to no longer be susceptible to the drugs, thus the need to research on developing antiretroviral double combination, both of equal potency and genetic barrier than its triple combination counterpart.
ii) Currently, there is no known antiviral that targets HIV-1 RNA as this is a potential target and it remains unexplored, therefore there is a need to search for inhibitors that will disrupt HIV RNA biological functions.
iii) Virus proteins such as Rev and Tat which are very crucial for the export of HIV RNA, increased transcription of HIV genes and spread of virus could serve as potential drug target as the disruption of their processes will arrest virus replication.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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