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Review Article

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Progress and Prospects in Therapeutics against HIV Infection

Manisha Mandal*

Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar-855107, India

ABSTRACT

Tremendous advances have occurred in recent decades in the development of safe and effective medications for the treatment of viral diseases. Currently there are more than 20 HIV drugs approved for use in humans. Combined chemotherapy has been found useful for a number of viral infections comprising drugs with different mechanism of action. HIV treatment guidelines recommend the use of two nucleoside reverse transcriptase inhibitors (NsRTIs) plus a third antiretroviral drug for treatment-naive patients with AIDS that include non-nucleoside reverse transcriptase inhibitors (NNRTIs; eg, efavirenz), ritonavir-boosted protease inhibitors (eg, darunavir, atazanavir), and integrase inhibitors (eg, raltegravir, dolutegravir). However emergence of drug resistant HIV variants and their dissemination impede proper treatment of AIDS. Preferred regimens as first-line treatment in patients with suboptimum adherence comprise drugs with improved potency, safety and infrequent selection for resistance-associated mutations. The present review stands for an update on facts and phenomena of present, past, and future use of antiretrovirals based upon the available documents in SCI and non-SCI journals.

Keywords: Antiretrovirals, reverse transcriptase inhibitors, viral protease inhibitors, viral coreceptor antagonist, fusion inhibitor, integrase inhibitors.

INTRODUCTION

The AIDS disease caused by HIV is a major health problem with an actively spreading pandemic outbreak that has infected 34 million people worldwide and led to 30 million deaths due to AIDS-related diseases. [1] Although significant progress for the treatment of AIDS have been made that can slow the course of the disease but presently no vaccine or medication is obtainable that can completely eradicate this infection. [2] This is on account of the extra ordinary genetic diversity of the virus attributed to the high mutation and RNA recombination rates, immune selection that altogether give rise to virus variants with great genetic heterogeneity, and development of mechanisms for evasion of the host immune response as well as acquisition of drug resistance through selection pressure or person to person transmission. [3-4]

Currently, there are 26 drugs approved for antiretroviral therapy and they are classified as reverse transcriptase inhibitors (nucleoside and nucleotide analogues), nonnucleoside reverse transcriptase inhibitors (NNRTI), cell entry inhibitors, integrase inhibitors and protease inhibitors (PI). [4] Combinatorial treatment, have displayed considerable potentiality in controlling HIV infection in the form of HAART regimen, comprising either a NNRTI or a ritonavir-boosted PI in combination with two NRTIs. [5] However antiviral drugs when administered in combination over prolonged periods have caused serious side effects leading to medication non-adherence and thus

***Corresponding author: Dr. Manisha Mandal,**
Department of Physiology, MGM Medical College & LSK Hospital, Kishanganj, Bihar-855107, India;
E-mail: debmanisha@rediffmail.com
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treatment failure. [6] Besides, many newer antiretroviral drugs approved for use in combination are quite costly, thus limiting their use in developing countries, where infection is most common. However, improvements in effectiveness, safety and dosage simplification have alleviated those complications. Therefore, in order to achieve effective control and to combat this disease efforts are on to develop newer drugs. The present article is an attempt to provide an overview of antiretroviral therapies, with an emphasis on studies that have been conducted in the past 5 years with recent advances in field; antiretrovirals under clinical development trials; newer potential antiretrovirals with greater efficacy and less toxicity compared to ubiquitously used drugs, while adding further insights into the mechanisms of antiretroviral drug resistance. The current review is due to an extensive search in the field from both SCI and non-SCI journals as well as from scientific websites. The antiretrovirals are classified based on their mechanism of action through inhibition of viral life cycle stages.

NUCLEOSIDE AND NUCLEOTIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI)

Nucleoside Reverse Transcriptase Inhibitors (NsRTI) are structural analogues of nucleosides which are converted into triphosphate (TP) derivatives that compete with usual deoxynucleoside triphospahates (dNTPs) for inclusion into the growing DNA chain causing competitive inhibition of HIV-1 reverse transcriptase (RT) for DNA polymerization due to the absence of 3/-OH group in the ribose ring. [4] The approved NsRTIs such as Zidovudine (AZT), Zalcitabine (ddC), Stavudine (d4T), Emtricitabine (FTC), Abacavir (ABC), Lamivudine (3TC), Didanosine (ddI), Tenofovir disoproxil fumarate (TDF) and the newer drugs such as Apricitabine (ATC), Amdoxovir, Racivir, Elvucitabine, Tenofovir alafenamide (TAF) and CMX-157 under clinical trial are depicted in Table 1. [7-27]

Table 1: Nucleoside Reverse Transcriptase Inhibitors (NsRTI)

Drugs [Reference]	Systematic name	Combination from same class	Side effects
Old NsRTI			
Zidovudine [7-9]	1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione	Lamivudine	Leukopenia, anemia, hepatotoxicity, cardiomyopathy, myopathy
Zalcitabine [10]	4-amino-1-((2R,5S)-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1H)-one	Zidovudine	Nausea, headache, peripheral neuropathy, oral ulcers, oesophageal ulcers
Stavudine [11-12]	1-((2R,5S)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione	Lamivudine Didanosine	Neuropathy, genotoxic, lipodystrophy
Abacavir [13-14]	{(1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-en-1-yl}methanol	Lamivudine Zidovudine	Fatal hypersensitivity
Lamivudine [15]	4-amino-1-[(2*R*,5*S*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one	Zidovudine, Stavudine	Long term use leads to emergence of resistant mutant
Didanosine [16]	9-((2R,5S)-5-(hydroxymethyl)tetrahydrofuran-2-yl)-3H-purin-6(9H)-one	Zidovudine Stavudine	Pancreatitis, neuropathy, hepatic dysfunction, myalgia, leucopenia, anemia, thrombocytopenia
Emtricitabine [17-18]	4-amino-5-fluoro-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one	Didanosine	Diarrhea, headache, nausea, rash, hyperpigmentation , hepatotoxicity, lactic acidosis
New NsRTI			
Apricitabine [19-21]	4-amino-1-[(2*R*,4*R*)-2-(hydroxymethyl)-1,3- oxathiolan-4-yl]pyrimidin-2(1*H*)-one	As monotherapy	Nasal congestion, muscle pain, headache
Amdoxovir [22]	[(2*R*,4*R*)-4-(2,6-Diaminopurin-9-yl)-1,3-dioxolan-2-yl]methanol	Zidovudine Lamivudine	Obstructive nephropathy, lens opacities
Racivir [23-25]	4-Amino-5-fluoro-1-[(2*S*,5*R*)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2(1*H*)-one	Enantiomeremtric itabine	Mild headache, fatigue
Elvucitabine [26-27]	4-Amino-5-fluoro-1-[(2*S*,5*R*)-5-(hydroxymethyl)-2,5-dihydrofuran-2-yl]pyrimidin-2-one	–	Bone marrow suppression

The drug Amdoxovir is converted to its active form dioxolane guanine triphosphate by deamination with a cellular enzyme adenosine deaminase, [28] and is currently in phase II clinical trials. [22] The oxothiolone Racivir is similar to FTC and 3TC, [23] and this NsRTI is currently in phase II clinical trials. [24] The effectiveness of Elvucitabine is withheld under phase II trial on account of some evidence of bone marrow suppression and rapid fall of CD4+ count. [28]

Nucleotide Reverse Transcriptase Inhibitors (NtRTI) are structural analogues of nucleotides and resemble nucleoside analogues. Adefovir was initially used for HIV infection but is not used at present. [29] Tenofovir (TFV) disoproxil fumarate or TDF is an acyclic nucleoside phosphonate administered as an esterase-sensitive prodrug. [4] Tenofovir alafenamide (TAF), a new prodrug of TFV is more efficient than TDF in terms of higher levels of TFV-DP (diphosphate) delivery into pheripheral blood mononuclear cells (PBMCs) at much lower TAF doses. TAF is under trial as fixed-dose combinations (similar to Stribild) with FTC and integrase inhibitor elvitegravir plus a pharmacoenhancer cobicistat to prolong the effect of elvitegravir. [30] Another recently developed NtRTI is CMX-157 which is under Phase I trial and is a phospholipid based prodrug of TFV with significant concentrations of TFV and its active metabolite in PBMCs. [30]

Several amino acid substitutions such as K65R, K70E, L74V, Y115F and M184I or M184V confer resistance to NRTIs by increasing the capacity of HIV-1 RT to differentiate the TP derivatives of NRTIs. K65R mutation is associated with TFV resistance [31] and interaction of Arg instead of Lys with the γ-phosphate of the incoming dNTP [32] restricts the conformational adaptability of the RT polymerase active site with a decreased nucleotide incorporation rate compared with the wild-type enzyme. [33-34] TDF resistance is associated with K65R and K70R mutations with the former substitution having greater impact on resistance while K65R and M184V patterns have greater impact on ABC resistance than L74V and Y115F patterns. [4] The HIV-1 RT polymerase with K65R mutation is capable of differentiating several NRTIs including TFV, ddI, ABC, 3TC, FTC and ddC. Mutation in K65R gene is associated with Apricitabine resistance with concomitant resistance to ddI and TFV. [21] 3TC and FTC resistance is related to amino acid substitutions M184I and M184V respectively containing β-branched amino acid that hinders the interaction of 3TC-TP or FTC-TP with the oxathiolane ring of the inhibitor due to a steric clash with NRTI. [35] Multi drug resistance is found in the Q151M complex comprising A62V, V75I, F77L, F116Y and Q151M mutations that decrease incorporation of AZTTP, d4TTP, ddATP, ddCTP or carbovir-TP [36-37] due to the loss of hydrogen bonding interactions altering the 3′-OH group of the dNTP ribose moiety. The Q151M complex confers resistance to FTC, AZT, 3TC, ABC inhibitors but not to TFV inhibitor. [4] Besides NRTI-associated resistance mutations, there is another mechanism by which HIV-1 becomes resistant to NRTIs through acquisition of thymidine analogue resistance mutations (TAMs) during treatment with AZT, d4T or TFV and involves M41L, D67N, K70R, L210W, T215F or T215Y, and K219E or K219Q amino acid substitutions. [4] AZT resistance due to TAM also causes cross-resistance to ABC and TFV. HIV-1 RTs having TAMs cause pyrophosphate donor (ATP)-mediated phosphorolysis of AZT, d4T and TFV to form the dinucleoside tetraphosphate product, finally forming dead-end complex (DEC) that inhibits the reaction when the 3′-OH of the primer occupies the P site and the complementary dNTP binds to the N site and remove 3′-terminal chain-terminators from blocked DNA primers. [4] ATC resistance occurs due to HIV-1 RT mutations such as L74V, M184V, K65R, V75I, M184V, Q151M, Q151M/M184V, TAMs including M41L and T215F/Y [38-39] while dexelvucitabine resistance is found with deletion of Ser68 in combination with K65R mutation. [40]

The 4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) is an NRTI under preclinical development and inhibits HIV-1 replication by blocking primer translocation after being inserted into the DNA chain as EFdA monophosphate. [41] EFdA is more active than AZT and is effective against a broad range of K65R, L74V, M41L/T215Y or the Q151M complex mutations bearing drug-resistant isolates. [42] EFdA resistance is due to M184V along with P119S and T165A mutation. [42-43] Another NRTI under preclinical development is GS-9148 [(5-(6-amino-purin-9-yl)-4-fluoro-2, 5-dihydro-furan-2-yloxymethyl)phosphonic acid], which is a phosphonate nucleotide analogue RTI effective against a broad range of K65R, L74V or M184V, combinations of 6 TAMs mutations bearing drug-resistant isolates. [44]

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTI)

Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) are small (<600 Da) hydrophobic compounds that directly bind at sites, about 10 A^{o} away, other than the substrate-binding site (where the NRTI bind) at a hydrophobic pocket adjacent to the viral RT active site in the RT p66 subunit and inhibit it non-competitively to induce conformational change in the enzyme which leads to its inactivation. [4, 45] They inhibit the RT of HIV-1 but not that of HIV-2. The approved drugs such as first generation NNRTI (nevirapine, delavirdine, efavirenz), next generation NNRTI (etravirine and rilpivirine) and the newer NNRTIs such as Lersivirine, difluoromethylbenzoxazole (DFMB) pyrimidine thioether derivatives, MK-6186, RO-0335, dapivirine, under preclinical development are depicted in Table 2. [46-57] Currently used drugs are Efavirenz, Nevirapine, Delavirdine and the newer agents in this group are Etravirine and Rilpivirine. Rilpivirine has completed phase III trials [58] and is recommended with a high-calorie meal for its good systemic distribution. [56]

Table 2: Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

Drugs (Approval date: US) [Reference]	Systematic name	Combination	Side effects
Old NNRTI			
Efavirenz (09/1998) [46-48]	(4*S*)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1*H*-3,1-benzoxazin-2-one	Two or more NRTIs and/or PI	Psychiatric symptoms, birth defects
Nevirapine (06/1996) [49-51]	11-cyclopropyl-4-methyl-5,11-dihydro-6*H*- dipyrido[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one	Three-drug regimens	Rash
Delavirdine (04/1997) [52-53]	*N*-[2-({4-[3-(propan-2-ylamino)pyridin-2-yl]piperazin-1-yl}carbonyl)-1*H*-indol-5-yl] methanesulfonamide	Zidovudine and didanosine and/or PI	Rash, fatigue, headache and nausea
Etravirine (01/2008) [2,54-55]	4-[6-Amino-5-bromo-2- [(4-cyanophenyl)amino] pyrimidin-4-yl]oxy-3,5-dimethylbenzonitrile	As a combination therapy in treatment experienced adults	Cutaneous reactions as a maculopapular rash
New NNRTI			
Rilpivirine (05/2011) [56-57]	4-{[4-({4-[(*E*)-2-cyanovinyl]-2,6-dimethylphenyl}amino)pyrimidin-2-yl]amino}benzonitrile	As a combination therapy with other NNRTI	Depressive disorders, insomnia, headache, rash

Rilpivirine is metabolised in liver by cytochrome CYP3A and Etravirine by cytochrome P450 CYP3A4, CYP2C9, CYP2C19 and have the potential for drug interactions with co-administered medications. [2]

Rash, central nervous system adverse effects, and hepatic amino transferase level elevations are generally associated with efavirenz-based regimens. [59]

The first generation NNRTIs nevirapine, delavirdine and efavirenz inhibit RTs by binding at catalytic sites of DNA polymerase, created through shifting of aromatic side-chains of Tyr181 and Tyr188, as well as through shifting of primer grip region (β12-β13-β14 strands) away from catalytic triad (β6-β10-β9 strands). [60] Nevirapine or efavirenz resistance is contributed by mutations that disrupt the stacking interactions such as Y181C, that limit the conformational flexibility of the NNRTI binding pocket (e.g. Y188L), or facilitate exit of the NNRTI from the binding site (e.g. K103N).

The next generation NNRTI etravirine is a diaryl pyrimidine compound that inhibits RTs by forming hydrogen bond with Lys101 and by placing its benzonitrile moiety in the binding pocket (formed by Val106, Pro225, Phe227, Leu234, Pro236 and Tyr318) with the dimethylcyanophenyl group oriented towards Tyr188, Phe227 and Trp229. [61-62] The amino acid substitutions related to Etravirine resistance are V90I, A98G, L100I, K101E/H/P, V106I,E138A/K/G/Q, V179D/F/T, Y181C/I/V, G190A/S and M230L amongst which at least two mutations associated with high etravirine resistance are V179F/Y181C, V179F/Y181I or Y181I/M230L. [61, 63-64] The other next generation NNRTI rilpivirine is also a diaryl pyrimidine compound that binds the NNRTI binding pocket in different conformations, positions and orientations due to the drug's torsional flexibility and compact design. [61] Rilpivirine resistance is associated with mutations such as V90I, L100I, K101E, V106A/I, V108I, E138G/K/Q/R,V179F/I, Y181C/I, V189I, G190E, H221Y, F227C and M230I/L out of which E138K and M184I are the most frequent mutations. [65-66] However, E138K has been found to be the major mutation associated with resistance to both rilpivirine and etravirine in the absence of NNRTI resistance mutations. [67]

Lersivirine, difluoromethylbenzoxazole (DFMB) pyrimidine thioether derivatives, MK-6186, RO-0335 and dapivirine are NNRTIs in preclinical development. Lersivirine (UK-453,061) is a pyrazole derivative that binds the RT in a conformation different from that shown by nevirapine or efavirenz, characterized by the rotation of Tyr181. Lersivirine is active against HIV-1 strains bearing single amino acid substitutions in the RT such as L100I, K101E, K103N, V106A, V108I, E138K, Y181C, Y181I, M184V, Y188C, F227L, E233V, L234I, and P236L. [68] High-level *in vitro* resistance to lersivirine is conferred by F227C as well as the double-mutants V106A/F227L and Y181I/Y188L. [68] Mutations found in patients failing therapy with lersivirine include K101E, V106M, V108I, Y188H, H221Y, F227C or L, and L234I. [69] Other NNRTIs in preclinical development that show excellent potency against K103N and Y181C mutants are difluoromethyl benzoxazole (DFMB) pyrimidine thioether derivatives [70], MK-6186 [4], RO-0335 [64], and dapivirine (TMC120), a DAPY analogue, developed to be used as a vaginal microbicide. [4] However, high-level resistance to dapivirine is conferred by mutations L100I/K103N and K103N/Y181C [4], and other mutations such as L100I, K101E, V106I, E138K, V179I, Y181C and G190A have been selected *in vitro* in the presence of the drug. [4] Not surprisingly, the mutational profile of dapivirine resembles the ones described for etravirine and rilpivirine.

NUCLEOTIDE-COMPETING REVERSE TRANSCRIPTASE INHIBITORS

There is a new class of HIV-1 RTIs, known as nucleotide-competing RT inhibitors (NcRTIs) that are competitive inhibitors of natural dNTPs and act by binding to the active site of DNA polymerase during reverse transcription. [4] The indolopyridinone compound such as INDOPY-1 represents a NcRTI.

CELL ENTRY INHIBITORS

HIV-1 infection occur after binding of gp120 viral envelope glycoprotein with CD4 receptor and CCR5, CXCR4 coreceptors which cause fusion of the viral and host cell membranes in presence of gp41 viral transmembrane glycoprotein. [4] Cell entry inhibitors include fusion inhibitors (e.g. enfuvirtide, albuvirtide and sifuvirtide), CXCR4 and CCR5 antagonists (e.g. bicyclams such as AMD3100, maraviroc, vicriviroc and cenicriviroc), attachment inhibitors (e.g.zintevir, chicoric acid derivatives, dextran sulfate, cyanovirin andlectins), CD4 binding inhibitors (e.g. azaindole derivatives such as BMS-378806 or BMS-599793), monoclonal antibodies against CD4, the coreceptor or the gp120/gp41 complex (e.g. ibalizumabor IgG1b12), and protease inhibitors. Amongst these drugs only maraviroc and enfuvirtide have been approved for clinical use. However enfuvirtide recommended as twice-daily subcutaneous injections, is used as salvage therapy against multidrug-resistant HIVs due to its high cost. [4] There are two types of cell entry inhibitors, viz. fusion inhibitors and co-receptor inhibitors.

FUSION INHIBITORS

The HIV fusion protein is a Class I trimeric protein consisting of three noncovalently attached gp120/gp41 heterodimers (a product of gp160 precursor protein), gp120 is the outer receptor binding subunit and gp41 is the fusion subunit. The gp41 subunit contains at the N-terminal a fusion peptide, a heptad repeat region or HR1, a linker region, and a heptad repeat region or HR2 at the C-terminal, a tryptophan rich juxtamembrane region, a transmembrane domain, and a cytoplasmic tail. The formation of six-helix bundle trimeric hairpin loop structure by gp41 is essential for

fusion to occur with three HR2 helices packing in an antiparallel fashion, in the grooves created by a central triple helical coiled-coil composed of three HR1 regions. [71] Enfuvirtide (Fuzeon approved on 03/2003) is a fusion inhibitor that blocks HIV cell entry. It is homologous to a segment of the peptide motif HR2 (heptad repeat) region of gp41 corresponding to amino acids 643-678. As a linear 36 amino acid peptide, enfuvirtide binds to the HR1 region of gp41 blocking the formation of the six-helix bundle hairpin structure necessary for fusion and thus enfuvirtide function by blocking the interaction between HR2-HR1, the binding of which is a prerequisite for fusion to occur. [72] Under drug selection pressure, the high replication rate of HIV and the low fidelity of the HIV reverse transcriptase enzyme cause resistance development to enfuvirtide. [72] The development of resistance is associated with single and double substitution mutation in a conserved region spanning amino acids 36-45 in gp41. [73-74] Enfuvirtide is not available in oral form, causes adverse reaction at the injection site, is very expensive, and is generally only considered for patients with very few remaining ARV options, as a salvage therapy in patients with multi-drug resistant HIV.

CO-RECEPTOR INHIBITORS

Co-receptor inhibitors include CCR5 receptor antagonist that belong to a relatively new class of HIV-1 entry inhibitors. CCR5 is a chemokine co-receptor used by T-cell-tropic HIV strains during attachment of HIV-1 virus before viral fusion and entry into host cells. [75] The HIV fusion occurs by conformational changes due to attachment of CD4 to a gp120 pocket so that the V3 loop obtrudes and β-bridging sheets are formed, causing better access of the coreceptor attachment site on the target cell. [76]

Maraviroc (MVC) [77] (Brand name: Selzentry, Celsentri; US approval date: 08/2007) is the only CCR5 antagonist that has been licensed for clinical use as an HIV-1 antiretroviral therapy. [78] Other CCR5 antagonist HIV-1 inhibitors that lack clinical efficacy and possess adverse side effects include vicriviroc, aplaviroc and cenicriviroc. [79] The CCR5 co-receptor antagonist drugs act by binding to the hydrophobic pocket formed by the transmembrane helices of CCR5 which leads to structural alterations in the extracellular loops, ECL (projecting from the helices) such that they are no longer recognized by gp120. The N terminus is unaffected by the binding of CCR5 antagonists to CCR5. Thus, CCR5 antagonists are not competitive inhibitors of CCR5; rather they are allosteric inhibitors of HIV-1 entry. [2] Resistance to MVC occurs due to a critical interaction of HIV-1 with the sulfated tyrosine residues at position 10 and 14 of the CCR5 N-terminus and an altered interaction with histidine residues of the CCR5 ECL1 and ECL2 regions such that the adaptive changes in the gp120 V3 loop causes the viruses to utilize the antagonist-modified form of CCR5; mutations in gp41 conferring this ability have also been observed. [79]

INTEGRASE INHIBITORS

Integrase inhibitors are potent agents against HIV which function to inhibit the integrase enzyme required in replication of HIV. [80] Raltegravir [Isentress: *N*-(2-(4-(4-fluorobenzylcarbamoyl)-5-hydroxy-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)propan-2-yl); US approval date: 10/2007] and Elvitegravir {EVG: 6-[(3-Chloro-2-fluorophenyl)methyl]-1-[(2*S*)-1-hydroxy-3-methylbutan-2-yl]-7-methoxy-4-oxoquinoline-3-carboxylic acid} are recently approved drugs in this class. Eron *et al.* (2013) demonstrated that raltegravir, the HIV-1 integrase strand-transfer inhibitor, with optimized background therapy showed reduction of viral loads to less than 50 copies per mL in 51% patients while mean CD4 cell count increase was 164 cells per Ml and found a favourable long-term efficacy (at week 240) and safety profile (the most common drug-related adverse events at 5 years were nausea, headache, and diarrhea) with raltegravir in treatment-naive and previously treated patients having triple-class resistant HIV in whom antiretroviral therapy is failing. [81] Mutation in genes encoding integrase is associated with resistance to integrase inhibitors. Resistance to raltegravir is due to a primary mutation at position 155, 148, or 143 with a concomitant secondary mutation at other positions in the integrase. [82]

PROTEASE INHIBITORS

Protease inhibitors are small peptidic or non-peptidic competitive inhibitors of HIV-1 proteases which are member of a highly conserved aspartic protease family consisting of a dyad of aspartate residues one in each lobe of the enzyme's bilobal structure to activate a nucleophilic water molecule and catalyze peptide cleavage. [83] The drugs prevent the HIV protease from cleaving the viral gag-pol polyprotein and carrying out proteolytic processing of precursor viral proteins into mature viral proteins. [84] This action prevents the production of infectious viral particles with the subsequent decrease of the viral load and the partial immune restoration. [85] There are three generation of PIs developed to improve the drug efficacy and/or properties. Currently, there are six approved PIs namely, darunavir, lopinavir, atazanavir, fosamprenavir, saquinavir and tipranavir all of which act by binding to the active site of the enzyme. Darunavir, lopinavir and atazanavir are mostly used, and except atazanavir all PIs are used with ritonavir-boosting. All PIs except tipranavir are peptidomimetics. Table 3 depicts the different types of protease inhibitors and their characteristics. [86-96] There are two types of mutations conferring resistance to protease inhibitors, primary and secondary resistance mutations. Primary mutations such as D30N, V32I, L33F, M46I/L, I47A/V, G48V, I50L/V, V82A/F/L/S/T, and I84A/V act

Table 3: Protease inhibitors

Protease inhibitors (Approval date: US)[Reference]	Systematic name	Common side effects
1st generation		
Saquinavir (12/1995) [84]	(2*S*)-*N*-[(2*S*,3*R*)-4-[(3*S*)-3-(*tert*-butylcarbamoyl)-decahydroisoquinolin-2-yl]-3-hydroxy-1-phenylbutan-2-yl]-2-(quinolin-2-ylformamido)butanediamide	Gastrointestinal symptoms
Ritonavir (03/1996) [85]	1,3-thiazol-5-ylmethyl *N*-[(2*S*,3*S*,5*S*)-3-hydroxy-5-[(2*S*)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl] methyl}) carbamoyl]amino} butanamido]-1,6-diphenylhexan-2-yl]carbamate	Hypercholesterolemia, hypertriglyceridemia, hyperglycemia
2nd generation		
Lopinavir/ritonavir (09/2000) [86-87]	(2*S*)-*N*-[(2*S*,4*S*,5*S*)-5-[2-(2,6-dimethylphenoxy)acetamido]-4-hydroxy-1,6-diphenylhexan-2-yl]-3-methyl-2-(2-oxo-1,3-diazinan-1-yl)butanamide	Gastrointestinal disturbances, lipid abnormalities, CSF penetration
Fosamprenavir (10/2003) [86, 88]	–	–
Atazanavir (06/2003) [86,89-91]	methyl *N*-[(1*S*)-1-{[(2*S*,3*S*)-3-hydroxy-4-[(2*S*)-2-[(methoxycarbonyl)amino]-3,3-dimethyl-*N*'-{[4-(pyridin-2-yl)phenyl]methyl}butanehydrazido]-1-phenylbutan-2-yl]carbamoyl}-2,2-dimethylpropyl]carbamate	Bilirrubin elevation, should not be used with proton pump inhibitors
3rd generation		
Tipranavir (06/2005) [92-93]	*N*-{3-[(1*R*)-1-[(2*R*)-6-hydroxy-4-oxo-2-(2-phenylethyl)-2-propyl-3,4-dihydro-2*H*-pyran-5-yl]propyl]phenyl}-5-(trifluoromethyl)pyridine-2-sulfonamide	Ddrug interactions, inhibition of CYP isoenzymes
Darunavir (06/2006) [94]	[(1*R*,5*S*,6*R*)-2,8-dioxabicyclo[3.3.0]oct-6-yl] *N*-[(2*S*,3*R*)-4- [(4-aminophenyl)sulfonyl- (2-methylpropyl)amino]-3-hydroxy-1-phenyl- butan-2-yl] carbamate	Diarrhoea, nausea, headache, upper respiratory tract infection, nasopharyngitis

Table 4: Combination HAART regimen (Menendez-Arias, 2013)[4]

Drugs (Approval date in US)
Fixed-dose combinations of nucleostide analogues
Lamivudine/Zidovudine (09/1997)
Abacavir/Lamivudine/Zidovudine (11/2000)
Abacavir/Lamivudine (08/2004)
Emtricitabine/Tenofovir DF (08/2004)
Fixed-dose combinations of nucleostide and non-nucleostide inhibitors
Efavirenz/Emtricitabine/Tenofovir DF (07/2006)
Emtricitabine/Rilpivirine/Tenofovir DF (08/2011)
Fixed-dose combinations of reverse transcriptase and Integrase inhibitors
Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF (08/2012)

through modification of residues present in the substrate-binding pocket or its vicinity. [97] Secondary mutations arise during treatment with PIs and occur at codons encoding amino acidsout of the enzyme active site. For example N88D mutation compensates impaired replication due to D30N mutation in presence of L90M mutation. [98] Other molecular mechanism of secondary mutation include mutation A431V at NC/p1 gag cleavage site and L449F mutation at p1/p6 gag cleavage site in presence of D30N, I50L or I84V mutations. [99]

The newly prescribed PIs show very high genetic barriers to resistance i.e., PIs require large number of mutations to overcome drug selection pressure. For e.g. darunavir or tipranavir require 20-30 mutations in the protease-coding region to exhibit decreased susceptibility in HIV-1 isolates whereas a high-level resistance to ritonavir-boosted lopinavir or darunavir usually requires a minimum of three to four mutations. [100] PIs such as darunavir-like compounds e.g. GRL-1398, are in preclinical trial phase and have shown efficacy against HIV variants resistant to PIs and that require 8 or several mutations such as A28S [101], or TMC310911 at codon 41 in the protease-coding region [102], seldom found in patients treated with currently sanctioned antiretroviral drugs.

Vidal *et al* (2013) reported a high rate of virologic and immunologic suppression with darunavir/ritonavir plus optimized background treatment among highly antiretroviral-experienced HIV-infected patients in Brazil with 83% patients showing HIV RNA level <50 copies/mL and the median CD4 cell count of 301 cells/mm^3 from a baseline HIV RNA >100000 copies/mL that was inversely associated with virologic success at week 48. [103] Gastrointestinal symptoms, lipid abnormalities, and hepatic amino transferase level elevations appear to be generally associated with protease inhibitor (PI)-based regimens. [59]

HAART regimen typically consist of two NRTIs (either Tenofovir and Emtricitabine or Abacavir and Lamivudine or Zidovudine and Lamivudine) combined with an NNRTI (Efavirenz) or a boosted PI (either Atazanavir/Darunavir or Lopinavir/ritonavir or Lopinavir and Saquinavir), and choosing which combination to use depends on considerations of drug potency, tolerability, potential for adherence, and resistance. [5] The choice between NNRTI and PI therapy is specific to the individual patient, determined based on which adverse events the patient is able or willing to tolerate. Raltegravir, the integrase strand transfer inhibitor, is also used for the treatment of naive patients.

Fixed-dose antiretroviral agents, as dual NRTIs are available in combinations of Tenofovir/Emtricitabine, Zidovudine/Lamivudine and Abacavir/Lamivudine while boosted PIs is available as Lopinavir/Ritonavir combination. [5] Combined chemotherapy with two NRTIs or NRTIs plus NNRTIs possess *in vitro* synergistic activity with a reported mechanism of enhanced formation of phosphorylated metabolites with emricitabine plus tenofovir combination. [104-105]

The triple drug combination of Rilpivirine/Emricitabine/Tenofovir exhibited a stronger synergistic effect compared to the Efavirenz/Emtricitabine/Tenofovir combination, the

proposed mechanism being the increased formation of dead end complexes with tenofovir-terminated DNA primer/template plus HIV-1 RT in the presence of emricitabine-triphosphate or rilpivirine, and by emricitabine-monophosphate-terminated DNA primer /template and HIV-1 RT in the presence of rilpivirine, as well as increased levels of the active metabolites tenofovir-diphosphate and emricitabine-triphosphate when the drugs are dosed together. [106] Thus HAART combinations have been simplified with single-tablet, once-daily, fixed-dose regimen to reduce pill burden and frequency of administration that can in turn affect adherence to the drug besides having improved efficacy and a higher barrier to resistance development. [107-108] Table 4 contains information on anti-HIV medication approved by Food and Drug Administration, USA. [4]

HIV VACCINE

Besides progress being made towards development of medicines against HIV, several efforts have been made to produce HIV vaccine that could either confer long-term immunity to HIV infection based on cellular or humoral immune response that eradicates the virus or that could control HIV viremia by vaccination. Despite several diligent efforts, no safe and effective vaccines have yet being produced that can completely eliminate the disease. However, some of these attempts show promising results that require continued research efforts. Several clinical trials to evaluate the safety and efficacy of therapeutic vaccine have been implemented some of which are depicted in Table 5. [109-111]

Nabel (2007) reported few HIV candidate vaccines involving canarypox and recombinant envelope (Env) glycoprotein in combination, an adenovirus vector encoding gag, pol and nef proteins from HIV clade B, and another involving recombinant adenovirus 5. [3] The first indication that a vaccine could protect against HIV-1 infection was made by RV144 study in which vaccination with an avipox vector, boosted with the bivalent gp120s showed 31% fewer HIV-1 infections in the vaccine arm compared to the placebo arm in volunteers. [112] The HVTN 505 Study Team tested the efficacy of a DNA prime-recombinant adenovirus type 5 boost (DNA/rAd5) vaccine regimen in persons at increased risk for HIV-1 infection in the United States; the 6-plasmid DNA vaccine (expressing clade B gag, pol, and nef and env proteins from clades A, B, and C) administered at weeks 0, 4, and 8, while the rAd5 vector boost (expressing clade B gag-pol fusion protein and env glycoproteins from clades A, B, and C) was administered at week 24, however, the DNA/rAd5 vaccine regimen did not reduce either the rate of HIV-1 acquisition or the viral-load set point in the population studied. [111] The HVTN 505 vaccine induced antibodies, nonspecific for HIV infection, which recognized HIV as well as intestinal microbiome, thus the HVTN 505 vaccine candidate did not perform well. [113]

The vaccines based on monoclonal antibodies (mAbs) bind to site within the V2-V3 region of the HIV-1 viral spike [114] and also in the functionally conserved region of gp120 that interacts with the host cell receptor CD4. [115-116] The variable loops V2 and V3 within gp120 involved with HIV-1 recognition of the α4β7-integrin, the gut-homing receptor for HIV-1 used by primary isolates during early stages of infection [117] are recognized by the mAbs, that were reported to be extremely strain-specific while other mAbs were able to neutralize 70%–80% of current circulating isolates. [114, 118] Recent progress in the identification of vaccines that elicit broadly neutralizing antibodies have provided new opportunities to advance the goal of producing vaccines that prevent infection and/or progression to AIDS. [119] Advances are been made to treat and prevent HIV infections based on improvement of known and novel broadly neutralizing antibodies using combination of two complementary antibodies, each targeting different sites on the HIV surface molecule. [120] Universal sterilizing HIV vaccine that elicit cross-reactive and broadly neutralizing antibodies showed promising results with the such antibodies appearing in 10 to 30% of infected persons. [121] Currently development are towards rational design of effective HIV vaccine with directed-antigen evolution to produce HIV-envelope immunogens that will strongly attach and activate the germline naïve B-cell receptors. [122]

Despite the risks in terms of drug toxicities involved with antiretroviral therapy for HIV infection, the treatment could outweigh the benefits of controlling the virus. The results of three large clinical trials, SMART study in 2006, HPTN 052 study in 2011 and START study in 2015 demonstrate the benefits of starting ART early in infection that outweigh any theoretical risk protecting the health of the infected individual while preventing HIV transmission to uninfected individuals. [123] Besides, preexposure prophylaxis (PrEP) is nowadays a promising interventions on HIV prevention, which involves initiation of antiretroviral drugs in HIV-negative persons before potential exposure to the virus, with either TDF or the combination of TDF and FTC has been shown to provide protection in men at high risk for HIV-1 infection. The combination of PrEP and prompt initiation of ART for infected individuals offers a promising suggestion, made by National Institute of Allergy and Infectious Diseases (NIAID) scientists, to bring about an end to the HIV/AIDS pandemic. [123] A significant progress have been made on the development of antiretrovirals based on the concepts of targeting different steps of the viral life cycle including genome replication (reverse transcriptase inhibitors), maturation (viral protease inhibitors), viral co-receptor antagonist (maraviroc), fusion inhibitor, (enfuvirtide) and integrase inhibitors (raltegravir and elvitegravir). [84] Formulations of the same are well established, but

formulations for Elvitegravir and the nonnucleoside reverse transcriptase inhibitor rilpivirine is now emerging. [4] There are several on-going clinical trials in 2015 and onwards for the implementation of antiretroviral medications among people with HIV infection some of which are indicated in Table 6. [124-129] The combined therapy of the old and the newer drugs

Table 5: Recent HIV vaccine clinical trials

Clinical Trials.gov Identifier [Reference]	Study Duration (Phase)	Vaccine	Purpose
NCT02413645 [109]	Jun 2015- Oct 2016 (Phase 1)	TriMix_100; TriMix_300; 600µg mRNA (300µg HIV mRNA+300µg TriMix mRNA; 900µg mRNA (600µg HIV mRNA+300µg TriMix mRNA; 1200µg mRNA (900µg HIV mRNA+300µg TriMix mRNA	To evaluate the safety and to establish the recommended dose of iHIVARNA-01 as a new therapeutic vaccine against HIV
NCT01627678 [110]	Sept 2012- Nov 2013 (Phase 1 and 2)	Vacc-C5/GM-CSF Vacc-C5/Alhydrogel	To investigate the formation of non-neutralizing antibody against C5 region by Vacc-C5, which is a single heterodimeric peptide-based HIV vaccine corresponding to the C5 region on gp120 and the external domain of gp41
NCT01859325 [111]	May 2013- Jan 2030 (Phase 1)	Attenuated recombinant vesicular stomatitis virus containing HIV-1 gag gene; plasmid DNA containing human IL-12 gene; plasmid DNA containing genes encoding multiple HIV-1 proteins	To evaluate the safety and efficacy of therapeutic vaccine (to augment immunologic control of HIV infection and obviate the need for chronic combination ART), namely, HIV-1 multiantigen plasmid vaccine prime in combination with an interleukin-12 plasmid DNA adjuvant delivered by *in vivo* electroporation followed by a recombinant vesicular stomatitis virus vector containing the HIV-1 gag gene booster vaccine in subjects on combination ART who started therapy during acute or early HIV infection

Table 6: Present on-going clinical trials in 2015

Clinical Trials.gov Identifier [Reference]	Study Duration (Phase)	Drugs	Purpose
NCT02206555 [124]	Nov 2014- Nov 2016 (Phase 4)	Emtricitabine/Tenofovir Disoproxil fumarate	Primary HIV prevention with pre exposure prophylaxis in sero-negative individuals at high risk for HIV infection
NCT02527096 [125]	Sept2015- Nov 2017 (Phase 2)	Ddolutegravir and Lamivudine	Evaluation of the antiviral efficacy of 48 weeks treatment with the two drugs combination Dolutegravir and Lamivudine in HIV-1 infected patients virologically suppressed with triple HAART.
NCT02514707 [126]	Jan2014-Jun 2025	Any combination including second line	Evaluation of the outcomes of HIV infected individuals after ten years on antiretroviral treatment
NCT02572947 [127]	Dec 2015- Sept 2016 (Phase 2)	Dolutegravir	To investigate the feasibility of Dolutegravir monotherapy in maintenance therapy in virologically suppressed patients for at least six months on conventional triple ART of Dolutegravir plus two NsRTIs
NCT02513901 [128]	July 2015- Dec 2015 (Phase 1 and 2)	Chidamide with ART	To evaluate the safety and efficacy of multi-dose Chidamide in combination with antiretroviral therapy in HIV-infected adults with suppressed viral load
NCT02469246 [129]	Jun 2015- Jun 2018 (Phase 3)	Emtricitabine/Tenofovir Aalafenamide or Abacavir/Lamivudine; both along with allowed 3rd pre-existing ARV regimen	To evaluate the efficacy, safety, and tolerability of switching Aabacavir/Lamivudine (ABC/3TC) to Eemtricitabine/Tenofovir Alafenamide versus maintaining ABC/3TC in HIV-1 infected adults who are virologically suppressed on regimens containing ABC/3TC.

with different mechanism of actions and belonging to different groups have improved the spectrum of treatment of AIDS. [130] However, adverse reactions to medication play major role in non-adherence to treatment; moreover, acquisition of drug resistance reduces the efficacy of treatment too. Hence, to make antiretroviral therapy suitable and to overcome the problem of drug resistance, the search for safe and efficient antiretroviral agent with improved bioavailability, pharmacokinetics with simplified dosing regimens, and the development of newer cost effective agents are required for preventive therapies. [5]

REFERENCES

1. UNAIDS. UNAIDS report on the global AIDS epidemic 2012. UNAIDS, Geneva, Switzerland. 2012.
2. Jaykare SC, Motghare VM, Padwal SL, Pise HN, Deshpande RP. Antiretroviral therapy- newer drugs on the anvil. Indian J Med Specialities 2013; 4:47-54.
3. Nabel GJ. Mapping the future of HIV vaccines. Nature Rev Microbiol 2007; 5: 482-84.
4. Menendez-Arias L. Molecular basis of human immunodeficiency virus type 1 drug resistance: Overview and recent developments. Antiviral Res 2013; 98:93-120.
5. Tremblay CL, Baril J, Fletcher D, Kilby D, MacPherson P, Shafran SD, *et al.* Challenges in initiating antiretroviral therapy in 2010 Can J Infect Dis Med Microbiol 2010; 21:1-15.
6. Cooper CL, Breau C, Laroche A, Lee C, Garber G. Clinical outcomes of first antiretroviral regimen in HIV/hepatitis C virus co-infection. HIV Med 2006; 7:32-37.
7. Mitsuya H, Weinhold K, Furman P, St Clair M, Li L, Lehrman S, *et al.* 3'-Azido-3'-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-

associated virus in vitro. Proc Natl Acad Sci USA 1985; 82:7096-100.
8. Sun R, Eriksson S, Wang L. Identification and characterization of mitochondrial factors modulating thymidine kinase 2 activity. Nucleosides, Nucleotides and Nucleic Acids. 2010; 29:382-5.
9. Scruggs ER, Dirks Naylor AJ. Mechanisms of zidovudine-induced mitochondrial toxicity and myopathy. Pharmacol 2008; 82:83-8.
10. Hivid (zalcitabine) tablets. Product information. (September 2002). Available from http://www.rocheusa.com/products/hivid/pi.pdf
11. Horwitz JP, Chua J, DaRooge MA, Noel M, Klundt IL. Nucleosides. IX. The formation of 2', 2'-unsaturated pyrimidine nucleosides via a novel beta-elimination reaction. J Org Chem1966; 31:205-11.
12. Wangsomboonsiri W, Mahasirimongkol S, Chantarangsu S, Kiertiburanakul S, Charoenyingwattana A, Komindr S, *et al*. Association between HLA-B*4001 and lipodystrophy among HIV-infected patients from Thailand who received a stavudine-containing antiretroviral regimen. Clin Infect Dis 2010; 50:597-604.
13. Hetherington S, Hughes AR, Mosteller M, Shortino D, Baker KL, Spreen W, *et al*. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. Lancet 2002; 359:1121-2.
14. Rauch A, Nolan D, Martin A, McKinnon E, Almeida C, Mallal S. Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study. Clin Infect Dis 2006; 43:99-102.
15. Fox Z, Dragsted UB, Gerstoft J, Phillips AN, Kjaer J, Mathiesen L, *et al*. A randomized trial to evaluate continuation versus discontinuation of lamivudine in individuals failing a lamivudine-containing regimen: The COLATE trial. Antivir Ther 2006; 11:761-70.
16. VIDEX (didanosine): chewable/dispersible buffered tablets; buffered powder for oral solution; pediatric powder for oral solution. Product information (July 2000). Available from http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm
17. Kimberlin DW. Antiviral therapies in children: Has their time arrived? Pediatr Clin N Am 2005; 52:837-67.
18. Oxenius A, Price DA, Günthard HF, Dawson SJ, Fagard C, Perrin L, *et al.* Stimulation of HIV-specific cellular immunity by structured treatment interruption fails to enhance viral control in chronic HIV infection. Proc Natl Acad Sci USA 2002; 99:13747-52.
19. Cahn P, Wainberg MA. Resistance profile of the new nucleoside reverse transcriptase inhibitor apricitabine. J Antimicrob Chemother 2010; 65:213-7.
20. Cahn P, Cassetti I, Wood R, Phanuphak P, Shiveley L, Bethel RC, *et al.* Efficacy and tolerability of 10-day monotherapy with apricitabine in antiretroviral-naive, HIV-infected patients. AIDS 2006; 20:1261-8.
21. Wainberg MA, Cahn P, Bethell RC, Sawyer J, Cox S. Apricitabine: a novel deoxycytidine analogue nucleoside reverse transcriptase inhibitor for the treatment of nucleoside-resistant HIV infection. Antivir Chem Chemother 2007; 18:61-70.
22. Ravichandran S, Veerasamy R, Raman S, Krishnan N, Agrawal RK. An overview on HIV-1 reverse transcriptase inhibitors. Digest J Nanomaterials Biostructures 2008; 3:171-87.
23. Albrecht MA, Wilkin TJ, Coakley EP, Hammer SM. Advances in antiretroviral therapy. Top HIV Med 2003; 11:97-127.
24. Herzmann C, Arasteh K, Murphy RL, Schulbin H, Kreckel P, Drauz D, *et al.* Safety, pharmacokinetics, and efficacy of (+/-)-β-2′,3′- dideoxy-5-fluoro-3′-thiacytidine with efavirenzand stavudine in antiretroviral-naive human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 2005; 49:2828-33.
25. Mehellou Y, de Clercq E. Twenty-six years of anti-HIV drug discovery: where do we stand and where do we go? J Med Chem 2010; 53:521-38.
26. Cihlar T, Ray AS. Nucleoside and nucleotide HIV reverse-transcriptase inhibitors: 25 years after zidovudine. Antiviral Res 2010; 85:39-58.
27. Barnhart M, Shelton J. A better state of ART improving anti-retroviral regimens to increase global access to HIV treatment. J AIDS HIV Res 2011; 3:71-8.
28. Otto MJ. New nucleoside reverse transcriptase inhibitors for the treatment of HIV infections. Curr Opin Pharmacol 2004; 4:431-6.
29. De Clercq E. Antiviral drugs in current clinical use. J Clin Virol 2004; 30:115-33.
30. Hodge RAV. Meeting report: 26th International conference on antiviral research. Antiviral Res 2013; 100:276-85.
31. Margot N, Isaacson E, McGowan I, Cheng AK, Schooley RT, Miller MD. Genotypic and phenotypic analyses of HIV-1 in antiretroviral experienced patients treated with tenofovir DF. AIDS 2002; 16:1227-35.
32. Huang H, Chopra R, Verdine GL, Harrison SC. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. Science 1998; 282:1669-75.
33. Das K, Bandwar RP, White KL, Feng JY, Sarafianos SG, Tuske S, *et al.* Structural basis for the role of the K65R mutation in HIV-1reverse transcriptase polymerization, excision antagonism, and tenofovir resistance. J Biol Chem 2009; 284:35092-100.
34. Barrioluengo V, Álvarez M, Barbieri D, Menéndez-Arias L. Thermostable HIV-1 group O reverse transcriptase variants with the same fidelity as murine leukaemia virus reverse transcriptase. Biochem J 2011; 436:599-607.
35. Gao HQ, Boyer PL, Sarafianos SG, Arnold E, Hughes SH. The role of steric hindrance in 3TC resistance of human immunodeficiency virus type-1 reverse transcriptase. J Mol Biol 2000; 300:403-18.
36. Deval J, Selmi B, Boretto J, Egloff MP, Guerreiro C, Sarfati S, *et al*. The molecular mechanism of multidrug resistance by the Q151M human immunodeficiency virus type 1 reverse transcriptase and its suppression using boranophosphate nucleotide analogues. J Biol Chem 2002; 277:42097-104.
37. Frangeul A, Busetta C, Deval J, Barral K, Alvarez K, Canard B. Gln151 of HIV-1 reverse transcriptase acts as a steric gate towards clinically relevant acyclic phosphonate nucleotide analogues. Antivir Ther.2008; 13:115-24.
38. Gu Z, Allard B, de Muys JM, Lippens J, Rando RF, Nguyen-Ba N, *et al. In vitro* antiretroviral activity and *in vitro* toxicity profile of SPD754, a new deoxycytidine nucleoside reverse transcriptase inhibitor for treatment of human immunodeficiency virus infection. Antimicrob Agents Chemother 2006; 50:625-31.
39. Cahn P, Wainberg MA. Resistance profile of the new nucleoside reverse transcriptase inhibitor apricitabine. J Antimicrob Chemother 2010; 65:213-17.
40. Schinazi RF, Massud I, Rapp KL, Cristiano M, Detorio MA, Stanton RA, *et al.* Selection and characterization of HIV-1 with a novel S68 deletion in reverse transcriptase. Antimicrob Agents Chemother 2011; 55:2054-60.
41. Michailidis E, Marchand B, Kodama EN, Singh K, Matsuoka M, Kirby KA, *et al.* Mechanism of inhibition of HIV-1 reverse transcriptase by 40-ethynyl-2-fluoro-20-deoxyadenosine triphosphate, a translocation defective reverse transcriptase inhibitor. J Biol Chem 2009; 284:35681-91.
42. Kawamoto A, Kodama E, Sarafianos SG, Sakagami Y, Kohgo S, Kitano K, *et al.* 20-deoxy-40-C-ethynyl-2-halo-adenosines active against drug resistant human immunodeficiency virus type 1 variants. Int J Biochem Cell Biol 2008; 40:2410-20.
43. Yang G, Paintsil E, Dutschman GE, Grill SP, Wang CJ, Wang J, *et al.* Impact of novel human immunodeficiency virus type 1 reverse transcriptase mutations P119S andT165A on 40-ethynylthymidine analog resistance profile. Antimicrob Agents Chemother 2009; 53:4640-46.

44. Cihlar T, Ray AS, Boojamra CG, Zhang L, Hui H, Laflamme G, *et al.* Design and profiling of GS-9148, a novel nucleotide analog active against nucleoside-resistant variants of human immunodeficiency virus type 1, and its orally bioavailable phosphonoamidate prodrug, GS-9131. Antimicrob Agents Chemother 2008; 52:655-65.
45. Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science 1992; 256:1783-90.
46. Sustiva (efavirenz) capsules and tablets. Product information (April 2005). Available from http://www.bms.com/cgi-bin/anybin.pl
47. Lowenhaupt EA, Matson K, Qureishi B, Saitoh A, Pugatch D. Psychosis in a 12-year-old HIV-positive girl with an increased serum concentration of efavirenz. Clin Infectious Dis 2007; 45:e128-30.
48. Ford N, Mofenson L, Kranzer K, Medu L, Frigati L, Mills EJ, Calmy A. Safety of efavirenz in first-trimester of pregnancy: A systematic review and meta-analysis of outcomes from observational cohorts. AIDS 2010; 24:1461-70.
49. Montaner JS, Reiss P, Cooper D. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA 1998; 279:930-7.
50. Podzamczer D, Ferrer E, Consiglio E, Gatell JM, Perez P, Perez JL, *et al*. A randomized clinical trial comparing nelfinavir or nevirapine associated to zidovudine/lamivudine in HIV-infected naive patients (the Combine Study). Antiviral Ther 2002; 7:81-90.
51. Wit FW, Kesselring AM, Gras L. Discontinuation of nevirapine because of hypersensitivity reactions in patients with prior treatment experience, compared with treatment-naive patients: the ATHENA cohort study. Clin Infect Dis 2008; 46: 933-40.
52. DHHS panel. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents (May 4, 2006). (Available for download from AIDS Info).
53. Rescriptor brand of delavirdine mesylate tablets. Product information. Available from http://www.pfizer.com/pfizer/download/uspi_rescriptor.pdf
54. de Bethune MP. Non-nucleoside reverse transcriptase inhibitors (NNRTIs), their discovery development, and use in the treatment of HIV-1 infection: a review of the last 20 years (1989-2009). Antiviral Res 2010; 85:75-90.
55. Rathbun RC, Liedtke MD. The next generation: Etravirine in the treatment of HIV-1 infection in adults refractory to other antiretrovirals. Virus Adaptation and Treatment. 2010; 2:91-102.
56. Ford N, Lee J, Andrieux-Meyer I, Calmy A. Safety, efficacy, and pharmacokinetics of rilpivirine: systemic review with an emphasis on resource limited settings. HIV/AIDS 2011; 3:35-44.
57. James C, Preininger L, Sweet M. Rilpivirine: a second generation non-nucleoside reverse transcriptase inhibitor. Am J Health Syst Pharm 2012; 69:857-61.
58. De Clercq E. Where rilpivirine meets with tenofovir, the start of a new anti-HIV drug combination era. Biochem Pharmacol 2012; 84:241-8.
59. Panel on antiretroviral guidelines for adults and adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. December, 2009:1-161.
60. Paris KA, Haq O, Felts AK, Das K, Arnold E, Levy RM. Conformational landscape of the human immunodeficiency virus type 1 reverse transcriptase non-nucleoside inhibitor binding pocket: lessons for inhibitor design from a cluster analysis of many crystal structures. J Med Chem 2009; 52:6413-20.
61. Das K, Clark Jr AD, Lewi PJ, Heeres J, De Jonge MR, Koymans LM, *et al.* Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J Med Chem 2004; 47:2550-60.
62. Lansdon EB, Brendza KM, Hung M, Wang R, Mukund S, Jin D, *et al.* Crystal structures of HIV-1 reverse transcriptase with etravirine (TMC125) and rilpivirine (TMC278): implications for drug design. J Med Chem 2010; 53:4295-9.
63. Tambuyzer L, Azijn H, Rimsky LT, Vingerhoets J, Lecocq P, Kraus G. Compilation and prevalence of mutations associated with resistance to non-nucleoside reverse transcriptase inhibitors. Antivir Ther 2009; 14:103-9.
64. Javanbakht H, Ptak RG, Chow E, Yan JM, Russell JD, Mankowski MK, *et al.In vitro* resistance development for RO-0335, a novel diphenyl ether nonnucleoside reverse transcriptase inhibitor. Antiviral Res 2010; 86:212-9.
65. Cohen CJ, Andrade-Villanueva J, Clotet B, Fourie J, Johnson MA, RuxrungthamK, *et al.* On behalf of the THRIVE Study Group, Rilpivirine versus efavirenz with two background nucleoside or nucleotide reverse transcriptase inhibitors in treatment-naive adults infected with HIV-1 (THRIVE): a phase 3, randomised, non-inferiority trial. Lancet 2011; 378:229-37.
66. Azijn H, Tirry I, Vingerhoets J, de Béthune MP, Kraus G, Boven K, *et al.* TMC278, a next generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. Antimicrob Agents Chemother 2010; 54:718-27.
67. Asahchop EL, Wainberg MA, Oliveira M, Xu H, Brenner BG, Moisi D, *et al.* Distinct resistance patterns to etravirine and rilpivirine in viruses containing NNRTI mutations at baseline. AIDS 2012; http://dx.doi.org/10.1097/QAD.0b013e32835d9f6d.
68. Corbau R, Mori J, Phillips C, Fishburn L, Martin A, Mowbray C, *et al.* Lersivirine, a nonnucleoside reverse transcriptase inhibitor with activity against drug-resistant human immunodeficiency virus type 1. Antimicrob Agents Chemother 2010; 54:4451-63.
69. Vernazza P, Wang C, Pozniak A, Weil E, Pulik P, Cooper DA, *et al.* Efficacy and safety of lersivirine (UK-453,061) versus efavirenz in antiretroviral treatment-naïve HIV-1-infected patients: week 48 primary analysis results from an ongoing, multicenter, randomized, double-blind, phase IIb trial. J Acquir Immune Defic Syndr 2013; 62:171-9.
70. Boyer J, Arnoult E, Médebielle M, Guillemont J, Unge J, Jochmans D. Difluoromethylbenzoxazole pyrimidine thioether derivatives: a novel class of potent non-nucleoside HIV-1 reverse transcriptase inhibitors. J Med Chem 2011; 54:7974-85.
71. Pohlmann S, Reeves JD. Cellular entry of HIV: Evaluation of therapeutic targets. Curr Pharm Des 2006; 12:1963-73.
72. Greenberg ML, Cammack N. Resistance to enfuvirtide, the first HIV fusion inhibitor. J Antimicrob Chemother 2004; 54:333-40.
73. Greenberg ML, Sista P, Miralles G. Melby T, Davison D, Jin L, *et al.* Enfuvirtide (T-20) and T-1249 resistance: observations from Phase II clinical trials of enfuvirtide in combination with oral antiretrovirals (ARVs) and a Phase I/II dose-ranging monotherapy trial of T-1249. Antivir Ther 2002; 7:S106.
74. Mink M, Greenberg ML, Mosier S, Janumpalli *S.* Impact of HIV-1 gp41 amino acid substitutions (positions 36-45) on susceptibility to T-20 (enfuvirtide) *in vitro*: Analysis of primary virus isolates recovered from patients during chronic enfuvirtide treatment and site directed mutants in NL4-3. Antivir Ther 2002; 7:S24.
75. Lederman MM, Penn-Nicholson A, Cho M, Mosier D. Biology of CCR5 and its role in HIV infection and treatment. JAMA 2006; 296:815-26.
76. Kwong P. Human immunodeficiency virus: refolding the envelope. Nature 2005; 433:815-6.

77. Wood A, Armour D. The discovery of the CCR5 receptor antagonist, UK-427, 857, a new agent for the treatment of HIV infection and AIDS. Prog Med Chem 2005; 43:239-71.
78. Gorry PR, Ellett A, Lewin SR. Maraviroc. In Kucers' The Use of Antibiotics. In: Grayson L, Crowe S, McCarthy J, Mills J, Mouton J, Norrby SR, Paterson D, Pfaller M, editors. 6th Ed. London: Hodder & Stoughton Ltd. 2010, pp. 2869-76.
79. Roche M, Salimi H, Duncan R, Wilkinson BL, Chikere K, Moore MS, *et al.* A common mechanism of clinical HIV-1 resistance to the CCR5 antagonist maraviroc despite divergent resistance levels and lack of common gp120 resistance mutations. Retrovirol 2013; 10:43-62.
80. Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, *et al.* Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Science 2000; 287:646-50.
81. Eron JJ, Cooper DA, Steigbigel RT, Clotet B, Gatell JM, Kumar PN, *et al.* Efficacy and safety of raltegravir for treatment of HIV for 5 years in the BENCHMRK studies: final results of two randomised, placebo-controlled trials. Lancet Infect Dis 2013; 13:587-96.
82. Delelis O, Thierry S, Subra F, Simon *F*, Malet I, Alloui C, *et al.* Impact of Y143 HIV-1 integrase mutations on resistance to raltegravir *in vitro* and *in vivo*. Antimicrob Agents Chemother 2010; 54:491-501.
83. Dunn BM. Structure and mechanism of the pepsin-like family of aspartic peptidases. Chem Rev 2002; 102:4431-58.
84. De Clercq E. Anti-HIV drugs: 25 compounds approved within 25 years after the discovery of HIV. Int J Antimicrob Agents 2009; 33:307-20.
85. Alfonso Y, Monzote L. HIV Protease Inhibitors: Effect on the opportunistic protozoan parasites. Open Med Chem J 2011; 5:40-50.
86. Vella S, Floridia M. Saquinavir. Clinical pharmacology and efficacy. Clin Pharmacokinet 1998; 34:189-201.
87. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 1998; 35:275-91.
88. Dieleman JP, Salahuddin S, Hsu YS, Burger DM, Gyssens IC, Sturkenboom MC, *et al.* Indinavir crystallization around the loop of henle: Experimental evidence. J Acquir Immune Defic Syndr 2001; 28:9-13.
89. Wensing AM, van Maarseveen NM, Nijhuis M. Fifteen years of HIV protease inhibitors: Raising the barrier to resistance. Antiviral Res 2010; 85:59-74.
90. Fernández Montero JV, Barreiro P, Soriano V. HIV protease inhibitors: recent clinical trials and recommendations on use. Expert Opin Pharmacother 2009; 10:1615-29.
91. Eron JJ, Haubrich R, Lang W, Pagano G, Millard J, Wolfram J, *et al.* A phase II trial of dual protease inhibitor therapy: Amprenavir in combination with indinavir, nelfinavir, or saquinavir. J Acquir Immune Defic Syndr 2001; 26:458-61.
92. Rivas P, Morello J, Garrido C, Rodríguez-Nóvoa S, Soriano V. Role of atazanavir in the treatment of HIV infection. Ther Clin Risk Manag 2009; 5:1-18.
93. Möbius U, Lubach-Ruitman M, Castro-Frenzel B, Stoll M, Esser S, Voigt E, *et al.*Switching to atazanavir improves metabolic disorders in antiretroviral-experienced patients with severe hyperlipidemia. J Acquir Immune Defic Syndr 2005; 39:174-80.
94. Piacenti FJ. An update and review of antiretroviral therapy. Pharmacother 2006; 26:1111-33.
95. Shen CH, Wang YF, Kovalevsky AY, Harrison RW, Weber IT. Amprenavir complexes with HIV-1 protease and its drug-resistant mutants altering hydrophobic clusters. FEBS J 2010; 277:3699-714.
96. McKeage K, Perry CM, Keam SJ. Darunavir: A review of its use in the management of HIV infection in adults. Drugs 2009; 69:477-503.
97. Johnson VA, Calvez V, Günthard HF, Paredes R, Pillay D, Shafer R, et al. 2011 update of the drug resistance mutations in HIV-1. Top Antivir Med. 2011; 19:156–164.
98. Sugiura W, Matsuda Z, Yokomaku Y, Hertogs K, Larder B, Oishi T, *et al.* Interference between D30N and L90M in selection and development of protease inhibitor-resistant human immunodeficiency virus type 1. Antimicrob Agents Chemother 2002; 46:708-15.
99. Fun A, Wensing AMJ, Verheyen J, Nijhuis M. Human immunodeficiency virus gag and protease: partners in resistance. Retrovirol 2012; 9:63.
100. Rhee SY, Taylor J, Fessle WJ, Kaufman D, Towner W, Troia P, *et al.* HIV-1 protease mutations and protease inhibitor cross-resistance. Antimicrob Agents Chemother 2010; 54:4253-61.
101. Ide K, Aoki M, Amano M, Koh Y, Yedidi RS, Das D, *et al.* Novel HIV-1 protease inhibitors (PIs) containing a bicyclic P2 functional moiety, tetrahydropyrano-tetrahydrofuran, that are potent against multi-PI-resistant HIV-1 variants. Antimicrob Agents Chemother 2011; 55:1717-27.
102. Dierynck I, van Marck H, van Ginderen M, Jonckers THM, Nalam MNL, Schiffer CA, *et al.* TMC310911, a novel human immunodeficiency virus type 1 protease inhibitor, shows *in vitro* an improved resistance profile and higher genetic barrier to resistance compared with current protease inhibitors. Antimicrob Agents Chemother 2011; 55:5723-31.
103. Vidal JE, Wan Songa AT, Matosa ML, Bartmanna D, dos Anjosa G, de Mirandaa EJP, *et al.* High rate of virologic suppression with darunavir/ritonavir plus optimized background therapy among highly antiretroviral-experienced HIV-infected patients: results of a prospective cohort study in São Paulo, Brazil. Braz J Infect Dis 2013; 17:41-7.
104. Feng JY, Ly JK, Myrick F, Goodman D, White KL, Svarovskaia ES, *et al.* The triple combination of tenofovir, emtricitabine and efavirenz shows synergistic anti-HIV-1 activity in vitro: a mechanism of action study. Retrovirol 2009; 6:44.
105. Schader SM, Colby-Germinario SP, Schachter JR, Xu H, Wainberg MA. Synergy against drug-resistant HIV-1 with the microbicide antiretrovirals, dapivirine and tenofovir, in combination. AIDS 2011; 25:1585-94.
106. Kulkarni R, Feng JY, Miller MD, White KL. Dead-end complexes contribute to the synergistic inhibition of HIV-1 RT by the combination of rilpivirine, emtricitabine, and tenofovir. Antiviral Res 2014; 101:131-5.
107. Llibre JM, Clotet B. Once-daily single-tablet regimens: a long and winding road to excellence in antiretroviral treatment. AIDS Rev 2012; 14:168-78.
108. Thompson MA, Aberg JA, Hoy JF, Telenti A, Benson C, Cahn P, *et al.* Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA Panel. JAMA 2012; 308:387-402.
109. A Phase I, open label dose escalation study to evaluate safety of iHIVARNA-01 in chronically HIV-infected patients under stable combined antiretroviral therapy. Available from https://clinicaltrials.gov/ct2/show/NCT02413645.
110. Immunotherapy of HIV-infected patients an open, dose-escalating assessment of Vacc-C5 with either GM-CSF or Alhydrogel as adjuvant in HIV-1-infected subjects on antiretroviral therapy (ART). Available from https://clinicaltrials.gov/ct2/show/NCT01627678.
111. A Phase I randomized, double-blind, placebo-controlled study of a multi-antigen DNA vaccine prime delivered by in vivo electroporation, rVSV booster vaccine in HIV-infected patients who began antiretroviral therapy during acute/early infection. Available from https://clinicaltrials.gov/ct2/show/NCT01859325.
112. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, et al.Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009; 361:2209-20.
113. Williams WB, Liao HX, Moody MA, Kepler TB, Alam SM, Gao F, et al. Diversion of HIV-1 vaccine-induced immunity by gp41-microbiota cross-reactive antibodies. Science 2015; 349(6249):aab1253. doi: 10.1126/science.aab1253.
114. Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, et al. Broad and potent neutralizing antibodies from

an African donor reveal a new HIV-1 vaccine target. Science 2009; 326:285-9.
115. Wu X, Yang ZY, Li Y, Hogerkorp CM, Schief WR, Seaman MS, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 2010; 329:856-61.
116. Zhou T, Georgiev I, Wu X, Yang ZY, Dai K, Finzi A, et al.Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science 2010; 329:811-7.
117. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, Wei D, et al. HIV-1 envelope protein binds to and signals through integrin $\alpha_4\beta_7$, the gut mucosal homing receptor for peripheral T cells. Nat Immunol 2008; 9:301-9.
118. Honnen WJ, Krachmarov C, Kayman SC, Gorny MK, Zolla-Pazner S, Pinter A. Type-specific epitopes targeted by monoclonal antibodies with exceptionally potent neutralizing activities for selected strains of human immunodeficiency virus type 1 map to a common region of the V2 domain of gp120 and differ only at single positions from the clade B consensus sequence. J Virol 2007; 81:1424-32.
119. Kwong PD, Mascola JR, Nabel GJ. Rational design of vaccines to elicit broadly neutralizing antibodies to HIV-1. Cold Spring Harbor Perspectives Med 2011; doi: 10.1101/cshperspect.a007278.
120. Ackerman M, Alter G. Mapping the Journey to an HIV Vaccine. N Engl J Med 2013; 369(4):389-391.
121. Mikell I, Sather DN, Kalams SA, Altfeld M, Alter G, Stamatatos L. Characteristics of the earliest cross-neutralizing antibody response to HIV-1. PLoS Pathog 2011; 7(1):e1001251.
122. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhniy O, McGuire A, et al. Rational HIV immunogen design to target specific germline B cell receptors. Science 2013; 340, 711-6.
123. AS Fauci and HD Marston. Ending the HIV-AIDS pandemic-Follow the science. N Engl J Med 2015; 373(23):2197-9.
124. Implementation of HIV pre exposure prophylaxis with antiretroviral medications among people at high risk for HIV infection: A demonstration project. Available from https://clinicaltrials.gov/ct2/show/NCT02206555.
125. A pilot trial evaluating maintenance therapy with lamivudine and dolutegravir in HIV-1 infected patients virologically suppressed with triple HAART - ANRS 167 lamidol. Available from https://clinicaltrials.gov/ct2/show/NCT02527096.
126. Outcomes of HIV infected individuals after ten years on antiretroviral treatment. Available from https://clinicaltrials.gov/ct2/show/NCT02514707.
127. An interventional, one-arm, open label pilot study to assess the feasibility of dolutegravir monotherapy in virologically suppressed patients on conventional triple antiretroviral therapy of dolutegravir plus two nucleoside reverse transcriptase inhibitors. Available from https://clinicaltrials.gov/ct2/show/NCT02572947.
128. Safety and efficacy of the histone deacetylase inhibitor chidamide in combination with antiretroviral therapy for eradication of the latent HIV-1 reservoir (CHARTER). Available from https://clinicaltrials.gov/ct2/show/NCT02513901.
129. A phase 3b, randomized, double-blind, switch study to evaluate F/TAF in HIV-1 infected subjects who are virologically suppressed on regimens containing ABC/3TC. Available from https://clinicaltrials.gov/ct2/show/NCT02469246.
130. Clotet B, Feinberg J, van Lunzen J, Khuong-Josses MA, Antinori A, Dumitru I, *et al.*Once-daily dolutegravir versus darunavir plus ritonavir in antiretroviral-naive adults with HIV-1 infection (FLAMINGO): 48 week results from the randomized open-label phase 3b study. Lancet 2014; 383:2222-31.

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