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Recent Trends in HIV-1 Drug Resistance

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Abstract

Once considered an inevitable consequence of HIV treatment, drug resistance is declining. This decline supports the hypothesis that antiretroviral therapy can arrest replication and prevent the evolution of resistance. Further support comes from excellent clinical outcomes, the failure of treatment intensification to reduce residual viremia, the lack of viral evolution in patients on optimal therapy, pharmacodynamics studies explaining the extraordinarily high antiviral activity of modern regimens, and recent reports of potential cures. Evidence supporting ongoing replication includes higher rates of certain complications in treated patients and an increase in circular forms of the viral genome after intensification with integrase inhibitors. Recent studies also provide an explanation for the observation that some patients fail protease-inhibitor based regimens without evidence for resistance.

Introduction

Not long after the introduction of the nucleoside analogue reverse transcriptase inhibitor (NRTI) zidovudine (AZT) as the first antiretroviral drug for the treatment of HIV-1 infection, resistance to AZT was detected in treated patients [1]. Since that time, resistance has been reported for each new antiretroviral drug introduced [2], and the field has been haunted by the specter of widespread and inevitable drug resistance, necessitating the continued development of new classes of antiretroviral drugs. However, recent clinical experience suggests that HIV-1 drug resistance is actually declining, and it is now becoming clear that resistance is not an inevitable consequence of HIV-1 treatment, but rather a reflection of suboptimal treatment. Optimal therapy appears to prevent the evolution of resistance, even over long time periods. This review will explore the theoretical basis for this remarkable development.

The modern era of HIV-1 treatment began in 1997 when two new classes of antiretroviral drugs were introduced, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and the protease inhibitors (PIs). Three drug combinations consisting of an NNRTI or a PI and two drugs from the NRTI class were tested in clinical trials [3-5]. In these trials, it was shown for the first time that combination antiretroviral therapy (ART) could reduce viremia to clinically undetectable levels. When patients start on an appropriate combination of three antiretroviral drugs, plasma virus levels fall within a few months from pre-therapy levels on the order of 10^4-10^5 copies of genomic viral RNA/ml to below the limit of detection of clinical assays (50 copies/ml). Clinically, the disappearance of detectable viremia is

associated with increases in or preservation of $CD4^+$ T cell counts and the reversal or prevention of immunodeficiency. Combination ART rapidly became the standard of care for HIV-1 infection (Figure 1). Newly revised US treatment guidelines [6**] call for the treatment of all infected individuals with ART regimens consisting of two NRTIs and either an NNRTI, a PI or an integrase strand transfer inhibitor (InSTI) [7,8]. Several recent studies suggest that patients who start modern ART regimens early in the course of disease have a near normal lifespan expectancy, even in resource limited settings [9*].

Although ART can suppress viremia to clinically undetectable levels, there is inevitably a rebound in viremia within several weeks after discontinuation of therapy [10]. The ability of the virus to persist despite ART is due at least in part to is ability to establish a state of latent infection in resting memory $CD4^+$ T cells [11,12]. This latent reservoir is extremely stable, even in patients on optimal ART [13-16], and is a likely source of viral rebound following interruption of therapy [17]. Another important indication of viral persistence during ART is the presence of trace levels of free virus in the plasma [18-21]. This residual viremia is detectable with RT-PCR assays that have single molecule sensitivity. All current antiretroviral drugs act by blocking new infection events rather than by blocking virus production by cells that already have an integrated provirus (Figure 1). Thus, the residual viremia may reflect the activation of latently infected cells or possibly the release of virus from other stable reservoirs [17] . The only exceptions to the rule of viral rebound after discontinuation of ART are rare cases of patients treated early in the course of infection who are able to control viral replication through unknown immunologic mechanisms [22,23**].

The decline in HIV-1 drug resistance

HIV-1 drug resistance is a result of random mutations introduced by the error prone HIV-1 reverse transcriptase when it converts the single stranded genomic viral RNA into double stranded DNA shortly after viral entry. On average, one mutation is introduced in the 10 kb genome for every three cycles of replication [24]. These mutations are typically base substitutions although insertions, duplications and recombination can also occur. Because resistance mutations arise during reverse transcription in newly infected cells, the evolution of resistance can in principle be arrested if new infection event are blocked by ART. Avoiding resistance has been a major guiding principle of ART, and recent studies suggest that this goal is actually achievable.

Clinical experience and recent observational studies indicate that the incidence and prevalence of HIV-1 drug resistance is actually declining [25**,26*,27*,28*,29*]. A comprehensive study of essentially all infected individuals in Sweden found a dramatic decrease in the prevalence of resistance between 2003 and 2007 with slower decreases thereafter [25**]. This decrease coincided with the phase out of older drugs and the introduction of newer classes of antiretroviral drugs. Most mutations were found in patients with a history of suboptimal treatment. The only worrying trend was a very slight recent increase in the prevalence of NNRTI resistance mutations, which was attributed to the infection with resistant viruses in low to middle income countries where NNRTI regimens are very common and resistance monitoring is absent. A large multicohort European study found a decrease in the prevalence of drug resistance, particularly multiclass resistance, beginning around 2005 and continuing through the study end date in 2008 [26*]. In a large study of French patients with virologic failure (two plasma HIV-1 measurements >50 copies/ml), the fraction of samples with common RT resistance mutations (M184V/I, K103N) declined in the period between 2005 and 2010, despite continued use of drugs that select those mutations (lamivudine and emtricitabine for M184V/I and efavirenz for K103N) [27*]. The authors attribute this decline to the use of single pill, once a day regimens which promote improved adherence and prevent differential adherence to components of a

regimen. Selection of the NRTI resistance mutation K65R has also been declining despite the widespread use of tenofovir, which selects this mutation [28*]. The prevalence of multidrug resistance has been declining in Portugal in the time between 2001 and 2013 [29*]. Overall, these studies suggest that improvements in ART, including convenient single pill regimens consisting of three relatively non-toxic drugs, have improved adherence and allowed the full potential of ART to become apparent.

The efficacy of modern ART regimens in suppressing viral replication and preventing the evolution of resistance is a major factor in recent change in HIV-1 treatment guidelines. The concern about resistance was often cited as a reason for delaying the initiation of ART. However, numerous studies have shown that early treatment is associated with better outcomes (reviewed in [6]), and with the high antiviral activity and improved toxicity profiles of modern regimens, there is no medical reason to delay treatment. Thus current US guidelines recommend treating all infected individuals with ART.

The debate over ongoing viral replication

The dramatic success of ART suggests that viral replication, and hence viral evolution, can be completely blocked in adherent patients. However, a vigorous debate over whether ART can completely inhibit viral replication has raged for several years. Table 1 lists the major arguments supporting and contradicting the hypothesis that ART can completely block HIV-1 replication and the evolution of resistance. Because of the large number of people currently being treated with ART, there is an enormous amount of clinical data that pertains to this question. Careful analysis by committees that set treatment guidelines has shown that early initiation of treatment is associated with the best outcomes in HIV-1 infection [6**]. There is no evidence that adherent patients spontaneously fail treatment with resistant virus. There is very strong evidence that in untreated patients, ongoing viral replication in each infected individual leads to diversification of a single infecting founder virus into a complex quasispecies that shows progressive divergence from the founder virus over time. The error prone nature of reverse transcriptase results in an inevitable linkage between viral replication and viral evolution. In response to the selective pressure exerted by the immune response or suboptimal drug treatment, escape or resistance mutations arise very rapidly (within weeks). The success of treatment together with the dramatic decline in resistance described above argue strongly that ART can halt clinically significant viral replication and hence viral evolution.

Interventional studies involving treatment intensification also support the conclusion that ART stops viral replication. As discussed above, patients on ART who have suppression of viremia to below the limit of detection of clinical assays do have trace levels of free virus in the blood that can be detected with extremely sensitive “single copy” assays [18-21]. If the residual viremia is a reflection of ongoing viral replication not fully suppressed by ART, then the addition of a fourth antiretroviral drug from a different class to an optimal ART regimen should produce a decline in residual viremia detectable with such assays. If, on the other hand, residual viremia simply represents release of virus from stable reservoirs, then intensification should not decrease residual viremia. As shown in Figure 1, none of the current antiretroviral drugs block release of virus from cells that have an integrated provirus. Thus the activation of a small fraction of the resting $CD4^+$ T cells that harbor latent HIV-1 could result in trace levels of viremia that would not be affected by treatment intensification. Over the last several years, numerous intensification studies have been carried out with different intensification drugs [30-33]. The results have been remarkable consistent. All studies show that intensification has no effect on residual viremia. This means that residual viremia is largely due to release of virus from stable reservoirs, and that if there is ongoing replication, it results in a level of viremia that is insignificant even compared to the trace

levels detected with the single copy assay. A recent study has shown that intensification also fails to reduce trace levels of HIV-1 RNA in the cerebrospinal fluid [34].

Direct studies of viral evolution in patients on ART are complicated by the very low levels of virus present in the blood but have generally not shown any evidence for evolutionary change even over long time intervals. In some cohort studies, a small subset of the patients show evidence of viral evolution [35,36], but it cannot be excluded that these were patients with poor adherence. No study has documented ongoing viral evolution in the setting of adequate drug levels, and most patients show a striking lack of evolutionary change in viral sequences. The residual viremia is composed of drug sensitive viruses that do not evolve over time [37-41]. In acute infection, the gut associated lymphoid tissue (GALT) is a major site of viral replication [42], and high levels of HIV-1 DNA are found in $CD4^+$ T cells in the GALT even in treated patients [43]. A recent study has shown that even in the GALT, there is no detectable viral evolution in patients on ART, while evolution is readily detectable in untreated patients [44**].

Recent studies of HIV-1 pharmacodynamics have provided a quantitative basis for understanding the ability of ART to inhibit viral replication. Pharmacodynamics refers to the relationship between drug concentration and drug effect. The most commonly used parameter pharmacodynamic parameter is the IC_{50}, the drug concentration at which there is 50% inhibition of some measure of viral replication. The fraction of infection events affected (inhibited) by a drug (f_a) at a given concentration D is related to the IC_{50} by the median effect equation [45]:

$$f_a = \frac{1}{1+\left(\frac{IC_{50}}{D}\right)^m}$$

where m is a measure of the slope or steepness of the dose response curve. This parameter has an exponential relationship to drug effect, and when the $D > IC_{50}$, slope values greater than 1 can produce extremely high levels of inhibition. The slope parameter is related to the Hill coefficient, a measure of cooperativity, and slope values >1 are typically associated with cooperative binding of ligands to a multivalent receptor. However, the molecular targets of most antiretroviral drugs are monovalent with respect to inhibitors, and thus high slope values were not expected for antiretroviral drugs. In 2008, Shen et al. showed that some classes of antiretroviral drugs, notably the NNRTIs and the PIs, show highly cooperative dose response curves with high slope values in the inhibition of infectivity [45]. These high slopes allowed clinical concentrations of these drugs to produce many logs of inhibition of viral replication. When the combined effects of multiple drugs used together in ART regimens were considered, it became clear that most ART regimens could produce levels of inhibition that were consistent with a complete block in ongoing replication [46*].

One particularly interesting argument supporting the efficacy of ART in blocking new infection involves recent reports of potential cures. The original report described the “Berlin patient”, an HIV-1-infected man who had sustained suppression of viremia on ART when he developed acute myeloid leukemia [47]. As part of the treatment for leukemia, he received two hematopoetic stem cell (HSC) transplants from a donor who was homozygous for a 32 base pair deletion in the HIV-1 co-receptor CCR5. ART was discontinued at the time of the initial transplant, and viremia never rebounded. A recent exhaustive study of multiple tissues from this patient five years after the initial transplant failed to uncover consistent evidence of viral persistence [48*]. In this case, donor cells were protected from infection by the absence of CCR5. Two additional cases of HSC transplantation have recently been reported, and in these cases the donors were wild type for CCR5 [49**]. In these cases, the donor

cells were protected from HIV-1 infection by ART, which was continued uninterrupted throughout the entire transplant period. HIV-1 became undetectable at the time when 100% chimerism was documented. It remains to be determined whether these two new cases actually represent cures, but if so, they would dramatically illustrate the ability of ART to prevent otherwise susceptible donor cells from HIV-1 infection.

Although the preponderance of evidence suggests that modern ART regimens can arrest HIV-1 evolution in adherent patients, there is some evidence that trace amounts of replication continue. Patients on ART do experience higher levels of immune activation and higher rates of some non-infectious complications [50]. There is concern that some level of replication continuing in particular compartments could contribute to these complications and account for differences in life expectancy between patients and uninfected individuals. However, studies of this kind are complicated by differences in other risk factors in patient populations, legacy effects persisting from the time before treatment, and by drug toxicity.

Another line of evidence supporting some degree of ongoing replication is a recent study of the effects of intensification with the InSTI raltegravir [51]. Although levels of residual viremia do not change with intensification, there is a transient increase in the level of 2LTR circles, especially in patients on PI-based regimens. The circles form in the nucleus of infected cells when integration fails and the reverse transcribed HIV-1 DNA undergoes an end to end intermolecular ligation. The increase in these circles suggests that there were some new infection events in which virions released from an infected cell successfully matured and infected another cell in which reverse transcription then proceeded to completion. It is currently unclear how to reconcile this result with the evidence against ongoing replication presented above.

Finally, a scenario in which replication could continue despite ART has recently be described by Sigal and Baltimore [52**]. These investigators have shown that the local spread of HIV-1 from an infected cell to a neighboring cell can involve very high multiplicities of infection, which from a simple probability standpoint, reduces the effectiveness of antiretroviral drugs. This finding raises the possibility of local burst of replication of new infection, perhaps initiated by reactivation of latently infected cells.

Failure without resistance

An interesting development in the analysis of HIV-1 drug resistance is the phenomenon of treatment failure without resistance [53,54*]. Some patients on PI-based regimens develop detectable viremia but do not have resistance mutations in HIV-1 protease. Of course, non-adherence is one simple explanation, but in many cases the patients do show resistance to other drugs in the regimens. Two recent studies have offered potential explanations for PI failure without resistance (Figure 2). Rosenbloom et al. used pharmacokinetic and pharmacodynamics parameters and data on the fitness cost of resistance mutations to show that the time spent in the mutant selection window (MSW) is short for PIs [55*]. The MSW refers to the time following treatment interruption during which a drug resistant mutant has both a positive growth rate and a selective advantage over wild type virus. In evolutionary terms a positive growth rate is indicated by $R_0 > 1$, where R_0 is the basic reproductive ratio, the number of new cells that are infected by the virus released from a single infected cell. At very high drug concentrations, the resistant virus will be inhibited to a lesser extent than wild type virus but may still be unable to grow ($R_0 < 1$). As the drug concentration falls with non-adherence, it will reach a level at which the mutant will have a positive growth rate ($R_0 > 1$). This defines one boundary of the MSW (Figure 2A). Most drug resistance mutations cause a reduction in viral fitness in the absence of drug, and therefore the drug concentration will fall to a point where the wild type virus has a higher replication rate than

the mutant virus. This defines the other boundary of the MSW. At drug concentrations within this window, the mutant virus will be selected. The time spent in the MSW after treatment is interrupted is a complex function of the drug half-life, and the effect of the resistance mutations on IC_{50}, *m*, and viral fitness. Recent studies indicate that for PIs, the time spend in the mutant selection window is extremely short [54]. With falling PI concentrations, wild type virus rapidly gains a selective advantage, and thus patients can fail due to non-adherence without developing resistance.

Recent studies have uncovered a second explanation for the phenomenon of failure without PI resistance [56*]. As discussed above, PIs are extremely effective antiretroviral drugs due to highly cooperative dose-response curves that are not fully explained by current pharmacodynamic theory [45,57]. The nature of these curves as well as the phenomenon of PI failure without resistance can both be explained through analysis of the effects of PIs on distinct steps in the life cycle (Figure 1). PIs do not affect virion release from infected cells but block entry, reverse transcription (RT), and post-RT steps [56]. The overall dose-response curves can be reconstructed by combining the curves for each step using the Bliss independence principle. Thus independent inhibition of multiple distinct steps in the life cycle generates the highly cooperative dose-response curves that make these drugs uniquely effective. Approximately half of the inhibitory potential of PIs is manifested at the entry step, likely reflecting interactions between the uncleaved Gag and the cytoplasmic tail (CT) of the Env protein [58**]. Sequence changes in Env, which are ignored in current clinical tests for PI resistance, can confer PI resistance, providing an explanation for PI failure without resistance. Mutations in the HIV-1 protease cleavage sites in the Gag polyprotein can also be selected for during PI failure [59**]. Thus some cases of PI failure without resistance mutations in the protease gene may actually represent the evolution of resistant viruses that have sequence changes in other genes that contribute to PI resistance. Because current clinical tests for PI resistance consider only the protease gene itself, this issue deserves additional exploration.

Conclusions

Recent clinical experience and experimental studies have clearly shown that HIV-1 infection can be successfully treated without the evolution of drug resistance. The underlying explanation for this development is the ability of current ART regimens to suppress viral replication to such a great extent that the evolution of drug resistance, which depends on new infection events, is essentially halted.

Acknowledgments

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77. An elegant imaging study demonstrating differential localization of Env trimers on the surfaces of immature and mature virus particles. The authors suggest that Gag cleavage by HIV-1 protease is necessary to allow Env trimers to cluster in a configuration that promotes the entry process.

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Highlights

- Recent studies suggest that the prevelance of HIV-1 drug resistance is declining.
- This reflects the ability of antiretroviral therapy to halt viral evolution.
- Recent studies explain treatment failure without protease inhibitor resistance.

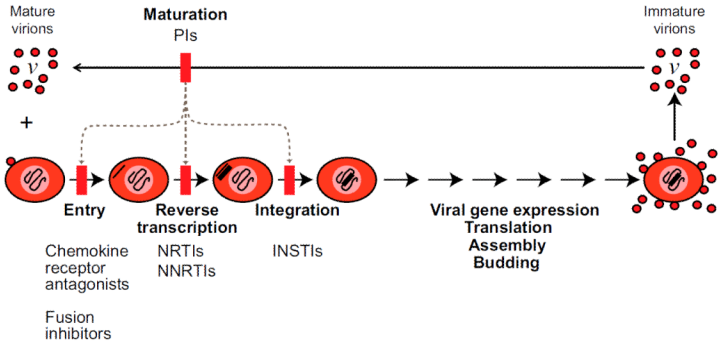

Figure 1.

Antiretroviral therapy for HIV-1 infection. The steps in the life cycle blocked by different classes of antiretroviral drugs are indicated. Current ART regimens consist of two NRTIs and either an NNRTI, a PI, or an InSTI. Inhibitors of HIV-1 entry, chemokine receptor antagonists and fusion inhibitors, can be used. Note that all current antiretroviral drugs act to prevent new cells from becoming infected. They do not block the production of virus particles by a cell that already carries an integrated provirus. The PIs prevent virus particles from maturing to an infectious form. Immature virus particles show defects at multiple downstream steps in the virus life cycle (dotted lines), including entry, reverse transcription, and integration. See text for references.

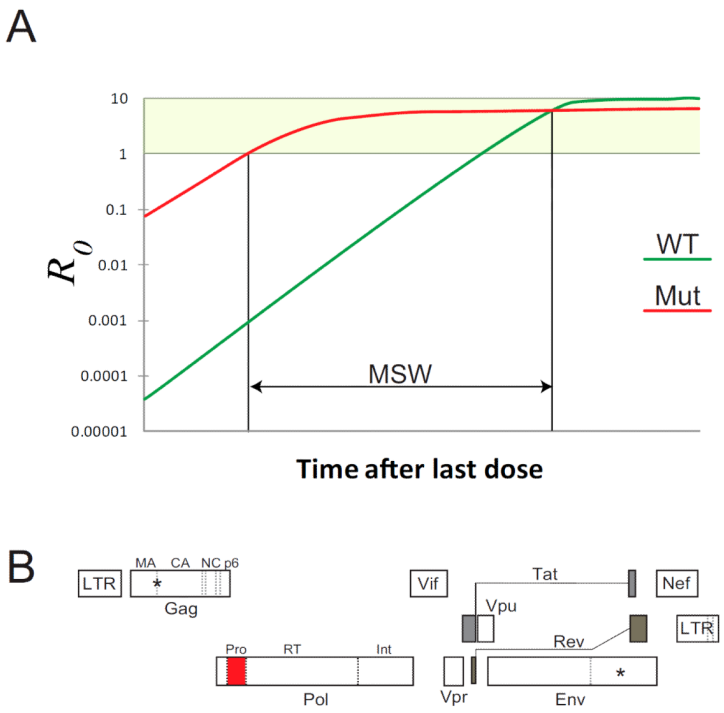

Figure 2.

Explanations for PI failure without resistance mutations in the protease gene. (A) Pharmacodyamic properties of PIs restrict the evolution of resistance. During periods of non-adherence, resistant viruses emerge. As drug concentrations fall, there is a mutant selection window (MSW) in which the mutant virus has both a positive growth rate ($R_0 > 1$) and a selective advantage over wild type virus. The length of the MSW depends on drug half-life, the properties of the dose-response curves for wild type (green) and resistant (red) viruses, and the fitness cost of the resistance mutation. For PIs, the time spent in the MSW is extremely short and with non-adherence, wild type virus rapidly emerges. Note that this form of treatment failure does not represent drug resistance, and suppression of viremia can be achieved by restoring adherence. (B) Mutations inside of (red) and outside of (*) the protease coding region can contribute to resistance. As shown in Figure 1, part of the inhibitory effect of PIs is due to effects on HIV-1 entry. Interactions between the uncleaved Gag precursor protein and the cytoplasmic domain of the Env protein inhibit entry, and thus PI-mediate inhibition of Gag cleavage results in inhibition of infectivity. Resistance to PIs may arise through mutations affecting the Gag-Env interaction. Mutations in the protease cleavage sites in Gag can also contribute to resistance. Current clinical assays for PI resistance examine only the protease gene and could miss other mutations contributing to resistance.

Table 1

Arguments for and against the hypothesis that ART can completely block HIV-1 replication and evolution (please see text for references).

For	Against
Success of ART in controlling viral replication and preventing immunodeficiency	Evidence for ongoing immune activation and inflammatory state in treated pateints
Failure of intensification to reduce residual viremia	Transient increase in 2LTR cirlecs with RAL intensification
Lack of evidence for sequence change over time in most patients	Sequence evolution in a subset of pateints
Pharmacodynamic data indicating extremely high antiviral activity of NNRTIs and PIs	Cell to cell spread reduces antiviral effect
Recent reports of cure	