

TIMELINE

HIV drug development: the next 25 years

Charles Flexner

Abstract | The development of drugs for HIV infection began soon after the virus was discovered 25 years ago. Since then, progress has been substantial, but numerous uncertainties persist about the best way to manage this disease. Here we review the current treatment options, consider novel mechanisms that can be exploited for existing drug targets, and explore the potential of novel targets. With a view to the next quarter century, we consider whether drug resistance can be avoided, which drug classes will be favoured over others, which strategies are most likely to succeed, and the potential impact of pharmacogenomics and individualized therapy.

Despite the availability of 23 approved anti-retroviral drugs (TABLE 1), there is continued interest in developing new agents for the treatment of HIV/AIDS for the following reasons:

- An effective vaccine or comparable preventative is unlikely to become available for years, and the epidemic will therefore be sustained in endemic areas and increase in new regions where the virus is introduced.
- There will be a need for better tolerated, more convenient and less expensive treatments.
- Increasing resistance to existing drugs will prompt the search for agents in classes that do not share cross-resistance, and for new classes of drugs that would not be affected by such resistance.

For the foreseeable future, HIV is likely to be one of the most common chronic infectious diseases on the planet. As antiretroviral drugs improve in tolerability, safety and long-term efficacy, there will be continued impetus to put more patients who are infected on treatment. This, in turn, will expand the market for existing and new products, and encourage innovation in discovery and development. Although future prospects are bright, important questions remain about the forces likely to drive HIV pharmacotherapy for the next 25 years.

Overview of present treatment options.

Published guidelines for developed countries now recommend that treatment offered to all persons infected with HIV with a CD4 lymphocyte count of <350 cells per mm^3 , regardless of their plasma HIV RNA concentration (also known as viral load)¹. All recommended regimens include a minimum of three active drugs; starting regimens generally consist of a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or HIV protease inhibitor combined with two nucleoside or nucleotide reverse-transcriptase inhibitors (NRTIs).

Possible regimens for treatment-experienced patients are more numerous, and include consideration of all potential agents to which the patient's virus is sensitive. One recent review of the records of a single large HIV clinic in the United States found that more than 800 unique regimens had been prescribed to several thousand patients within a decade (M. Saag, University of Alabama at Birmingham, personal communication).

As many patients modify their anti-retroviral regimen at least once during the first 3 years of therapy², a substantial fraction of the antiretroviral drugs prescribed in the developed world are used by treatment-experienced patients. For various reasons, treatment-experienced patients are

themselves at increased risk of regimen failure³. Resistance therefore continues to serve as a major impetus for new drug development, as patients harbouring drug-resistant strains of virus are a large group in need of new treatment options.

Of note, present guidelines already place millions of patients worldwide in the treatable category. This number is likely to grow as regimens become better tolerated, more convenient and more widely available, especially in developing countries. Whether starting regimens that are easier to take will produce fewer long-term failures, and therefore reduce the need for new drugs with better resistance profiles, is an important unanswered question.

Opportunities for drug discovery

As shown in FIG. 1, there are many steps for potential pharmacological intervention in the replication cycle of HIV. Despite this, approved drugs attack only three targets: reverse transcriptase; protease; and viral entry (TABLE 1; FIG. 1). TABLE 2 provides a list of promising investigational oral agents in clinical development. Two new drugs — maraviroc (Celsentri/Selzentry; Pfizer), a chemokine (C-C motif) receptor 5 (CCR5) chemokine receptor antagonist, and raltegravir, a HIV integrase inhibitor — have novel mechanisms of action. Maraviroc was FDA-approved in August 2007 and raltegravir is likely to be approved in the United States before the end of 2007.

Although new integrase and entry inhibitors have generated a great deal of excitement (see below), many of the drugs that are currently in advanced clinical development belong to existing classes. For example, two new NNRTIs — rilpivirine (also known as TMC-278) and etravirine (also known as TMC-125) — are expected candidates for approval within the next few years because there is no cross-resistance development to existing drugs in this class and they may be better tolerated. New HIV protease inhibitors with improved resistance profiles, better tolerability and convenience may also be approved in the near future. Motivation to develop new NRTIs could diminish, as existing agents in this class already possess better long-term safety and tolerability than older agents.

Table 1 | Licensed antiretroviral drugs, United States, 2007

Name	Trade name	Company	Launched
Nucleoside or nucleotide reverse-transcriptase inhibitors			
Zidovudine	Retrovir	GlaxoSmithKline	1987
Didanosine	Videx	Bristol-Myers Squibb	1991
Zalcitabine	HIVID	Roche	1992
Stavudine	Zerit	Bristol-Myers Squibb	1995
Lamivudine	Epivir	GlaxoSmithKline, Shire Pharmaceuticals	1998
Abacavir	Ziagen	GlaxoSmithKline	1999
Tenofovir disoproxil fumarate	Viread	Gilead	2001
Emtricitabine	Emtriva	Gilead	2003
Non-nucleoside reverse-transcriptase inhibitors			
Nevirapine	Viramune	Boehringer Ingelheim	1996
Efavirenz	Sustiva, Stocrin	Bristol-Myers Squibb, Merck	1998
Delavirdine	Rescriptor	Pharmacia & Upjohn, Agouron, Pfizer	1999
Protease inhibitors			
Saquinavir	Invirase	Hoffmann-La Roche	1995
Indinavir	Crixivan	Merck	1996
Ritonavir	Norvir	Abbott, GlaxoSmithKline	1996
Nelfinavir	Viracept	Agouron, Pfizer	1997
Amprenavir	Agenerase, Prozei	Vertex	1999
Lopinavir + ritonavir	Kaletra, Aluvia	Abbott	2000
Atazanavir	Reyataz, Zrivada	Bristol-Myers Squibb, Novartis	2003
Fosamprenavir	Lexiva, Telzir	Vertex, GlaxoSmithKline	2003
Tipranavir	Aptivus	Boehringer Ingelheim	2005
Darunavir	Prezista	Tibotec	2006
Entry inhibitors			
Enfuvirtide	Fuzeon	Trimeris, Roche	2003
Maraviroc	Celsentri, Selzentry	Pfizer	2007

Although the major viral targets — envelope, reverse transcriptase, integrase and protease — are already marked by drugs approved or nearing approval, there are a limitless number of new inhibitory mechanisms available for each of these viral proteins. For example, the maturation inhibitor bevirimat (also known as PA-457), which has shown anti-HIV activity in 10-day clinical trials, inhibits proteolysis by binding directly to a specific cleavage site in the gag polyprotein, rather than by binding to protease⁴. Novel mechanisms can therefore be exploited for existing targets.

Novel antiretroviral targets

Integrase. The integrase inhibitor raltegravir appears to be as potent as any previously developed antiretroviral drug in terms of its short-term⁵ and 24-week anti-HIV effects^{6,7}.

It is expected to become a mainstay of second-line therapy, if not an eventual candidate for first-line use. The advantage of this drug target is that integrase is an essential and highly conserved enzyme. However, one disadvantage is that moderate-level to high-level resistance to this and other integrase inhibitors can follow after only one or two amino-acid mutations⁸. A second integrase inhibitor, elvitegravir, is also in advanced clinical development. In September 2007, an independent FDA advisory committee voted for accelerated approval of raltegravir.

CCR5. The CCR5 antagonist maraviroc has excellent short-term anti-HIV activity, and is associated with substantial efficacy after 24 weeks of treatment when combined with antiretroviral nucleosides in treatment-experienced patients^{9,10}. The attraction of

this chemokine co-receptor target is that virtually all individuals are initially infected with the CCR5-trophic virus, and maraviroc is the first approved oral drug in the broad category of entry inhibitors. One drawback, however, is that maraviroc has little or no activity against viruses that use the chemokine (C-X-C motif) receptor 4 (CXCR4) as a co-receptor, or have dual/mixed tropism. Patients will need to be screened for virus co-receptor use with a commercial tropism assay before receiving this drug.

A second CCR5 antagonist, vicriviroc, has efficacy in Phase II trials in treatment-experienced patients¹¹, and is entering Phase III trials.

CXCR4. Two CXCR4 antagonists have demonstrated anti-HIV activity in small clinical studies, although changes in the plasma concentration of CXCR4-tropic viruses have been inconsistent, with some subjects having no measurable response^{12–14}. Both drugs cause a transient elevation in circulating neutrophil, lymphocyte and monocyte cell counts in treated subjects, which suggests a role for CXCR4 in peripheral trafficking of these cells¹⁵. In fact, one of these drugs, AMD3100 (plerixafor), is currently being used for adjunctive mobilization of stem cells in patients with various malignancies. The CXCR4 receptor and its native ligand, stromal cell-derived factor 1 (SDF1), also appear to play a critical role in central and peripheral axon migration and nervous system development¹⁶; *Cxcr4*-knockout mice have severely malformed brains. It is expected that every antagonist of this receptor will be teratogenic in humans. Whether these agents will also deter peripheral nerve regeneration in adults, or accelerate HIV-associated peripheral neuropathy, will require further study.

An important unanswered question about chemokine receptor antagonists is the potential long-term toxicity that might be associated with using an anti-infective drug that targets a host protein. CCR5 and CXCR4 have physiological roles in mediating host defences, and CXCR4 plays an important role in fetal development. Although humans who are homozygous for a 32-amino-acid deletion mutation that disables CCR5 are phenotypically normal under most circumstances, and are protected from infection by HIV-1 (REF. 17), recent studies indicate that inactivation of this receptor greatly increases the risk of severe encephalitis after West Nile virus infection¹⁸. One study with the CCR5 antagonist vicriviroc reported four cases of lymphoma in 86

treated patients during the first 48 weeks of study, although a causative role for the drug in these malignancies has yet to be proven¹¹. So far there appears to be no significant association between maraviroc treatment and lymphoma or other malignancies.

HIV regulatory proteins. HIV encodes several regulatory proteins whose functions range from transcriptional transactivation of the virus genome to antagonism of host defences (FIG. 1). As critical virus-encoded proteins, these represent potentially important drug targets; however they have failed to attract as much interest as virus elements already mentioned for several reasons. First, although these regulatory proteins exist to maximize replicative efficiency, most are not essential for the virus to reproduce. For example, the *nef* protein, which inhibits apoptosis in infected cells, can be deleted to produce a mutant virus of reduced virulence in primates. Still, *nef* deletion mutants are perfectly capable of propagating and infecting other animals, and can cause disease in humans¹⁹. A chemical *nef* antagonist is likely to quickly select for resistance, as its target is non-essential, and might not produce detectable reductions in plasma HIV RNA to the same extent as existing drugs.

A second possible difficulty faced by inhibitors of regulatory proteins is mechanistic. As the targets are usually protein–protein or protein–nucleic acid interfaces, rather than enzymes, there are pharmacological hurdles to impeding interactions involving such large surfaces. Parallel models for successful drug development in other diseases are few.

Perhaps as a case in point, two drugs targeting HIV regulatory proteins had no significant antiretroviral activity in previous clinical studies. A selective HIV *tat* antagonist had no anti-HIV activity in 96 patients treated for 12 weeks²⁰, and mifepristone, a progesterone analogue that interferes with *vpr* function *in vitro*, had no detectable anti-HIV activity at doses up to 225 mg per day when given to 56 HIV-infected subjects for up to 28 days²¹.

Other host proteins. Successful replication of HIV requires the participation of several host proteins²². Any of these could become potential antiviral targets. However, most cellular processes that are essential to the virus are also essential to the host, and targeting these pathways in a way that is selectively toxic to the virus may be difficult.

More promising are strategies to enhance naturally occurring host defences.

Table 2 | Promising investigational antiretroviral drugs, 2007*

Name	Target	Company	Phase
Raltegravir	HIV integrase	Merck	Preregistration
Elvitegravir	HIV integrase	Japan Tobacco, Gilead	II
Vicriviroc	CCR5 chemokine co-receptor	Schering–Plough	III
INCB009471	CCR5 chemokine co-receptor	Incyte Corporation	II
AMD-070	CXCR4 chemokine co-receptor	AnorMED	II
Bevirimat	Polyprotein maturation	Panacos Pharmaceuticals	II
Amdoxovir	NRTI	RFS Pharma	II
Apricitabine	NRTI	Avexa	II
Racivir	NRTI	Pharmasset	II
Reverset	NRTI	Incyte	Discontinued [‡]
Etravirine	NNRTI	Tibotec	Preregistration
Rilpivirine	NNRTI	Tibotec	III
UK-453,061	NNRTI	Pfizer	II
Breacanavir	HIV protease inhibitor	GlaxoSmithKline	Discontinued [§]

*Table includes oral agents producing a >0.5 log mean decrease in HIV plasma RNA concentration when given for a minimum of 10 days to infected patients; in the case of AMD-070, this standard applies to concentrations of chemokine (C-X-C motif) receptor 4 (CXCR4)-tropic virus only. [†]In 2006 owing to increases in grade 4 hyperlipasemia in patients receiving reverset. [‡]In 2006 owing to formulation issues. CCR5, chemokine (C-C motif) receptor 5; NNRTI, non-nucleoside reverse-transcriptase inhibitor; NRTI, nucleoside or nucleotide reverse-transcriptase inhibitor.

For example, *APOBEC3G*, a cellular enzyme of the cytidine deaminase family, is incorporated into virus particles and is capable of inactivating HIV RNA or DNA. The HIV *vif* protein facilitates *APOBEC* degradation and thus counteracts its effects²³. Pharmacological strategies that neutralize *vif* or upregulate *APOBEC* expression might therefore protect cells from infection.

Strategies for boosting host immunity have also been tested. Results of therapeutic vaccination trials have been disappointing to date; whether new, more sophisticated vaccine approaches will have antiviral efficacy in established infection is speculative. Cytokine therapy remains of interest to some investigators. Intermittent therapy with interleukin 2 (*IL2*) certainly boosts CD4⁺ lymphocyte counts, but has no beneficial impact on viral RNA concentrations²⁴. Given its expense, toxicity and need for parenteral administration, *IL2* is unlikely to prove cost-effective for most patients. Other non-specific immune-based therapies, including systemic corticosteroids and cyclosporine A, have failed to produce consistent benefit in short-term trials, and possess long-term side effects that render them unattractive.

The next 25 years

Will some drug classes be favoured over others? As convenience and tolerability improve, and the number of highly active agents expands, long-term drug safety becomes increasingly important. One must

assume that any agent — or combination — sustaining plasma HIV RNA concentrations below the detection limit will have an equivalent mortality and morbidity benefit for subjects infected with HIV²⁵. The major factors distinguishing one regimen from another are likely to be toxicity and tolerability. Several existing agents — for example, lamivudine (Epivir; GlaxoSmithKline/Shire Pharmaceuticals), emtricitabine (Emtriva; Gilead) and tenofovir (Viread; Gilead) — have absent, or nearly absent, long-term side effects, and this list is expected to grow in the future. An important obstacle to creating safer drugs is the lack of cell lines or animal models that precisely predict long-term drug safety in humans.

Some current regimens are associated with an increased risk of cardiovascular events such as myocardial infarction or stroke²⁶. To what extent this is a consequence of direct toxic effects of the agents involved, a reflection of the ageing population under study, or an unavoidable metabolic complication of reversing established HIV infection, will require further study. Several different drugs have been associated with increased cardiovascular risks, which suggests a generalized metabolic mechanism. However, certain agents, including the widely used protease inhibitor ritonavir (Norvir; Abbott), have a greater propensity to elevate blood lipids such as cholesterol and triglycerides²⁶. As these markers represent increased risk of morbidity and mortality,

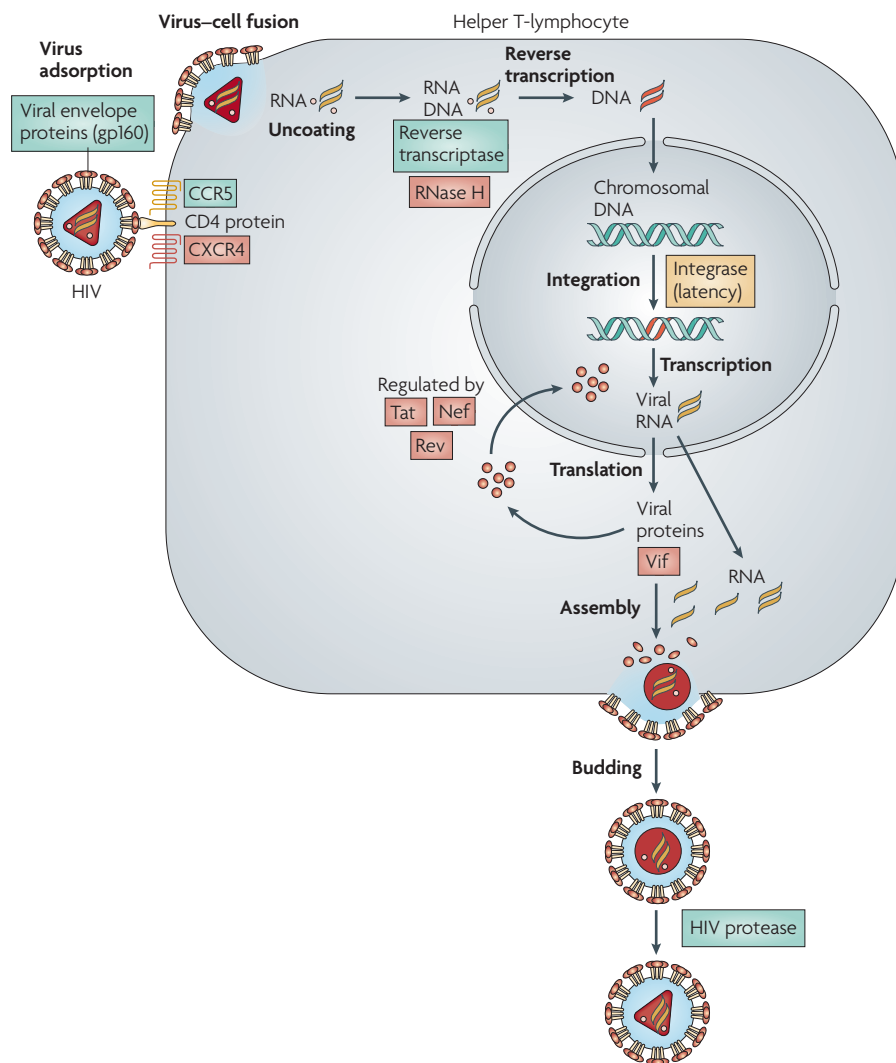


Figure 1 | Replication cycle of HIV with current and possible targets for antiviral intervention. Proteins that are targets for approved drugs are coloured green: gp160, reverse transcriptase, HIV protease and chemokine (C-C motif) receptor 5 (CCR5). Integrase (yellow) is a target in advanced clinical development. Proteins that are more speculative drug targets are coloured red: chemokine (C-X-C motif) receptor 4 (CXCR4), RNase H, tat, rev, nef and vif.

agents associated with such problems are likely to be shunned once alternatives become available.

The possible global impact of HIV-2 and its comparative resistance to some classes of antiretroviral drugs is an issue that has been underappreciated. All approved NNRTIs are inactive against HIV-2, whereas NRTIs and protease inhibitors appear to be equally effective against both families of the virus. Although HIV-2 is currently restricted in distribution to parts of West Africa²⁷, increasing prevalence of this virus might sway drug development decisions towards more broadly active agents. Drug resistance issues related to co-infection with HIV and hepatitis B virus are also likely to assume greater importance (BOX 1).

Is drug resistance unavoidable? Current dogma dictates that all effective anti-retroviral agents can and will select for drug-resistant virus. This view is driven by an understanding of the huge reservoir of replicating virus (1×10^{10} infected cells in an average patient), and its mutational propensity (about three mutations per virion per round of replication)²⁸. Combining agents without cross-resistance promotes long-term efficacy. No treatment strategy has violated this paradigm, although some agents with measurable anti-HIV effects in patients, such as interferon- α (IFN α), have not yet been shown to promote drug-specific resistance²⁹. In the case of IFN α , this may simply reflect the nonselective or pleuripotent mechanism of action of the drug.

One paradoxical drug development strategy involves promotion of mutagenesis during HIV replication, using ribonucleoside analogues, so that no progeny virus can survive³⁰. In effect, the virus mutates itself out of existence. Whether HIV can develop resistance to such a mechanism remains unclear.

Approaches targeting host proteins, for example chemokine receptors, might seem less prone to select for drug resistance. Experience so far suggests otherwise. In one instructive example, HIV can become resistant to the CCR5 antagonist maraviroc by modifying its envelope glycoprotein, gp160, so that it can bind to the host chemokine receptor even with inhibitor in place³¹.

Although drug resistance may be inevitable, treatment failure due to resistance is not. Strategies in which single active drugs are added to failing regimens are no longer acceptable because they predictably produce drug resistance and another round of treatment failure. The increasing number of approved and investigational antiretroviral drugs should help eliminate this scenario. Future trials for heavily treatment-experienced patients will come with the expectation that every regimen will contain at least two active drugs³² (BOX 2).

Ultimately, the best way to avoid resistance is to treat the patient with an effective combination of drugs, regardless of the individual agent's barrier to resistance. It should not be forgotten that efavirenz (Sustiva/Stocrin; Bristol-Myers Squibb), which is only a single point-mutation removed from complete resistance, is one of the most widely used and effective of antiretroviral agents. Its susceptibility to resistance is offset by high antiviral potency, an excellent pharmacokinetic profile — including a long elimination half-life — and a reasonable side-effect profile in most patients.

Which strategies are most likely to succeed?

For regimens without substantial toxicity, treatment failure is the consequence of non-adherence or pre-existing resistance. Baseline resistance testing reduces the chance that patients will receive drugs to which they are resistant. But the most successful treatment strategies minimize or tolerate occasional non-adherence. Imperfect adherence is widespread and nearly impossible to prevent, especially in cases where a drug combination will be taken for years. FIGURE 2a indicates the prevalence of missed and late doses in patients taking clarithromycin every 12 hours for life-threatening *Mycobacterium avium* infection. As shown, the range of adherence is wide and unpredictable.

Box 1 | The HIV/HBV overlap dilemma

Although it is estimated that about 5% of patients infected with HIV in the United States and Europe are co-infected with *hepatitis B virus* (HBV), the prevalence of co-infection is higher in the developing world, reaching 10% or higher in China and Southeast Asia. The RNA-dependent DNA polymerase (reverse transcriptase) of HIV and the DNA polymerase of HBV have functional similarity, and several antiviral nucleosides inhibit both enzymes and have clinical activity against both viruses. The list of drugs with overlapping specificity includes tenofovir, adefovir, lamivudine and emtricitabine.

One recent case series found that the anti-HBV nucleoside entecavir exerted anti-HIV activity in three co-infected patients being treated with entecavir alone, and promoted emergence of the M184V nucleoside resistance mutation in the HIV reverse transcriptase gene⁴⁵. These patients had a >1 log drop in plasma HIV RNA while taking no other anti-HIV drugs. This example raises concerns about using antiviral drugs with broad-spectrum activity in patients harbouring two or more chronic viral infections, which could promote drug resistance. An intriguing solution would be to require that anti-HIV regimens contain effective anti-HBV drugs, especially in areas where the prevalence of co-infection is high. Conversely, all patients being treated for chronic HBV might also need to receive drugs that will effectively block HIV replication. This strategy may be more attractive in the future if safe, effective and inexpensive overlap regimens can be developed. One possible outcome is that nucleoside and nucleotide reverse-transcriptase inhibitors could be avoided in regions with a high prevalence of HIV/HBV co-infection.

The RNA polymerase of *hepatitis C virus* bears little similarity to the HIV reverse transcriptase, and, aside from interferon- α (IFN α), there are, as yet, no approved drugs with overlapping clinical activity against these two viruses.

randomized clinical trials^{33,34}, there is a simplicity advantage to once-a-day drugs, especially in patients who require other medications for intercurrent conditions.

Two antiretroviral drugs — zidovudine and didanosine — are already available as generics in the United States. Generic copies of several other antiretrovirals are available in developing countries through accepted circumvention of intellectual property law. The impact of generic antiretrovirals represents a major unknown in future prescribing practices. To date, most treatment decisions in the developed world take place without regard to cost. As effective generic combination regimens and co-formulations (BOXES 3,4) become available in the next 25 years, prescribers may be forced to accept more inconvenient generic drugs (for example a twice-daily, multiple pill regimen) in place of more expensive but more attractive alternatives.

Pharmacogenomics and individualized therapy.

Although genetic constitution is an important determinant of drug disposition and response, there are few examples today of treatment decisions being driven by results of genetic tests. In HIV pharmacotherapy, one important and life-threatening toxicity, abacavir hypersensitivity syndrome (HSS), is strongly associated with *HLA-B*5701*, an uncommon human leukocyte antigen (HLA) genotype, with possible additional contributions from an ancestral haplotype

Past treatment failure is often a surrogate marker for past non-adherence. A non-adherent patient is more likely to fail a new regimen regardless of the number of active agents³. Unfortunately, second-line agents often lack the convenience and tolerability of first-line drugs. For example, the entry inhibitor enfuvirtide (Fuzeon; Trimeris/Roche) is generally active in heavily treatment-experienced patients, but must be injected subcutaneously twice a day. These drug traits exacerbate non-adherence, and this is an agent that would never be given as part of initial therapy to a treatment-naïve patient.

As past non-adherence may be a marker for future non-adherence, at least as much attention needs to be paid to improving adherence in such patients as to monitoring resistance and finding active drugs. Over the next several decades, drug development should focus on agents for treatment-experienced patients that are as safe and convenient as agents meant for treatment-naïve patients.

Favourable pharmacokinetics, including predictable oral bioavailability and long elimination half-life, are characteristic of the most useful antiretroviral drugs. However, inter-individual and intra-individual pharmacokinetic variability for many of these agents is high (FIG. 2b). Possible explanations include variable food effects, intercurrent medications and drug interactions, and other genetic and environmental influences (see below). The most successful agents must be able to tolerate this degree of variability in drug concentrations, or risk an unacceptably high rate of failure.

As a practical matter, all current antiretrovirals are administered once or twice-daily. Three approved drugs — dideoxycytidine (zalcitabine/HIVID; Roche), delavirdine (Rescriptor; Agouron) and indinavir (Crixivan; Merck) without pharmacokinetic enhancement by ritonavir (Norvir; Abbott) — must be given three times a day, and are rarely used as a consequence. Although once-daily and twice-daily regimens perform equivalently in most

Box 2 | Are two drugs enough? Or perhaps one?

Based on nearly two decades of clinical trial results, all recommended anti-HIV regimens include a minimum of three active agents. Recent studies have investigated the possibility of using two, or in some cases even a single potent antiretroviral agent, in place of traditional three-drug regimens. Usually these studies have involved simplification strategies in which treatment-naïve patients with a stable and undetectable plasma HIV RNA of <50 copies per ml are randomized to continue a three-drug regimen or receive a single active agent in its place. The most common simplification regimens involve the HIV protease inhibitors atazanavir or lopinavir combined with a low dose of ritonavir for pharmacokinetic benefit. In most cases, single-agent maintenance therapy is as good or nearly as good as triple therapy, at least in the short-term⁴⁰. However, some patients have experienced virologic failure, with new resistance mutations, while on single-agent protease inhibitor therapy⁴¹.

Few published studies initiated treatment with only one or two drugs. In one large randomized trial, a combination of two active drugs (lopinavir/ritonavir plus efavirenz) was about as effective as lopinavir plus two nucleosides or efavirenz plus two nucleosides, but with more toxicity⁴². Two small pilot studies have evaluated starting therapy with lopinavir/ritonavir alone, and most treatment-naïve patients with a baseline viral load of <100,000 copies per ml remain suppressed after 6 months of treatment with such a regimen⁴³.

Combination chemotherapy for chronic HIV infection is based on prevention of resistance and not on pharmacological synergy. Mathematical models suggest that three drugs are the minimum needed to prevent resistance with a high degree of certainty, as a function of the number of mutations required to become resistant⁴⁴. Whether one or two potent drugs — especially those with high genetic barriers to resistance — could achieve the same effect is unproved. The desirability of one- or two-drug regimens may diminish as one-pill-daily, three-drug co-formulations become more widely available (BOXES 3,4).

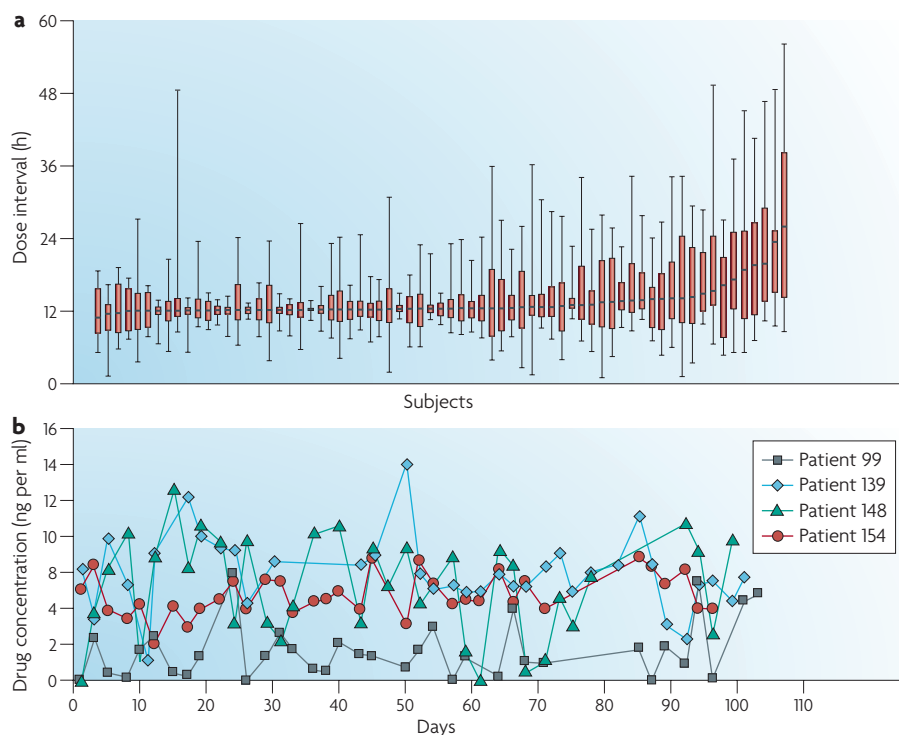


Figure 2 | **Adherence to clarithromycin (for *Mycobacterium avium* infection) in HIV-infected patients, and variability in pharmacokinetics of lopinavir (for HIV infection).** **a** | Patterns of adherence in HIV-infected patients taking a twice-daily drug. Box (25th–75th percentile plus median) and whisker (5th–95th percentile) plots of measured dose intervals for 69 patients infected with HIV taking clarithromycin every 12 hours for the treatment of *Mycobacterium avium* infection. Dose intervals were measured using Medication Event Monitoring System (MEMS) devices. Figure courtesy of D. Noe and C.F.; unpublished observations from a randomized prospective treatment trial⁴⁷. **b** | Measured intra-subject variability in concentrations of lopinavir dosed 400 mg every 12 hours. Participants had undetectable plasma HIV RNA on treatment for at least 3 months, and were seen in the clinic 3 times a week for up to 4 months. Blood for lopinavir concentration analysis was collected at approximately the same time of day at each visit, as described previously⁴⁷. Figure adapted with permission from REF. 48 © (2006) The University of Chicago Press.

that includes *HSP70-HOM* (also known as *HSPA1L*), a locus encoding a heat shock protein³⁵. In a homogenous Caucasian population, the presence of these two markers had a positive predictive value for HSS of 93.8% and negative predictive value of 99.5%. Because of this strong association, many patients are now pre-screened for *HLA-B57* genotype before prescribing abacavir. One older survey reported that *HLA-B57* was present in only 46% of HSS cases³⁶. However, several recent studies using abacavir sensitivity skin testing indicate that many of the B57-negative cases may represent misdiagnosis or a distinct, milder form of the syndrome.

Other host genetic markers that are predictive of antiretroviral treatment response or toxicity are more weakly associated with outcomes. In one study, patients harbouring a single base-pair polymorphism at position 516 of the cytochrome P450 2B6 gene (*CYP2B6*; *G516T*) had median efavirenz concentrations that were twofold to threefold higher than other variant genotypes³⁷. Individuals who were homozygous T/T at this locus had higher median efavirenz concentrations, but also had median central nervous system toxicity questionnaire scores that were more than twice those of G/G homozygotes after the first week of treatment³⁶. Despite these group differences, the range of drug concentrations and side effects overlapped substantially between patients of different genotypes, and the CNS toxicity difference did not persist beyond

week one. This interesting association is therefore unlikely to lead to genetically guided treatment decisions.

Genomic screening holds promise in the diagnosis and understanding of some important pharmacological effects, as shown

with abacavir hypersensitivity. Additionally, advances in cardiovascular genetics could help guide regimen selection for some groups of patients, for example by avoiding those drugs most strongly associated with cardiac risk. Common adverse drug effects

Box 3 | Formulations and co-formulations

The approval of Atripla (Bristol-Myers Squibb/Gilead) — a single tablet, once-a-day co-formulation of efavirenz, tenofovir and emtricitabine — in July 2006, signalled the start of a new era in antiretroviral therapy. Co-formulations greatly simplify treatment, and put HIV infection in the same class as hypercholesterolaemia, hypertension or gastroesophageal reflux disease — all chronic conditions that can be controlled with a single pill taken once daily. Similar comments apply to the Triomune co-formulation of generic nevirapine, stavudine and lamivudine, which is now one of the most widely used anti-HIV regimens in the world, albeit a twice-daily regimen.

Atripla may be the new standard for initial treatment. However, co-formulations come with a price. First, regimens containing drugs from more than one manufacturer may require months or years of negotiation just to agree that such a product can be developed. Co-formulations can involve drugs with chemical incompatibilities, and may require different excipients. Such products include only a single fixed dose of each component; this makes dose individualization — for example, in young children or patients with renal insufficiency — difficult or impossible.

New formulations often improve the pharmacokinetic properties and extend the patent life of existing drugs. This strategy has figured prominently in the marketing of drugs for other chronic diseases, as new formulations, often sustained-release formulations, frequently appear when the patent expires on an existing product.

The development and approval of the tablet formulation of the lopinavir/ritonavir co-formulation Kaletra (Abbott) using melt-extrusion technology, is an example of the kind of improvements we can expect in the future. This formulation reduced the pill burden from six capsules to four tablets per day, and improved the temperature stability of the components, allowing room temperature storage rather than refrigeration. As a consequence, lopinavir may now be made available in areas without reliable refrigeration.

Radical modifications in formulation, such as the development of a depot-like, once-a-month, injectable or transdermal antiretroviral, are beyond the reach of current drugs. As potency and pharmacokinetic properties improve, however, such approaches may appear on the treatment horizon.

Box 4 | As good as it gets?

Standard combination regimens for treatment-naive patients are capable of producing optimal virologic benefits (plasma HIV RNA maintained at <50 copies per ml) in nearly 90% of patients at or beyond 48 weeks⁴⁶. The most rigorous analyses of such data centre on intention-to-treat, and count as failures patients who drop out or modify treatment. Given the nature of clinical trial participation, it is unlikely that one could design a trial with fewer than 10% drop-outs or regimen modifications during the first year of therapy. It may not be possible then to develop an initial antiretroviral regimen with better than 90% virologic responses after 1 year. As efficacy may have reached its asymptotic maximum, new antiretroviral regimens for treatment-naive patients will be expected to have equivalent efficacy to existing regimens, but better convenience and tolerability.

Response rates in treatment-experienced patients are currently much lower, with optimal virologic benefits obtained in only 40–60% of patients during the first year of a new regimen³. With the availability of additional potent drugs, especially those in new classes such as HIV integrase inhibitors and entry inhibitors, outcomes in treatment-experienced patients should approach those achieved in treatment-naive patients. Regimens unable to reach and sustain such treatment goals are likely to fall out of favour.

Charles Flexner is at Johns Hopkins University, Divisions of Clinical Pharmacology and Infectious Diseases, Departments of Medicine, Pharmacology and Molecular Sciences, and International Health, Osler 503, 600 North Wolfe Street, Baltimore, Maryland 21287-5554, USA.

e-mail: flex@jhmi.edu

doi: 10.1038/nrd2336

Published online 12 October 2007

and likelihood of response to any given regimen are likely to be genetically complex and susceptible to environmental influences. Even if access to multi-allelic haplotypes becomes routine over the next 25 years, it is unlikely that many HIV treatment decisions will be guided by genetics. Drug approval tied to a requirement for prior genetic screening could even be a disadvantage for some future antiretrovirals, given the wide array of agents already on the market without such restrictions.

Will special populations drive drug development?

Several patient groups have characteristics that merit unique approaches to treatment. This includes infants and young children, pregnant women and, increasingly, the elderly. To some extent, HIV treatment decisions are already being tailored to the special needs of these groups. Efavirenz, which is teratogenic, is avoided in women who intend to become pregnant. Special formulations have been developed to allow individualized dosing of antiretrovirals in paediatric patients.

Whether any of these special populations will ever be large enough to warrant development of drugs mainly targeted to their needs is an open question. Certainly, the number of paediatric patients infected with HIV is large in developing countries. However, this number is expected to shrink as effective prevention of mother-to-child transmission is practiced more uniformly, as has happened in the United States, Europe and Australia.

Will global demographics drive drug discovery?

As the majority of HIV-infected patients will continue to live in some of the poorest parts of the world, it is logical to consider whether this market will become more attractive for those who discover and develop new antiretroviral drugs. One immediate

implication of these changing demographics is that new parenteral antiretrovirals — unless their properties are extraordinary — will probably attract few proponents. Early screening for oral bioavailability in human subjects is already a pivotal step for most investigational agents, and will remain so in the future. Human microdosing studies with radiolabelled candidates can rapidly identify compounds with optimal pharmacokinetic properties such as oral bioavailability, making this process more efficient³⁸.

Although some drugs will continue to be marketed in the developed world regardless of cost, other agents could be selected on the basis of decreased manufacturing expense and reduced sales price. For example, there could be renewed interest in peptide-based HIV protease inhibitors that could be produced cheaply in recombinant bacteria or plants, especially if problems of oral bioavailability can be solved.

Last, it should be pointed out that response rates to antiretroviral therapy have improved dramatically in the past 5 years, probably as a consequence of better drugs and better knowledge of how to use them^{2,39} (BOX 4). As treatments improve, so will the impetus to treat more infected individuals. Prospective studies could identify those at greatest risk of HIV-associated morbidity and mortality, for example due to tuberculosis co-infection, leading to targeted campaigns to deliver antiretrovirals to those likely to experience the greatest benefit.

With 40 million people infected with HIV alive today, and a higher number expected in the future, the first prospective study to demonstrate the benefits of earlier intervention, say at a CD4-count threshold of 500 cells per mm³, will increase the number of patients eligible for treatment by millions. This kind of statistic will encourage the development of new antiretroviral drugs for decades to come.

1. Department of Health and Human Services (DHHS). Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. *AIDSinfo web site* [online], <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf> (2006).
2. Moore, R. D., Keruly, J. C., Gebo, K. A. & Lucas, G. M. An improvement in virologic response to highly active antiretroviral therapy in clinical practice from 1996 through 2002. *J. Acquir. Immune Defic. Syndr.* **39**, 195–198 (2005).
3. Struble, K. *et al.* Antiretroviral therapies for treatment-experienced patients: current status and research challenges. *AIDS* **19**, 747–756 (2005).
4. Li, F. *et al.* PA-457: a potent HIV inhibitor that disrupts core condensation by targeting a late step in Gag processing. *Proc. Natl Acad. Sci. USA* **100**, 13555–13560 (2003).
5. Markowitz, M. *et al.* Antiretroviral activity, pharmacokinetics, and tolerability of MK-0518, a novel inhibitor of HIV-1 integrase, dosed as monotherapy for 10 days in treatment-naive HIV-1-infected individuals. *J. Acquir. Immune Defic. Syndr.* **43**, 509–515 (2006).
6. Cooper, D. *et al.* Results of BENCHMRK-1, a Phase III study evaluating the efficacy and safety of MK-0518, a novel HIV-1 integrase inhibitor, in patients with triple class resistant virus. Abstract 105aLB. *14th Conference on Retroviruses and Opportunistic Infections web site* [online], <http://www.retroconference.org/2007/Abstracts/30687.htm> (2007).
7. Stiegbigel, R. *et al.* Results of BENCHMRK-2, a Phase III study evaluating the efficacy and safety of MK-0518, a novel HIV-1 integrase inhibitor, in patients with triple class resistant virus. Abstract 105bLB. *14th Conference on Retroviruses and Opportunistic Infections web site* [online], <http://www.retroconference.org/2007/Abstracts/30688.htm> (2007).
8. Jones, G. *et al.* Resistance profile of HIV-1 mutants *in vitro* selected by the HIV-1 integrase inhibitor, GS-9137 (JTK-303). Abstract 627. *14th Conference on Retroviruses and Opportunistic Infections web site* [online], <http://www.retroconference.org/2007/Abstracts/29251.htm> (2007).
9. Nelson, M. *et al.* Efficacy and safety of maraviroc plus optimized background therapy in viremic, ART-experienced patients infected with CCR5-tropic HIV-1 in Europe, Auastralia, and North America: 24-week results. Abstract 104aLB. *14th Conference on Retroviruses and Opportunistic Infections web site* [online], <http://www.retroconference.org/2007/Abstracts/30636.htm> (2007).
10. Lalezari, J. *et al.* Efficacy and safety of maraviroc plus optimized background therapy in viremic, ART-experienced patients infected with CCR5-tropic HIV-1, 24-week results of a Phase 2b/3 study in the U. S. & Canada. Abstract 104bLB. *14th Conference on Retroviruses and Opportunistic Infections* [online], <http://www.retroconference.org/2007/Abstracts/30635.htm> (2007).
11. Gulick, R. M. *et al.* Phase II study of the safety and efficacy of vicriviroc, a CCR5 inhibitor, in HIV-1-infected, treatment-experienced patients: ACTG 5211. *J. Infect. Dis.* **196**, 304–312 (2007).
12. Hendrix, C. W. *et al.* Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J. Acquir. Immune Defic. Syndr.* **37**, 1253–1262 (2004).
13. Moyle, G. *et al.* CXCR4 antagonism: proof of activity with AMD11070. Abstract 511. *14th Conference on Retroviruses and Opportunistic Infections* [online], <http://www.retroconference.org/2007/Abstracts/29173.htm> (2007).
14. Saag, M. *et al.* Proof of concept of ARV activity of AMD11070 (an orally administered CXCR4 entry inhibitor): results of the first dosing cohort A studied in ACTG Protocol A5210. Abstract 512. *14th Conference on Retroviruses and Opportunistic Infections* [online], <http://www.retroconference.org/2007/Abstracts/30166.htm> (2007).

15. Stone, N. D. *et al.* Multiple dose escalation study of the safety, pharmacokinetics, and biologic activity of oral AMD070, a selective CXCR4 receptor inhibitor, in human subjects (ACTG A5191). *Antimicrob. Agents Chemother.* 23 Apr 2007 (doi:10.1128/AAC.00013-07).
16. Gilmour, D., Knaut, H., Maischein, H. M. & Nusslein-Volhard, C. Towing of sensory axons by their migrating target cells *in vivo*. *Nature Neurosci.* 7, 491–492 (2004).
17. Huang, Y. *et al.* The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nature Med.* 2, 1240–1243 (1996).
18. Glass, W. G. *et al.* CCR5 deficiency increases risk of symptomatic West Nile virus infection. *J. Exp. Med.* 203, 35–40 (2006).
19. Greenough, T. C., Sullivan, J. L. & Desrosiers, R. C. Declining CD4 T-cell counts in a person infected with nef-deleted HIV-1. *N. Engl. J. Med.* 340, 236–237 (1999).
20. Haubrich, R. H. *et al.* A randomized trial of the activity and safety of Ro 24-7429 (tat antagonist) versus nucleoside for HIV infection. *J. Infect. Dis.* 172, 1246–1252 (1995).
21. Para, M. F. *et al.* in *Program and Abstracts of the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Abstract H-256 (ASM Press, Herndon, Virginia, 2006).
22. Greene, W. C. & Peterlin, B. M. Charting HIV's remarkable voyage through the cell: basic science as a passport to future therapy. *Nature Med.* 8, 673–680 (2002).
23. Bishop, K. N., Holmes, R. K., Sheehy, A. M. & Malim, M. H. APOBEC-mediated editing of viral RNA. *Science* 305, 645 (2004).
24. Mitsuyasu, R. *et al.* The virologic, immunologic, and clinical effects of interleukin 2 with potent antiretroviral therapy in patients with moderately advanced human immunodeficiency virus infection: a randomized controlled clinical trial — AIDS Clinical Trials Group 328. *Arch. Intern. Med.* 167, 597–605 (2007).
25. Mellors, J. W. *et al.* Plasma viral load and CD4⁺ lymphocytes as prognostic markers of HIV-1 infection. *Ann. Intern. Med.* 126, 946–954 (1997).
26. The DAD Study Group. Class of antiretroviral drugs and the risk of myocardial infarction. *N. Engl. J. Med.* 356, 1723–1735 (2007).
27. Rowland-Jones, S.L. & Whittle, H. C. Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? *Nature Immunol.* 8, 329–331 (2007).
28. Coffin, J. M. HIV population dynamics *in vivo*: implications for genetic variation, pathogenesis, and therapy. *Science* 267, 483–489 (1995).
29. Marucco, D. A. *et al.* Antiretroviral activity of pegylated interferon α 2a in patients co-infected with HIV/hepatitis C virus. *J. Antimicrob. Chemother.* 59, 565–568 (2007).
30. Loeb, L. A. *et al.* Lethal mutagenesis of HIV with mutagenic nucleoside analogs. *Proc. Natl Acad. Sci. USA* 96, 1492–1497 (1999).
31. Westby, M. *et al.* Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. *J. Virol.* 81, 2359–2371 (2007).
32. De Gruttola, V. *et al.* Drug development strategies for salvage therapy: conflicts and solutions. *AIDS Res. Hum. Retroviruses* 22, 1106–1109 (2006).
33. Eron, J. J. *et al.* Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naïve HIV-positive patients: a 48-week randomized clinical trial. *J. Infect. Dis.* 189, 265–272 (2004).
34. Johnson, M. A. *et al.* A once-daily lopinavir/ritonavir-based regimen provides noninferior antiviral activity compared with a twice-daily regimen. *J. Acquir. Immune Defic. Syndr.* 43, 153–160 (2006).
35. Martin, A. M. *et al.* Predisposition to abacavir hypersensitivity conferred by HLA-B*5701 and a haplotypic HSP70-HOM variant. *Proc. Natl Acad. Sci. USA* 101, 4180–4185 (2004).
36. Hetherington, S. *et al.* Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *Lancet* 359, 1121–1122 (2002).
37. Haas, D. W. *et al.* Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS* 18, 2391–2400 (2004).
38. Lappin, G. & Garner, R. C. Big physics, small doses: the use of AMS and PET in human microdosing of development drugs. *Nature Rev. Drug Discov.* 2, 233–240 (2003).
39. Lampe, F. C. *et al.* Changes over time in risk of initial virological failure of combination antiretroviral therapy: a multicohort analysis, 1996 to 2002. *Arch. Intern. Med.* 166, 521–528 (2006).
40. Swindells, S. *et al.* Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy after sustained virologic suppression. *JAMA* 296, 806–814 (2006).
41. Karlstrom, O., Josephson, F. & Sonnerborg, A. Early virologic rebound in a pilot trial of ritonavir-boosted atazanavir as maintenance monotherapy. *J. Acquir. Immune Defic. Syndr.* 44, 417–422 (2007).
42. Zuger, A. Report from the XVI International AIDS Conference. ACTG 5142 compares class-sparing regimens in treatment-naïve patients. *AIDS Clin. Care* 18, 98 (2006).
43. Zuger, A. Report from the XVI International AIDS Conference. Lopinavir/ritonavir monotherapy. *AIDS Clin. Care.* 18, 99–100 (2006).
44. Muller, V. & Bonhoeffer, S. Mathematical approaches in the study of viral kinetics and drug resistance in HIV-1 infection. *Curr. Drug Targets Infect. Disord.* 3, 329–344 (2003).
45. McMahon, M. A. *et al.* The HBV drug entecavir — effects on HIV-1 replication and resistance. *N. Engl. J. Med.* 356, 2614–2621 (2007).
46. Gallant, J. E. *et al.* Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. *N. Engl. J. Med.* 354, 251–260 (2006).
47. Benson, C. A. *et al.* A prospective, randomized trial comparing the efficacy and safety of clarithromycin in combination with either ethambutol, rifabutin or both for the treatment of disseminated *Mycobacterium avium* complex (MAC) disease in persons with AIDS. *Clin. Infect. Dis.* 37, 1234–1243 (2003).
48. Nettles, R. E. *et al.* Marked intraindividual variability in antiretroviral concentrations may limit the utility of therapeutic drug monitoring. *Clin. Infect. Dis.* 42, 1189–1196 (2006).

Acknowledgements

This manuscript includes previously unpublished data from the AIDS Clinical Trials Group (ACTG) Protocol 223 (Figure 2a), and was supported in part by NIH grants AI-27668, AI-068636 and AI-069465.

Competing interests statement

The author declares competing financial interests: see web version for details.

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
[CY2B6](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) | [HLA-B](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) | [HSPA1L](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene)
HIV-1 Protein Interaction: <http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/index.html>
[nef](http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/index.html) | [tat](http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/index.html) | [vif](http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/index.html) | [vpr](http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/index.html)
OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
[Hepatitis B virus](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) | [hepatitis C virus](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) | [HIV-1](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM)
UniProtKB: <http://ca.expasy.org/sprot>
[APOBEC3G](http://ca.expasy.org/sprot) | [CCR5](http://ca.expasy.org/sprot) | [CXCR4](http://ca.expasy.org/sprot) | [IL2](http://ca.expasy.org/sprot) | [SDF1](http://ca.expasy.org/sprot)

FURTHER INFORMATION

HIV Databases: <http://www.hiv.lanl.gov/content/index>

ALL LINKS ARE ACTIVE IN THE ONLINE PDF