Review Article

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Obstacles to successful antiretroviral treatment of HIV-1 infection: problems & perspectives

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Mutations in human immunodeficiency virus type 1 (HIV-1) are a major impediment to successful highly active antiretroviral therapy (HAART) and the design of anti-HIV vaccines. Although HAART has made long-term suppression of HIV a reality, drug resistance, drug toxicity, drug penetration, adherence to therapy, low levels of continued viral replication in cellular reservoirs and augmentation of host immune responses are some of the most important challenges that remain to be sorted out. Continuing viral replication in the face of HAART leads to the accumulation of drug resistance mutations, increase in viral loads and eventual disease progression. Patients who fail therapy have minimal options for their clinical management. Therefore, a clear understanding of the pathogenesis of drug-resistant HIV-1, and all of the issues that influence the success of HAART is urgently needed. In the present article, we discuss various obstacles to HIV therapy, and provide perspectives relating to these issues that are critical in determining the success or failure of HAART.

Key words Drug resistance - drug toxicity - HAART - HIV-1 - protease inhibitors - reverse transcriptase inhibitors - viral reservoirs

Human immunodeficiency virus type 1 (HIV-1) is now well documented as the aetiologic agent of the acquired immune deficiency syndrome (AIDS) and its related disorders[1,2]. The first case report of AIDS appeared in 1981 with the identification of previously healthy homosexual men from the USA who presented with *Pneumocystis carinii* pneumonia and Kaposi's sarcoma[3]. The virus was first isolated by Barre-Sinoussi and colleagues in 1983[1], and was the second human retrovirus discovered following the isolation of human T-cell leukemia/lymphoma virus type 1 (HTLV-1) in 1981[4]. A second related virus that also caused the full spectrum of syndromes associated with HIV-1 was isolated from West African patients in 1985[5].

One feature that distinguishes lentiviruses such as HIV from the other retroviruses is the complexity of the lentiviral genome. Many retroviruses contain only three structural genes (*gag, pol* and *env*), however lentiviruses typically contain 3-6 additional accessory/regulatory genes. In the case of HIV, these are *vif, vpr, vpu* (accessory) and *nef, tat, rev* (regulatory). Despite this increased genomic complexity, HIV still has only a small number of genes presenting a limited drug target repertoire. The introduction of highly active antiretroviral therapy (HAART), which typically includes a minimum of two nucleoside reverse-transcriptase inhibitors (NRTI), and one protease inhibitor (PI) and/or a non-nucleoside reverse-transcriptase inhibitor (NNRTI), in most cases results in a reduction in plasma viral load to below the limit of detection. However, prolonged treatment with antiretroviral drugs results in the selection of HIV-1 variants with resistance to NRTIs, NNRTIs or PIs, leading to disease progression and AIDS. In

addition, there are now an increasing number of cases involving the transmission of resistant viruses to newly infected persons[6,7]. With this in mind, there is now a strong focus on research into new drugs targeting other aspects of the HIV life cycle, including viral entry (fusion inhibitors), and integration into the host genome (integrase inhibitors).

Current targets of HIV antiretroviral drugs

Inhibitors directed at the reverse transcriptase (RT) and protease proteins (Pr) were the first introduced into clinical practice, initially as mono or dual antiretroviral therapy (ART). However, the presence of millions of mutant forms of HIV within a single infected individual means that no single drug is able to successfully suppress the virus. Indeed, it was quickly recognized that monotherapy was invariably accompanied by drug resistance. However, therapy with potent combinations of three or more antiretroviral drugs (HAART) has been shown to rapidly reduce circulating levels of plasma HIV to below detectable levels (BDL) for periods of several years or more[8]. Currently there are 18 drugs approved for the treatment of HIV infection (Table I) and some of these are available in combination form. However, these agents almost exclusively target the protease and reverse transcriptase enzymes (with the exception of enfuvirtide), and the high mutation rate of HIV has resulted in the selection of viral strains with resistance to these antiretrovirals. There has also been a strong focus on the HIV-1 envelope gene as an antiretroviral target due to its role in mediating viral entry. The fusion inhibitor Enfuvirtide (T-20) has recently been licensed in the USA and is now available for use as a component in combination HAART regimens (Table I).

Pol proteins as drug targets

The polymerase protein (Pol) of HIV-1 is synthesised as a Gag-pols ($Pr^{160\ Gag\text{-}Pol}$) fusion polyprotein[9,10]. The pol gene precursor polypeptide appears to be generated by translational frame shifting as ribosomes read the full length viral transcript from the gag open reading frame (ORF) through to the pol ORF[11]. The pol gene precursor is cleaved to produce three viral enzymes: protease, reverse trancriptase, and integrase. To date, the majority of all antiretroviral drugs in clinical practice target the protease and reverse transcriptase proteins.

The HIV-1 protease enzyme plays a critical role acting to specifically cleave Gag and Pol precursor polypeptides into functionally active proteins. HIV protease is an

Table I. Different classes of licensed HIV-1 antiretroviral agents

Class	Drugs		Viral target	Mode of action
Nucleoside reverse transcriptase inhibitors (NRTIs)	Zidovudine	(AZT)	Reverse transcriptase	Phosphorylated by cellular enzymes. Competitively inhibits viral DNA synthesis or causes chain termination. TFV is a nucleotide analogue
	Didanosine	(ddl)		
	Zalcitabine	(ddC)		
	Stavudine	(d4T)		
	Lamivudine	(3TC)		
	Abacavir	(ABV)		
	Tenofovir	(TFV)		
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Nevirapine	(NVP)	Reverse transcriptase	Not phosphorylated. Non-competitive inhibition of viral DNA synthesis. Binds directly to enzymes
	Delavirdine	(DLV)		
	Efavirenz	(EFV)		
Protease inhibitors (PIs)	Saquinavir	(SQV)	Protease	Binds to protease active site, thereby inhibiting enzyme function
	Indinavir	(IDV)		
	Ritonavir	(RTV)		
	Nelfinavir	(NFV)		
	Amprenavir	(APV)		
	Lopinavir	(LPV)		
	Atazanavir	(ATV)		
Fusion inhibitors	Enfuvirtide	(T-20)	Envelope gp41	Binds to HR1 region of the gp41 envelope glycoprotein

aspartic proteinase, and is responsible for cleavage of the Gag (p55) and Gag-Pol (p160) polyprotein products, yielding the functional core proteins (p17, p24, p7, p6) and essential enzymes (reverse transcriptase, integrase, protease) required to produce mature HIV[12,13]. HIV protease comprises 2 identical structures which are 99 amino acids long and are C-shaped in symmetry, with the active site sequence at positions 25 to 27 in each chain[14]. When HIV protease is chemically blocked, the formation of these core proteins is disrupted and assembled virions are immature and non-infectious.

The HIV-1 reverse transcriptase enzyme (RT), is an Mg^{2+}- requiring, RNA-dependent DNA polymerase, that is responsible for replicating the RNA genome[15]. The RT enzyme converts the single-stranded virion into double-stranded DNA for subsequent integration into the host cell genome. RT is derived from a Gag-Pol precursor that is processed by protease to yield a heterodimeric enzyme composed of a 66 kDa protein (p66) and a 51 kDa protein (p51)[11]. The p66 kDa protein (p66) may be degraded to p51 and p15. Polymerase activity resides within the p51 fragment, and RNase activity is associated with the presence of p15[16]. Although the monomeric forms of the enzymes (p51 or p66) may exhibit RT activity, both subunits are required for optimal polymerase activity[17].

Integrase is a 31kDa protein produced from the C terminal portion after the processing of $Pr^{160\ Gag\text{-}Pol}$, and is required for integration of a double-stranded DNA copy of the viral RNA genome into the host chromosome. Since this proviral integration is essential for HIV replication, it represents an important target for future antiviral drug design. Integrase defective HIV-1 mutants have also been shown to reduce viral replication, Tat protein activity, and the stability, packaging and processing of the Gag-Pol polyprotein[18].

Envelope protein as a drug target

HIV fusion and entry occur via the interaction of the trimeric envelope gp160 spike (gp120 and gp41), with receptor molecules on the surface of target cells[19]. The fusion process is a vital step in the viral replicative cycle, making it an attractive target for antiretroviral drugs. Fusion inhibitors, such as enfuvirtide (T-20), target this crucial fusion step of the viral life cycle. Enfuvirtide is administered parenterally and inhibits fusion of the viral and cell membranes by binding to a portion of the gp41 molecule[20]. Clinical trials (Phase II and III) of T-20 have shown significant antiviral activity in a majority of patients[21,22]. T-20 however shows limited activity toward HIV-2, a factor attributed to the differences in the envelope sequence.

Emergence of drug resistance during HAART

Protease inhibitors (Pls) and drug resistance

The HIV protease enzyme is a dimeric aspartyl protease required for the post-translational cleavage of precursor Gag-Pol polyproteins during virion maturation, to generate building blocks required for assembly of new virus particles[23-28]. The activity of this protein is essential for virus infectivity, rendering it a major drug target. Many protease inhibitors (PIs) are currently available (Table I), and there are others in the clinical or pre-clinical stages of development.

Resistance mutations in the protease gene may result from amino acid substitutions at or near the active site interfering with binding of the inhibitor because of conformational perturbations, or to amino acids lying outside the active region. The latter frequently compensate for the deleterious effects of primary mutations[29-31], and involve mutations in the cleavage sites in the Gag-Pol polyprotein precursor, lying outside the protease gene domain[32]. Cleavage site mutations do not produce drug resistance in themselves, but compensate for alterations in protease that result from primary and secondary mutations. Resistance to PIs emerges rapidly when these inhibors are administered at inadequate doses or as part of suboptimal regimens[33]. Generally, high-level resistance to PIs results from the sequential accumulation of amino acid substitutions in the Pr gene, along pathways that usually vary between different PI drugs[34,35]. The selective advantages conferred by PI resistance mutations depend upon the nature of the drug, its local concentration and the impact of the mutation on infectivity. Due to the observation that drug concentrations *in vivo* are subject to uneven tissue distribution and can vary over time, parameters leading to the emergence of PI-resistant strains remain poorly defined[36].

The first approved PIs were saquinavir (SQV) and indinavir (IDV). Generally these drugs are intrinsically potent and require the emergence of multiple mutations

before high-level resistance occurs. However, resistance to SQV often confers resistance to IDV and vice versa[37,38]. When viral suppression to below the limit of detection is not achieved in a dual NRTI plus PI regimen, early mutations often occur to lamivudine (3TC), followed by mutations associated with resistance/cross resistance to other NRTIs[39]. Nelfinavir (NFV) has a lower genetic barrier to resistance than SQV and IDV, and is susceptible to the single D30N and L90M mutations which can develop quickly in PI-naive patients. The presence of two or more mutations including D30N, G48V, 150V, V82A/F/T/S, 184V and L90M generally confers cross resistance to all three of these PIs[40,41], whereas a single mutation at codon 30 does not infer cross resistance to other PIs[42]. Amprenavir (APV), when used as a first-line PI is inclined to select for the I50V mutation conferring cross-resistance to lopinavir (LPV)[37]. LPV is a more recently approved PI that is co-formulated with the cytochrome P450 inhibitor RTV to increase LPV levels. RTV may also be combined with APV, SQV and IDV to increase pharmacologic effects. LPV has a high genetic barrier to resistance[43], but a recent study has suggested that APV-selected resistance may confer cross-resistance to LPV[44]. Atazanavir (ATV) is the most recently approved PI. It has minimal effects on lipids, but when combined with other PIs in patients without underlying PI resistance, it is susceptible to the signature I50L mutation. However, unlike the I50V mutation observed with APV, this mutation is not associated with cross-resistance to other PIs. In patients who have previously been on PI-based therapy before commencing with ATV, a number of both primary and secondary mutations can be associated with ATV resistance[37,45].

Interpretation of Pr mutants is complicated by the extensive polymorphisms found in the Pr gene of HIV-1 isolates from untreated patients. In one study, variation was noted in nearly 48 per cent of PR codons compared with the consensus (wild-type) sequence[46]. The significance of these polymorphisms in determining treatment outcome remains uncertain, since most studies have not found any correlation between the presence of these polymorphisms and virologic response, or the rate at which PI resistance emerges. Some HIV-1 subtypes have naturally occurring polymorphisms or mutations that are associated with resistance. For example, M36I is very common in subtype C and other non-subtype B isolates. Accumulation of multiple mutations can contribute to cross-resistance, and an understanding of the mechanisms involved is important for establishing effective treatment strategies in patients who cease responding to an initial PI-containing regimen[47,48]. In one report, four amino acid substitutions associated with indinavir therapy, M46I, L63P, V82T and I84V, were required for cross-resistance to other PIs, including saquinavir and amprenavir[41]. Another study involving more than 6000 clinical samples showed phenotypic cross-resistance in 59-80 per cent of samples with HIV-1 resistance to at least one PI[38]. The predominant genotypic change in viruses with resistance to at least one PI involved codons 10, 36, 46, 54, 71, 77, 82 and 90 of the Pr gene. Viruses that were cross-resistant to all four PIs displayed higher frequencies of changes at these positions, and also at positions 48 and 84[38]. Thus, along with single amino acid changes coding for drug resistance, cross-resistance with protease and RT inhibitors presents a complex challenge to antiretroviral therapy.

Reverse transcriptase inhibitors (RTIs) and drug resistance

The NRTI components of HAART are crucial to the success of combination antiretroviral therapy. AZT (3'-azido-2',3'-dideoxythymidine) was the first medication introduced to combat HIV infection through inhibition of the reverse transcriptase enzyme, and its success heralded the development of an array of other nucleoside and non-nucleoside RTIs, with many more currently under development.There are currently ten approved RTIs, including six NRTIs and four NNRTIs. Despite the success of RTIs and their pivotal role in HAART, specific amino acid mutations are associated with resistance to several different RTIs, and mutational complexes conferring broad cross-resistance within this class have also been observed. Thus, similar to the situation with PIs, RTIs also have associated problems with drug resistance, and this must be carefully monitored during the course of therapy.

(i) Nucleoside reverse-transcriptase inhibitors (NRTIs) – NRTIs were the first class of effective antiretroviral compounds, and zidovudine (AZT) was the first drug to reach clinical practice[49]. NRTIs inhibit the reverse transcriptase enzyme by competing with endogenous nucleosides for incorporation into the DNA chain generated by reverse transcription of HIV RNA.

All nucleoside analogues must be triphosphorylated within the cell, enabling inhibition of reverse transcriptase activity and premature chain termination. Resistance to NRTIs develops from nucleotide changes within the RT gene and the subsequent generation of amino acid substitutions in the RT enzyme[50,51]. Each NRTI induces a predictable set of genetic alterations, generally with primary mutations arriving first, and secondary mutations developing during continued therapy[52]. For example, resistance to zidovudine develops with the sequential selection of specific mutations in the RT gene, including codons 41 (M41L), 67 (D67N), 70 (K70R), 210 (L210W), 215 (T215Y) and 219 (K219Q)[53,54]. These mutations are known as thymidine analogue mutations (TAMs) or nucleoside analogue mutations (NAMs), and with ongoing viraemia there is a progressive accumulation of these mutations resulting in resistance to didanosine (ddl), zalcitabine (ddC), tenofovir (TDF) and abacavir (ABC). Mutations associated with didanosine resistance involve codons 65, 74, and 184, while resistance mutations for zalcitabine include those at codons 65, 69, 74, and 184[55]. However, some overlap in mutations occur between different antiretroviral agents of the same class, and are the reason for cross-resistance among the different agents. The number of mutations required to induce resistance and cross-resistance also varies among agents[53,55]. Multiple RT mutations are required for high level phenotypic resistance to AZT, whereas lamivudine resistance occurs in the presence of a single mutation at codon 184. The relation between individual drug induced resistance mutations and viral replication capacity also influences the pathogenic course. For example, when the M184V mutation develops in the presence of thymidine analogue mutations, the M184V mutation desensitizes the virus to the inhibitory effects of zidovudine[53,56].

The increasing use of sequential, alternating and combination nucleoside analogue regimens can select HIV variants with mutations that confer resistance to all the currently available NRTIs. Two sets of mutations have been described; the Q151M complex and the T69S insertion mutations. Q151M is a two base pair change in a conserved RT region that is close to the first nucleotide of the single-stranded nucleotide template[57,58]. A primary codon change, Q151M emerges first and confers partial resistance to all the current approved NRTIs[59]. In addition to Q151M, this complex is often associated with secondary mutations at codons 62 (A62V), 75 (V75I), 77 (F77L) and 116 (F116Y) which further reduces the sensitivity to NRTIs and perhaps more importantly improves the fitness of the Q151M mutants[59]. The prevalence of Q151M ranges from 2 to 6 per cent in cohorts of treatment experienced patients[60].

The T69S-S-S or T69S-S-A insertion mutations arise after prolonged treatment with multiple nucleosides and confer high level resistance to all the currently available NRTIs as well as the nucleotide analogue tenofovir. However, they appear more likely to confer multi-nucleoside resistance when accompanied by secondary mutations such as A62V, or a background of zidovudine associated mutations (TAMs) such as M41L, D67N, K70R, and more particularly L210W, T215Y/F and K219Y[37,61,62].

The most common mutations occurring in clinical HIV-1 samples obtained from patients receiving NRTIs were originally identified for their role in conferring zidovudine resistance. Various combinations of these mutations at codons 41, 67, 70, 210, 215 and 219[50,54,63] have been shown to mediate ATP-dependent hydrolytic removal of a dideoxy nucleotide monophosphate (ddNMP) from a terminated cDNA chain and to possibly cause a compensatory increase in RT processivity[64]. Studies have also shown that nucleotide excision mutations are associated with clinical resistance not just to zidovudine, but also to stavudine, abacavir, and to a lesser extent didanosine, zalcitabine and tenofovir[65].

(ii) Non-nucleoside reverse transcriptase inhibitors (NNRTIs) – Non-nucleoside reverse transcriptase inhibitors (NNRTI) are non-competitive inhibitors of HIV-1 RT and bind to a hydrophobic cavity near the active site of reverse transcriptase, causing a conformational change in the enzyme. There are currently three approved NNRTIs, nevirapine (NVP), delavirdine (DLV) and efavirenz (EFV). NNRTI binding sites are largely restricted to beta-sheets comprising codons 100-110 and 180-190[66]. In contrast to many of the NRTIs, a single mutation can cause high level resistance to NNRTIs.

The most common mutations in HIV selected by NNRTIs are L100I, K103N, V106A, V108I, Y181C/I, Y188C/L, G190A/E/S, P225H and P236L[67]. These signature mutations often emerge during therapy when

plasma HIV RNA is not maintained below the limits of detection[68]. Y181C and K103N are associated with significant cross-resistance between NVP, DLV and EFV[37,62]. The Y181C mutation confers a high level of resistance to nevirapine and delavirdine, but not to efavirenz[69,70]. Y181C reduces NNRTI binding affinity leading to drug resistance, while the K103N mutation acts by preventing the formation of the hydrophobic binding pocket, thus reducing binding affinity indirectly. Y181C has also been reported to reverse AZT resistance when introduced to isolates carrying major AZT resistance mutations[71]. Experiments have also shown that viruses with resistance to AZT and/or ABV have increased susceptibility to NNRTIs. As the three currently available NNRTIs bind to the same site, cross-resistance is common.

Virologic failure of NNRTIs is characterized by a rapid rebound in HIV RNA levels and the emergence of high level phenotypic drug resistance. One study observed the emergence of resistance to nevirapine in as little as 1 to 2 wk, confirming that viral turnover can still be rapid and dynamic when using this class of inhibitors[68]. The rapid induction of resistance has been observed most strongly during NNRTI monotherapy, and rapidly emerging resistant virus may often completely replace wild type strains within 2 to 4 wk[72].

Resistance to the fusion inhibitor T-20

Resistance mutations to T-20 have been observed *in vitro* and *in vivo* and appear to involve mutations mainly in the Env gp41 region[73]. The most significant substitutions occurred near the N-terminus of the HR-1 region, in the highly conserved GIVQQQ sequence known to be critical for fusion. Drug resistance mutations in the RT and protease regions have no influence on T-20 activity *in vivo*.

Causative factors of HIV-1 drug resistance and the failure of HAART

With sustained major declines in opportunistic infections and malignancies, HIV infection is becoming an increasingly chronic disease in countries where antiretroviral drugs are available, and the continued long-term success of antiretroviral therapy relies on an increasing range of drugs. Despite the decrease in morbidity and mortality associated with HAART regimens, and the significant increase in the life expectancy of treated HIV-infected individuals, the eventual failure of therapy and progression to AIDS is still almost inevitable. The failure of HAART most likely arises from a combination of viral and host factors that facilitate the emergence of HIV variants with resistance to multiple antiretroviral drugs. These factors are outlined in the Fig. The emergence of drug resistance in patients receiving HAART can be primarily attributed to the high spontaneous mutation rate and high rate of HIV turnover within infected individuals, selective pressures arising from antiretroviral therapy, pharmacokinetic characteristics of antiretroviral drugs, patient tolerance/adherence to antiretroviral regimens and the existence of viral reservoirs. Each of these factors is discussed in detail below.

HIV genetic heterogeneity: an impediment to successful antiretroviral therapy

Genetically, HIV-1 is highly variable virus. Characterization of the first HIV-1 isolates revealed a range of variation between 1-8 per cent at the nucleotide sequence level, with an even larger variation at the amino acid level[74]. HIV-1 infection is also characterized by a high degree of genetic variability within infected persons, with the population present at a certain time point within an infected person consisting of a complex mixture of

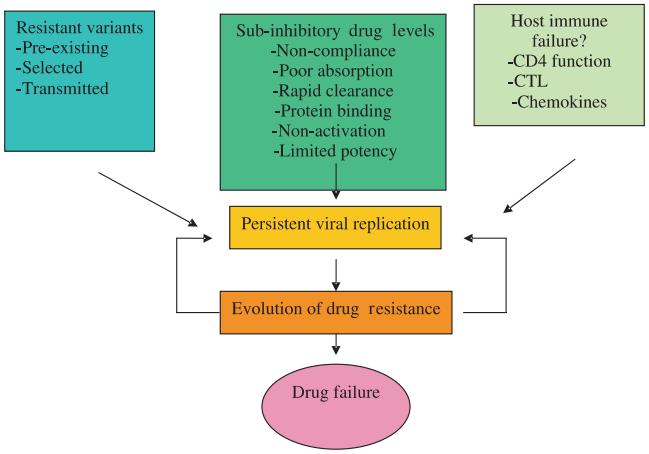

Fig. Evolution of HIV drug resistance and pathways leading to the failure of therapy. The net effects of each of these factors are persistent virus replication and immunologic decline, which in turn leads to clinical disease progression and AIDS. CTL, cytotoxic T lymphocyte.

heterogeneous strains termed "quasispecies"[75]. Quasispecies generally differ in their antigenic and phenotypic properties and compete among themselves for survival and propagation[76]. The subsequent overgrowth or dominance of a certain viral strain over another is largely determined by its relative adaptation to a given intra-host environment, a factor particularly relevant for the emergence of drug resistant variants.

Error-prone reverse transcriptase enzyme and rapid viral turnover

The molecular basis of HIV-1 variability is a highly error-prone reverse transcriptase enzyme[77]. RNA viruses, including retroviruses such as HIV-1, have mutation rates approximately one hundred times higher than those of DNA viruses, bacteria or other eukaryotes[78]. The rate of nucleotide substitutions introduced by reverse transcriptase is approximately 10^{-4} per nucleotide per cycle of replication, which is equal to one nucleotide substitution per genome during a single replication cycle[79]. Insertions, deletions, and duplications also contribute to the genetic heterogeneity of HIV-1[80]. HIV-1 has a rapid turnover, and it is estimated that approximately 10^9 virions per day are generated in an infected individual. The composite lifespan of plasma virus and virus-producing cells is very short with a half-life of approximately two days, and an almost complete replacement of wild-type strains by drug resistant virus occurs in plasma within 2-4 wk[72]. During antiretroviral treatment, rapid viral turnover in combination with a high mutation rate is a primary factor behind the emergence of HIV variants with antiretroviral drug resistance.

Genetic recombination influencing HIV-1 diversity

Genetic recombination is another important strategy by which HIV generates genetic diversity[80], and this process contributes strongly to high level multiple drug resistance[81-83]. Each retrovirus particle contains a dimeric RNA genome and a reverse transcriptase enzyme that can switch templates during proviral synthesis. Recombination may link drug resistant mutations in HIV-1, leading to increased resistance to a particular drug[84], or the generation of multi-drug resistant variants[82]. In addition, recombination may lead to the acquisition of mutations that compensate for a loss in viral fitness or replicative capacity due to previous acquisition of resistance mutations. Recombinant virus can easily be selected for in *in vitro* experiments[82,84,85]. Recent studies have directly shown the development of multiply drug resistant strains through homologous recombination between two distinct starting plasmids or viruses[82,86]. Because recombination can create a multiple drug resistant virus out of two single drug resistant strains, it is generally believed that the capacity of the virus to recombine facilitates the evolution of drug resistance[82, 84-87], and this rapid evolution of drug resistance in HIV remains a major obstacle for HIV therapy. Further, the precise selective advantage of retroviral recombination and its relationship with the evolution of drug resistance remains unclear. A high frequency of recombination has been observed in areas where multiple genetic forms of HIV-1 circulate. The uncontrolled use of antiretrovirals in these areas is therefore of particular concern, as it may result in the emergence and widespread circulation of multi-drug resistant strains generated through recombination.

Recombination is a strategy for viral rejuvenation, and it is likely that recombination between HIV strains may lead to the evolution of fitter forms and viral strains acquiring drug resistance to all major classes of HIV-1 inhibitors. Alternatively, a different scenario could be that just as recombination can create fitter virus by recombining parts of two parental genomes with lesser fitness, so it can also create less fit virus by breaking up favourable combinations of mutations in the parental genomes. This interaction between recombination, mutations and viral fitness is highly intricate, but nonetheless, recombination and its mechanisms, especially at the level of diverse subtypes, warrant further investigation. Given that *pol* is the major target gene for all major classes of anti-HIV drugs and most HIV strains show hotspots for recombination in *gag-pol* and *env* regions, further studies on the evolution of drug resistance in concert with viral evolution are worthwhile.

Selective pressures imposed by antiretroviral drugs

The effectiveness of all currently prescribed HIV antiretroviral drugs is limited by the emergence of drug resistant variants, which frequently show extensive cross-resistance within each drug class[52,88,89]. The large number of virions within infected individuals even during successful therapy means that some variants will survive

and give rise to an infection that is resilient to antiretroviral drugs. In the absence of drugs, drug resistant HIV strains generally have reduced fitness compared to wild-type counterparts[90]. The impact of drugs on HIV-1 mutation rates has been investigated extensively. Usually, initial decreases in viral fitness are accompanied by the emergence of primary mutations that confer direct drug resistance. Continued drug selective pressure then allows the virus to select secondary mutations that compensate for the primary mutations allowing restoration of wild-type enzymatic activity of the enzyme (Pr or RT) targeted by the drug. This continual evolution eventually leads to a recovery in fitness to similar or sometimes higher levels than that of the wild-type virus[55,91-94].

Even prior to the commencement of HIV antiretroviral therapy, variants exist within the population that are naturally resistant to some extent to a particular drug, although these usually exist only as minority sub-populations. The presence of an antiviral drug alters the selective pressure on the viral population. Naturally occurring mutants including those with a measure of drug resistance will continue to replicate and increase their population relative to the drug suppressed wild-type. Over time, these escape mutants accumulate additional mutants, which either increase their level of resistance or compensate for their reduced fitness. The eventual result is an outgrowth of the resistant strain, which appears to be fitter in the presence of the drug promoting drug failure. When drugs that only partially inhibit HIV-1 replication are administered, the resulting evolutionary pressure selects for resistant strains. Resistance emerges at a rate that is proportional to the frequency of pre-existing variants and their relative growth advantage in the presence of drug[90]. In the laboratory and clinical settings, drug resistant HIV variants generally only accumulate to readily detectable levels in the ongoing presence of antiretroviral drugs. However, the use of drug combinations is designed to limit the emergence of multiply drug resistant variants and may suppress plasma viraemia more effectively. Despite this, escape mutants will continue to replicate and gain additional mutations leading to an eventual outgrowth of multi-drug resistant strains.

Recent studies have shown that drugs targeted against reverse transcriptase and strains with resistance to NRTIs can increase HIV-1 mutation frequencies. The effect of AZT, 3TC and AZT/3TC conferring resistance mutations on the HIV-1 mutation rate has been recently investigated[95,96]. AZT was found to increase the HIV-1 mutation rate by a factor of 7.6 in a single round of replication, and 3TC was found to cause a 3.4 fold increase[95]. HIV-1 replication with AZT-resistant reverse transcriptase was also found to increase the mutational rate by as much as 4.3 fold. Analysis of the combined effects of drug and drug-resistant virus showed much larger scale increases in mutational activity (up to 24 fold)[96]. The correlation of increased HIV-1 mutation rates with the emergence of antiretroviral drug resistance suggests that drug failure could increase the chances of further resistance evolving from subsequent drug regimens.

Drug pharmacokinetics

Broadly speaking, the pharmacokinetics (PK) of a given antiretroviral drug are considered satisfactory when levels are maintained above the IC_{50} of the virus (the concentration of the drug required to inhibit *in vitro* growth by 50%). As HIV replicates within cells, PIs and RTIs must enter cells to inhibit viral replication. Thus, the penetration of individual drugs into cells and the mechanisms involved in their clearance are important issues. Suboptimal drug concentrations result in several undesirable effects, including the emergence and propagation of drug resistant HIV variants[97-99]. The genetic barrier to resistance, which is the number of mutations required to confer clinically relevant increases in the IC_{50} and the cost of those mutations to replicative fitness of the mutant strain, also impact on the development of drug resistance.

The pharmacological activity of antiretroviral drugs is ultimately dependent on unbound drug entering cells that harbour HIV, and multi-drug combination therapy requires an understanding of the pharmacokinetics of all drugs in a regimen. The PK of orally administered antiretrovirals involves absorption, first-pass metabolism in the intestine and liver, systemic distribution, metabolism and removal (excretion)[100]. When a drug enters the systemic circulation, it distributes into tissues according to the relative affinity of a given tissue compared to plasma. Although this drug distribution is generally influenced by passive diffusion gradients, some particular cell types (*e.g.,* those of the blood-brain barrier) also contain active efflux mechanisms which keep the drug concentrations lower than in surrounding plasma. Many antiretrovirals bind to plasma proteins, influencing uptake

into cells, as only unbound drugs in plasma can pass across the cell membrane efficiently[100]. Important PK parameters to consider include the volume of distribution (concentration of drug in plasma for a given amount of drug in the body), rate of clearance (efficiency of drug excretion), drug half-life (determines the course of accumulation of the drug in the body in chronic dosing), and the degree of fluctuation within a dosing interval. These parameters can vary significantly between patients, resulting in differences in drug absorption, drug metabolic and excretory activity, drug distribution and the overall efficacy of drug regimens. Failure to carefully consider the pharmacokinetic properties of any drug regimen may result in suboptimal drug concentrations leading to treatment failure and the selection of drug-resistant variants. Alternatively, drug concentrations which are too high may induce toxicity and therefore reduce patient compliance.

In addition to the pharmacokinetic parameters described above, the limited penetration of antiretroviral drugs into certain viral sanctuary sites including the central nervous system (CNS) and other cellular compartments also affects the efficacy of drugs *in vivo*. HIV also rests latently in long-lived memory $CD4^+$ T cells, avoiding detection and elimination by antiretroviral regimens and the host immune system[101-104]. Thus, the HIV populations can remain concealed in such "preferred" compartment sites, and may re-emerge when antiretroviral regimens fail[90,105]. Differences in the intracellular metabolism of nucleoside analogues between resting and activated cells may also result in incomplete suppression of apparently sensitive viruses. Drug interactions that interfere with absorption or enhance elimination of antiretroviral agents are another potential cause of drug failure.

The central nervous system: an example of suboptimal drug penetration

There is considerable evidence that unique anatomical structures limit the distribution of antiretroviral drugs into the CNS. These are the blood-brain barrier located between the blood and brain tissue, and the blood-CSF barrier primarily formed by the choroid plexus. High plasma protein binding of protease inhibitors and their unidirectional efflux by P-glycoprotein membrane proteins in the blood-brain barrier limit CNS penetration and absorption of antiretrovirals[106]. Thus, the CNS represents a site where ongoing viral replication may occur in the absence of antiretroviral suppression. It has been shown that most PIs are substrates of P-glycoprotein (P-gp), which acts as an efflux pump limiting the extent of the PI distribution in the CNS, a finding further confirmed by mouse models[107]. A clearer understanding of how drug resistant strains develop in compartments with limited antiretroviral drugs penetration such as the CNS, may offer insights into the mechanisms behind the emergence of drug resistance in the mainstream HIV population. The development of more efficacious antiretroviral drugs is therefore of paramount importance to achieve and maintain consummate therapeutic drug levels in both the brain and the blood.

Patient adherence, tolerance and drug toxicity

Patient adherence is a highly important factor in the administration of effective antiretroviral regimens. Recent trials have suggested that during the maintenance phase, early and late virologic failures appeared to be related more to adherence issues and the subsequent potency of treatment rather than the emergence of drug resistant viruses[108,109]. Patient adherence to antiretroviral regimens affects the evolution of viral variants with different degrees of sensitivity to drugs[110]. Theoretically, total adherence should prevent the emergence of resistant strains, but incomplete patient adherence coupled with an array of other pharmacologic factors result in the presence of a heterogeneous population, and the possibility of selecting for viral resistance.

Many factors influence the degree of patient adherence to therapy. In developed countries issues such as the side effects of drugs (toxicity), the simplicity of the regimen, and the existence of social support systems for the patient all play a role. However, the situation in developing countries is much different, where the high costs of antiretroviral regimens and the lack of infrastructure needed to monitor their use makes access to these medications extremely difficult for most HIV-infected individuals. Poor access to healthcare providers and counselling, and broader issues such as poverty and poor literacy add to difficulties in patient adherence in these areas. Drug-use, high-risk behaviours, and depression also contribute to poor adherence.

The incidence of adverse effects associated with the administration of antiretroviral agents is dependent on a number of factors including the ethnic origin of the patient, use of additional medications, and host factors. Adverse reactions to antiretroviral therapy are common, and profoundly affect its clinical efficacy through adherence problems. Adverse effects associated with antiretroviral agents include mitochondrial toxicity, hypersensitivity, lipodystrophy, dyslipidaemia and type 2 diabetes. Mitochondrial toxicity arises from NRTI inhibition of mitochondrial DNA polymerase activity, leading to impaired synthesis of mitochondrial enzymes that generate ATP by oxidative phosphorylation[111-113]. This causes elevated plasma lactate production and gluconeogenesis, resulting in lactic acidosis and secondary diabetes. Other effects include cardiomyopathy, peripheral neuropathy and pancreatitis. NRTIs and PIs also cause lipodystrophy, a subcutaneous peripheral lipoatrophy of the limbs, buttocks and face, and central accumulation of fat[114-118]. Metabolic features associated with lipodystrophy include hypertriglyceridaemia, hypercholesterolaemia, insulin resistance and lactic acidaemia[111,119-123].

PIs are associated strongly with gastrointestinal effects including diarrhoea, nausea and vomiting. Dyslipidaemia at levels associated with increased cardiovascular disease are also common in patients receiving HAART, and it has been shown that protease inhibitors can directly induce dyslipidaemia independent of HIV infection[124,125]. Insulin resistance and diabetes have in most cases been identified in protease inhibitor recipients, and drug hypersensitivity with ABV and NVP in HIV-infected patients manifesting as severe rash is over 100 times more common than in the general population[126]. Efavirenz has adverse effects on the central nervous system, causing insomnia, dizziness, poor concentration and nightmares and even psychosis[127-129]. In addition, all antiretroviral drugs are associated with liver dysfunction through either direct or indirect mechanisms. Cardiovascular disease has also been firmly linked with antiretroviral therapy and is exacerbated by smoking and other risk factors[130].

Viral reservoirs

Anatomical reservoirs of HIV occur in tissues that are immunologically sheltered or separated by a barrier from the blood and lymphoid systems. The CNS, and male genital tract are two well-characterized examples. Some anatomical sites may be non-permissive to immune surveillance and effective drug penetration, thus serving as potential sites of persistent HIV replication (*e.g.*, the respiratory, gastrointestinal and reproductive tracts). These reservoirs may be established early in the course of HIV infection, or during the course of HAART[131].

Cellular reservoirs of HIV-1

Cellular reservoirs of HIV-1 arise from the ability of HIV to infect a variety of immune cell types including monocytes, macrophages, NK cells and T lymphocytes, in addition to other non-immune-based cells. Cellular latency may be established in these compartments by several different molecular mechanisms.Cellular reservoirs of HIV include memory CD4+T lymphocytes, blood monocytes and macrophages/cells of macrophage lineage. These reservoirs are characterised by their stability, and are believed to act as sanctuaries from the effects of drugs and host immune responses during HAART, significantly contributing to viral persistence. Recently, we have demonstrated that during HAART a number of cell types (monocytes, macrophages, CD8+ and CD4+ T cells) may harbour proviral integrants with different degrees of antiretroviral drug resistance in the same patient. These data support the ongoing cell-specific selection of viral variants during HAART in different cellular and cell-free compartments *in vivo*[132].

Persistence of HIV in vivo during HAART in memory cell subsets

Resting memory CD4+ T cells containing integrated provirus are the most significant cellular reservoir of HIV-1[101-104, 133]. The integration of HIV-1 into the genome of resting memory CD4+ T cells was confirmed by Chun and colleagues[133] and is a highly stable event that may last for the entire life span of these cells. Latent replication-competent HIV-1 provirus has been observed largely in resting memory (CD45RO) CD4+T lymphocytes, but persistent low level HIV replication has also been demonstrated in naïve CD45RA CD4+T cells of patients receiving HAART. These cell subsets are of considerable importance if complete eradication of HIV is to be achieved with conventional HAART.

Current methods for detecting HIV-1 drug resistance

Resistance to current antiretroviral drugs is determined by mutations in the genes that encode the Pr and RT enzymes. Primary mutations are those that alter binding of the drug to its target and result in an increase in the amount of drug necessary to inhibit the enzyme. Secondary mutations increase the level of resistance by improving the fitness of viruses carrying primary mutations. Although HIV-1 drug resistance is usually acquired during anti-HIV drug therapy, drug resistance can also be transmitted between individuals and recent studies also suggest that transmitted HIV-1 drug resistance is gradually increasing[134]. Prospective studies have shown that patients whose physicians have access to drug resistance data, particularly genotypic resistance data, respond better to therapy than control patients whose physicians do not have access to the same information[135].

Currently available tests for HIV-1 drug resistance

Antiretroviral resistance is determined by showing reduced susceptibility of HIV-1 to the given drug. Standarized assays are now available allowing quick assessment of genotypic or phenotypic drug resistance in plasma HIV-1.

Genotypic testing – Genotypic assays use either nucleic acid sequencing methods to detect all mutations within the RT and Pr genes, or novel methods such as line-probe or chip-based assays to identify select nucleotide changes known to be associated with drug resistance[136]. All such assays depend upon amplification of the Pr and RT genes from viral RNA in plasma (by means of RT-PCR) or from proviral DNA derived from peripheral blood mononuclear cells (PBMCs). Automated sequencing provides the most comprehensive data as the entire Pr and RT genes are accounted for, however this may be more than is required in many clinical situations.

Genotypic testing is now inexpensive and readily available in many laboratories, however there are limitations to this approach of assessing drug susceptibility which include variable reproducibility and increased potential for laboratory error. The interpretation of drug resistance profiles through sequence-based analysis is also an issue, and inconsistencies in this area are evident between the various internet resources. In addition, current assays only detect viruses representing 5-20 per cent of the total population, and resistance present in minor subpopulations is often missed. More sensitive methods have recently been developed to detect minority resistance strains[137].

Phenotypic testing – The functional characteristics and growth properties of a viral isolate are referred to as the viral phenotype. Phenotypic testing is only performed in a few laboratories, takes longer to report results and is considerably costlier than genotypic testing. In the context of drug resistance, phenotyping measures the susceptibility of the virus to inhibition by a particular drug. Direct testing of clinical isolates in PBMC from seronegative donors has been used, but is time consuming. Moreover, PBMC from different donors vary in their ability to support the growth of HIV-1, leading to significant inter-assay variation.

Many of these problems have been overcome with the availability of commercial recombinant assays such as AntiVirogram (Virco) and PhenoSense (ViroLogic)[138]. These assays rely on the incorporation of plasma-derived HIV-1 RT and Pr genes into the backbone of an HIV-1 reference strain. The recombinant is then tested *ex vivo* to measure the IC_{50} and to measure a fold-change in susceptibility compared to wild type virus. Limitations of these assays include interpretation problems, as biological or virologic cut-offs measured by the assays do not incorporate achievable drug levels and can thus over or under-estimate the likelihood of a clinical response to a given drug. Similar to genotypic assays, minor subpopulations may also be missed, and the early emergence of drug resistant variants, which are rapidly selected for with continued drug pressure may not be detected.

PBMCs as a predictor of drug resistance

Circulating plasma virions are considered to be the best marker for assessing the efficacy of therapy. This is due to the fact that plasma-derived strains represent the most recently produced HIV variants from productively infected cells. However, it is important to consider the role of cellular reservoirs in the assessment of drug resistance mutations, particularly those, which are latently infected[101-104,133]. Importantly, latently

integrated HIV in cellular reservoirs always remains a significant threat because this provirus remains unaffected by antiretrovirals. A constant trafficking between cell-free (plasma) and cell-associated virus encourages mixing between the two populations, and different levels of drugs in different compartments may induce differential selection pressures on HIV populations. This selection pressure may significantly impact on the distribution of viral variants and also influence the nature of continuously evolving virodemes *in vivo*, many of which may harbor critical drug resistance mutations.

Several reports have demonstrated that PBMCs contain different drug resistance profiles to those of circulating HIV strains[105,139]. Although provirus within circulating lymphocytes is often "archived", the capacity for these variants to emerge upon changes in therapy or drug failure remains to be assessed. One recent study revealed that the degree of drug resistance also differs between individual leukocyte populations within the same patient[132]. The impact of the cellular compartmentalization of drug resistant HIV variants may further complicate the design of effective drug regimens, and impact on the successful suppression of HIV during HAART.

Interpretation of drug resistant genotypes

Genotypic tests are used more commonly in clinical settings because of their wider availability, lower cost and shorter turn-around time. The most common approach to genotype interpretation are "rule-based" and are available in both commercial kits and free online databases. The rule-based method involves the identification of key resistance mutations for individual drugs based on an algorithm developed by a panel of experts. These algorithms are the basis of automated computer generated reports. One example is the "TruGene assay", which incorporates expert opinion and the latest clinical research for interpretation of genomic mutations, providing a comprehensive summary of the patient's resistance status. Alternatively, numerous online databases are available which offer genotype interpretation (Table II). These have the obvious advantage of being a free internet resource, and are the most commonly used tools.

Ongoing sequences from global isolates, and patients treated with different anti-HIV drug combinations are needed to continue to identify the spectrum of genetic changes selected by drug therapy. An important point is that algorithms used for interpretation require frequent updating as new information becomes available. A reference database also allows researchers to rapidly compare all new RT and Pr sequences to those of a patient. Some examples of rule-based methods include the Stanford University Database[140], the Los Alamos National HIV Sequence Database, Retrogram, the Resistance Collaborative Group (RCG)[141], GuideLines (Visible Genetics), Virtual Phenotype (Virco), and the French National Agency for AIDS Research (ANRS)[142]. It should be noted that these systems may vary in their interpretation of mutations as they use different algorithms to generate drug resistant profiles.

Table II. Key web resources for HIV drug resistance genotyping

Database name	Web address	Features
Los Alamos National Laboratory HIV Sequence Database	http://hiv-web.lanl.gov	Searchable HIV related data bases including a database of drug resistance mutations
Standford HIV RT and Protease Sequence Database	http://hivdb.standford.edu/hiv	Comprehensive database of HIV RT and protease sequences linked to treatment data and phenotypic drug susceptibility data
International AIDS Society USA	http://hivinsite.ucsf.edu	Contains the most recently published guidelines along with simplified diagrams of key drug resistance mutations

New drugs in the pipeline

New RT inhibitors

The efficacy and tolerability of several new RT inhibitors are under investigation for future use in antiretroviral regimens. These include TMC 125, amdoxovir, DPC 083 and emtricitabine (FTC). TMC 125 is an NNRTI which has shown promising results in recent pilot studies in both NNRTI naïve and NNRTI experienced subjects, and has also shown favourable results in recent long-term trials[143,144]. Amdoxovir, a guanosine analogue, is deaminated to dioxolane guanine *in vivo*, a compound with anti-HIV activity[145]. This drug has been shown to have activity against some nucleoside-resistant viruses *in vitro* including those with the codon 69 insertion for multinucleoside resistance[146], but is susceptible to the K65R and L74V resistance mutations. DPC 083, a derivative of efavirenz, has shown increased activity against RT mutants with K103N, G190S and K101E *in vitro*, and in contrast to currently available NNRTIs, resistance to DPC 083 requires more than one substitution[147,148]. FTC, an investigative analogue of cytosine has anti-HIV activity[149] and is incorporated significantly more efficiently than lamivudine during the reverse transcription stage. However, like lamivudine, resistance to FTC is also incurred by the M184V mutation[150]. The pharmacokinetic profile of FTC is compatible with a single daily dosage, and trials using FTC as part of triple combination therapy have shown that satisfactory HIV suppression can be achieved with this compound[151].

New protease inhibitors

Currently, new investigative protease inhibitors include TMC 114, and tipranavir. Tipranavir has shown potent anti-HIV effects *in vitro*, including inhibition of isolates with phenotypic resistance to currently available PIs[152]. Further trials are assessing the efficacy of this compound. TMC 114, a nonpeptidic PI, is an analogue of TM 126, and has *in vitro* activity against isolates with high-level drug resistance to current PIs. Atazanavir, a protease inhibitor approved recently in America, has been shown to have *in vitro* activity against some variants with PI resistance mutations[153,154]. However, studies have shown a propensity for cross-resistance with other PIs in patients with previous PI treatment.

Inhibitors of HIV entry

With the emergence and transmission of resistant virus and the durability of HAART in the long-term proving to be a considerable challenge, efforts have also focussed on the development of antiviral agents that interfere with different processes in the HIV life cycle. These studies have led to the identification of agents that interfere with mechanistically distinct events in the HIV entry process, and several of these have entered into clinical evaluation stages[155-158]. PRO-542 is a fusion protein specifically designed with four CD4 elements to increase overall affinity for gp120[159]. Tolerability and absence of side effects has been established in a recent Phase I trial[160], and others have shown the compound has potent antiviral activity[161]. SCH-351125 a CCR5 antagonist, which was the first to be advanced to clinical efficacy studies, shows potent antiviral activity and pharmacokinetics which support twice daily dosaging[162]. Resistance to this compound does not involve a switch to CXCR4 variants. A trial administering SCH-351125 as monotherapy twice-daily reduced plasma viraemia by greater than $0.5\log_{10}$[163], and further testing at higher dosages is underway.

The recent approval of T-20 for clinical use in the USA has encouraged further research into fusion inhibitors. Recently, a second HIV fusion inhibitor T-1249 has been shown to display up to 100 times more antiviral activity than T-20 *in vitro*, and a preliminary study involving treatment-experienced patients using monotherapy demonstrated HIV RNA changes of up to $-1.4\log_{10}$ cpm[164]. Furthermore, resistance to RTIs did not affect this response.

HIV integrase inhibitors

The HIV integrase enzyme catalyses integration of the double-stranded viral DNA into the host genome, through a stepwise process of viral DNA complex assembly, viral DNA complex processing and strand transfer linking viral/host DNA. A number of potential integrase inhibitors have been identified, including the diketo analogue S-1360[165], the diketo acid L-708,906[165] and a novel integrase inhibitor V-165[166]. S-1360 has shown strong antiviral activity *in vitro*. Phase II trials using this compound were commenced in October 2002, and launch is expected in 2004/2005[167]. The development of antiviral resistance to L-708, 906 was recently studied

by growing HIV-1 strains in the presence of increasing concentrations of the compound resulting in the emergence of the resistance mutations T66I, L74M, and S230R. Viruses with all three mutations showed 10-fold less sensitivity to the compound, and phenotypic cross-resistance to S-1360 was observed for all strains[168]. Other inhibitors in the developmental pipeline include L-870812 and L-870810. These compounds were developed by Merck and prevent the step in the viral life cycle known as strand transfer.

Zinc finger inhibitors

Zinc fingers are a chain of amino acids found in the nucleocapsid of HIV, a viral core protein that is involved in binding and packaging of viral RNA into new virions budding from an infected cell. They may also play a role in reverse transcription. ACH-126, 443 (beta-LFd4C) and helioxantin, by Achillon Pharmaceuticals Inc; azodicarbonamide (ADA) by Hubriphar, a Belgian Company, and MC-135 are three zinc finger inhibitors under development[169].

Future directions and new strategies in the use of antiretroviral therapy

Currently, there are 18 drugs approved for treatment of HIV infection, and treatment guidelines recommend combinations of these agents. Despite a significant decrease in HIV-related morbidity and mortality in the developed world as a result of the implementation of HAART, current regimens face increasing problems with drug resistance, cross-resistance between different classes of antiretrovirals, and long-term toxicity. Therefore, in addition to further improving currently available antiretroviral drugs (*i.e.*, stronger antiviral activity, reduced toxicity, increased convenience), there is a clear and increasingly urgent need for the development of new drugs that target different aspects of the HIV-1 life cycle. The most promising developments in this area have been made with inhibitors of viral entry, and inhibitors of the viral integrase enzyme. Used in combination with currently available drugs, the implementation of these additional new classes of antiretrovirals will provide a much stronger impediment to the emergence of antiretroviral drug resistance.

To reduce the long-term toxicities of antiretroviral drugs, and to preserve future treatment options for as long as possible, various therapeutic strategies have been proposed apart from the usual HAART. There has been a swing away from the "hit hard hit early" approach to therapy. Due to the fact that no available regimen can eradicate HIV-1, currently effective regimens may cause undesirable and sometimes life-threatening toxic effects. Multi-drug resistance can develop unless regimens are strictly adhered to, and it has been suggested that a more balanced approach to the administration of HAART could prove beneficial[170]. Drug conservation strategies, for example where HAART is commenced when the CD4+ T cell count is around 250 cells/µl and ceased when it rises above 350 cells/µl in an episodic manner, are in clinical trial[170]. Other strategies include the use of induction therapy followed by maintenance with less intensive or simpler regimens, cycling of drug combinations, and use of immune stimulants (*e.g.*, interleukin 2, therapeutic vaccines) to boost CD4+T cell counts. The role of structured treatment interruptions (STI) has been controversial as there are a range of possible risks, including CD4+ T cell loss with the appearance of opportunistic infections, emergence of resistance mutations (and their seeding of viral reservoirs)[171,172], and rebound of plasma viral load. However, possible advantages of STI include the reduction of antiretroviral drug toxicity and use, thus improving quality of life and perhaps decreasing costs.

Conclusions

As the global pandemic of HIV continues to spread, the need for effective treatment has become increasingly pressing. Despite large scale efforts and dedication of resources into vaccine research and development world-wide, the production of an agent with the ability to prevent HIV infection still seems a long way off. In contrast, dramatic improvements in the mortality and morbidity of HIV-infected individuals have been achieved as a result of the implementation of HAART. Continued advances in currently prescribed RT and PR inhibitors, along with the inclusion of new antiretroviral agents in combination therapies targeting additional elements of the HIV life cycle, could facilitate the successful long-term survival and management of HIV-infected individuals well beyond current limits. Despite optimism about the future of HIV antiretroviral therapies, the issues of drug resistance, cross-resistance, pharmacokinetics and patient adherence, drug toxicity, and the augmentation of host immune

responses remain challenging issues in the management of HIV infection. Accordingly, future research into HIV antiretroviral drug treatments must also focus on these problems, allowing the design of more effective long-term treatment strategies.

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