Biomed & Pharmacother 1999 ; 53 : 93-105

Dossier: AIDS intensive virostatic therapy

HIV disease treatment in the era of HAART

P. Bonfanti, A. Capetti, G. Rizzardini

1st Department of Infectious Diseases and Allergy, Luigi Sacco Hospital, via GB Grassi, 74 Milan, Italy

Summary – In the last three years basic science and clinical research have radically changed the therapeutical approach to HIV disease. Recent guidelines suggest that treatments to HIV disease should be early and aggressive, with the use of new potent antiretroviral drugs. This approach has been defined as HAART (highly active antiretroviral therapy). In this review we will discuss the main stages of antiretroviral therapy focusing on the acquisitions about results as well as problems of triple therapy. © 1999 Elsevier, Paris

HAART / HIV disease / protease inhibitors

In the past three years basic scientific and clinical research have radically changed the therapeutical approach to HIV disease. The new acquisitions that have principally influenced the use of anti-retrovirals include: a) studies on pathogenesis demonstrating the dynamic characteristics of HIV infection, a pattern that is present even during clinical latency [1-3]; b) the development of new techniques for the determination of the 'viral burden', which every year becomes more sensitive, thus allowing measurement of HIV replication rates [4]; c) the demonstration of the high predictive value of these techniques in terms of prognosis and response to treatment [5-7]; and, d) the availability of new classes of drugs, such as protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs), which are extremely potent and effective as shown by several clinical trials [8-13]. These developments led experts to suggest new guidelines where the therapeutic approach to HIV disease would be early and aggressive [14-16], thus starting the era of highly active antiretroviral therapy (HAART).

In those countries where HAART is affordable, it has led to significant decreases in new AIDS cases, opportunistic infections, and finally reducing deaths by AIDS [17, 18]. On the other hand, new data reported this year, in particular results which show that HIV can survive in extremely long-living cells and be reactivated even after years of potent antiretroviral therapy [19, 20], has somewhat cooled the initial enthusiasm for HAART. Furthermore, the study of the mechanisms through which HIV becomes insensitive to antiretrovirals and develops a broad cross-resistance towards drugs of the same class, has proven that in fact there exists only very limited options after HAART failure. In this review we will discuss the main stages of antiretroviral therapy, focusing on the acquisitions on results and the problems of triple therapy.

BRIEF HISTORY

Antiretroviral therapy began in the mid-1980s with the first study vs. placebo case (BW02) conducted by Fischl et al. on the use of zidovudine (AZT) in 282 subjects affected with AIDS or AIDS-related complex. An interim analysis at six months showed a reduction in the frequency of opportunistic infections and increased survival among the treated patient groups [21], so that patients on placebo were switched to therapy for ethical reasons. Given the need to obtain information quickly, a trial team (the AIDS Clinical Trial Group, ACTG) was instituted in the United States, coordinating various clinical trials on the employment of all available antiretrovirals. Two of these trials, ACTG 002 and ACTG 016, showed that low doses of zidovudine were more effective and less toxic than those initially employed and that it was also possible to achieve a delay in disease progression, at least for a short period, in patients at relatively early stages [22, 23]. Based on these and other results the first 'state-of-the-art' conference organized by the NIH [24] recommended, at least in the United States, that therapy should be offered to all patients, symptomatic or not,

who presented a CD4+ cell count of less than 500 /mm^3. This was the first big step in the history of antiretroviral therapy. In the following years however, other trials brought doubt and a critical consideration of the results which had been acquired up to that point. The most important of these trials (or at least the one that had the heaviest impact) was the Concorde study, a controlled French-English trial testing the benefits of early AZT therapy vs. delayed treatment. The results of this study, although questionable under a methodological profile, showed a favorable trend in the early treated group after a short period of time. The results at 3 years however, showed no evidence for either a delayed progression of the disease, nor an increased survival rate [25].

The 'time effect' of early monotherapy (at least with zidovudine) was confirmed by Volberding et al. through the publication of data from the ACTG 019 study: early treatment of patients having more than or equal to 500 CD4+ cells/mm^3 did not prolong the disease-free interval, nor did it demonstrate an increase in survival as compared to patients who started treatment when they had less than 500 CD4+ cells/mm^3 [26]. Other studies on drug alternatives to zidovudine, such as the first trial on combination therapy in advanced disease (ACTG 155) [27], gave disappointing results. In light of this situation came the second 'state-of-the-art' conference that confirmed the doubts and uncertainties on antiretroviral therapy [28].

A new phase in antiretroviral therapy was initiated by the results of the ACTG 175 and Delta trials in which a combination of zidovudine/didanosine (ddI) and zidovudine/zalcitabine (ddC) combination regimens in antiretroviral-naive subjects showed a reduction on both mortality rates and the progression to AIDS. These were the first studies where an increase in survival was clearly shown. The ACTG 175 trial compared monotherapy (AZT or ddI) with regimens in which drugs were combined (AZT/ddI or AZT/ddC) in naive patients with CD4+ lymphocytes between 200 and 500 /mm^3 [10]. Similar results were obtained from the Euro-Australian trial Delta-1, which compared combination treatments (AZT/ddI or AZT/ddC) and AZT monotherapy in patients having CD4+ T-lymphocytes lower than 350 /mm^3, and showed the superiority of the AZT/ddI regimen [11].

The CAESAR study has subsequently demonstrated the efficacy of the zidovudine/lamivudine (3TC) combination. In this study the addition of lamivudine to AZT-naive and AZT-pretreated patients having less than 250 CD4+ T-lymphocytes/mm^3 reduced mortality and delayed disease progression [12].

In contrast, the results of the Delta-2 or Community Programs for Clinical Research on AIDS (CPCRA) 007 studies, investigating combination therapy with two nucleoside analogues in pretreated patients, were not as satisfactory. Many patients however, had been pretreated with AZT for more than 2 months and it was found that the risk of disease progression and death increased proportionally with the duration of previous zidovudine treatment [11, 29].

THE ERA OF TRIPLE THERAPY

The enthusiasm that followed the first success with two-drug combination therapies grew with the introduction in clinics of new classes of antivirals, such as non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). The main characteristics of the principal components of these two groups of antivirals are summarized in *tables I* and *II*.

PIs are the drugs that have shown the best results and at present, triple combinations of drugs including a protease inhibitor are the gold standard of antiretroviral therapy. The HIV protease, encoded by the *pol* gene and composed of 99 amino acids, is responsible for the cleavage of the non-functional polyprotein precursor. Its inactivation (due to interactions with the active site) causes the production of immature virions, incapable of infecting new cells. The subsequent elucidation of the crystal structure of the HIV protease led to the computer-guided design of candidate drugs in order to

Table I. Characteristics of protease inhibitors.

	Indinavir	*Ritonavir*	*Saquinavir-HGC*	*Saquinavir-SGC*	*Nelfinavir*
Dosing recommendations	800 mg tid	600 mg bid	600 mg tid	1,200 mg tid	750 mg tid
Oral bioavailibility	60–70% (take without food)	60–90% (take with food)	4% (take with large meal)	12% (take with large meal)	20–80% (take with food)
Metabolism	cytochrome P450 3A4 > 2D6	cytochrome P450 3A4	cytochrome P450 3A4	cytochrome P450 3A4	cytochrome P450 3A4
Storage	room temperature	refrigerate capsules	room temperature	room temperature	room temperature

tid: three per day; bid: two per day.

Table II. Characteristics of non-nucleoside reverse transcriptase inhibitors.

	Nevirapine	*Delavirdine*	*Efavirenz*
Dosing recommendations	200 mg qd for 2 weeks than 200 mg bid	400 mg tid	600 mg qd
Oral bioavailability	90%	85%	not available
Metabolism	cytochrome P450 3A4	cytochrome P450 3A4	cytochrome P450 3A4
Storage	room temperature	room temperature	room temperature

tid: three per day; qd: quotidian.

obtain drugs which adapt to the tri-dimensional binding hollow of the enzyme, with the aim of achieving competitive binding with the active site [30]. Initially these molecules were used in monotherapy and showed a much stronger antiviral potency as compared to all other antivirals employed in HIV therapy. These molecules also allowed the famous studies on the replication dynamics of HIV to be performed [2, 3].

The first protease inhibitor to be approved by FDA and registered for clinical practice was saquinavir (SQV). One of the most relevant studies with this drug is the ACTG 229. This trial compared the efficiency of saquinavir in combination with one or two nucleoside analogues (AZT and ddC) vs. the double-nucleoside combination alone. The study was not however powerful enough to detect differences among the three arms as far as the clinical endpoints were concerned; the efficacy was thus evaluated in terms of surrogate markers. The viral load decrease at 24 weeks was found to be higher in the triple combination branch than in the double combinations ($P < 0.003$) and still remained below baseline levels at 48 weeks [31]. The drug was available in the hard gel capsule formulation which was scarcely absorbed after oral administration, showing 4% bioavailability; this determined its lower antiviral potency as compared to the other protease inhibitors. A new formulation, soft gel capsules, was subsequently approved by FDA, which showed higher bioavailability and efficacy [32].

Ritonavir (RTV) and indinavir (IDV) are the two protease inhibitors which have been approved. The efficacy of ritonavir has been assessed in a multicentre randomized double-blind trial. The study recruited individuals with $CD4^+$ cell counts of 50–550 cells/mm^3 and a viral load greater than 25,000 copies/mL; the majority of the patients had received prior antiretroviral treatment. After four weeks therapy, plasma HIV RNA load was significantly reduced, and 38% of patients were below the level of detection by week 12. In addition, at week 4 there was a significant increase in $CD4^+$ cell counts [33].

The efficacy of ritonavir has also been evaluated in studies having clinical end-points. In a trial involving 1,090 patients with baseline $CD4^+$ cell counts lower than 100 per cubic millimeter, ritonavir, in combination with nucleoside therapy, reduced the combined end points of new opportunistic diseases and death by 53%, and reduced the end point of death alone by 43%, as compared with placebo [34]. Scores for quality of life declined during the first four weeks of ritonavir treatment, but then improved significantly as compared with baseline values. The patients in the placebo group had a gradual decline in the quality of life [35].

For trials involving indinavir, the results of two very important studies have been published in the last two years. Data from trial ACTG 320 demonstrated highly significant clinical benefit from adding indinavir to zidovudine/lamivudine when compared to the dual nucleoside combination alone in terms of reducing the rate of progression to AIDS or death in advanced HIV infection: 11% in zidovudine/lamivudine group vs. 6% in the triple combination group ($P = 0.001$) [36]. The most relevant published data relating to the efficacy of protease inhibitor-based triple therapy comes from the Merck 035 trial. This study enrolled 97 patients with prior zidovudine experience with a viral load greater than 20,000 copies/mL and $CD4^+$ cell counts between 50 and 400 cells/mm^3. At 24 weeks, 90% of individuals receiving the triple combination had undetectable viremia, compared with 43% of those receiving indinavir monotherapy and 0% of those receiving zidovudine/lamivudine dual therapy. At 100 weeks, the initial triple combination suppressed HIV RNA load in 78% of contributing patients [37]. The limit of HIV RNA detection used in this study was 500 copies/mL. Nelfinavir (NFV) was the last inhibitor registered for clinical use and it also appears to have great antiviral potency [38]; in an ongoing study greater than 70% of adults receiving a nelfinavir based combination regimen showed plasma HIV RNA levels below the limit of detection (< 400 copies/mL) after 84 weeks [39].

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are also known as 'HIV-1 specific reverse transcriptase inhibitors', which is a more correct definition since some types of these drugs indeed have a nucleosidic base. Drugs belonging to this class are very heterogeneous compounds that show common proper-

ties in that they inhibit the reverse transcriptase with a different mechanism than that of the nucleoside analogues. They are active at nanomolar concentrations, they have a high therapeutic/toxic dose ratio and they act exclusively against HIV-1 [40]. These compounds also rapidly select for resistant strains, cross-reacting with other NNRTIs despite the structural differences between them [41]. However the association of these drugs with nucleoside analogues tends to delay the development of resistance mutations.

The drugs presently available in many western countries are nevirapine, delavirdine and efavirenz. Nevirapine was approved by the FDA on the basis of the results obtained on surrogate markers from two important trials: the ACTG 241 and the INCAS study. The first study enrolled 398 patients with more than six months' prior therapy and compared zidovudine/didanosine/nevirapine with zidovudine/didanosine treatments. At 48 weeks, the reduction in viral load was more significant in the triple combination group ($P = 0.028$) [42]. The INCAS trial was a double-blind, placebo-controlled study in which adult naive patients were randomized to three arms of combination therapy: nevirapine/zidovudine/didanosine vs. zidovudine/didanosine vs. nevirapine/zidovudine. After 52 weeks of triple therapy, 51% of the patients maintained undetectable levels of HIV RNA in the blood (< 20 copies/mL), compared to less than 12% in the two drugs arms ($P < 0.001$) [43].

Delavirdine should not be employed within suboptimal regimens because it rapidly selects for resistance. In a recently published study its efficacy in addition to indinavir-based triple combination was assessed in patients whose therapy was failing. After 6 months, viral load declined in 33% of subjects to below the limit of detection (< 400 copies/mL) [44]. Efavirenz (EFV) is the latest compound approved by the FDA and has proved effective in various associations: together with nelfinavir in the DMP 266-024 study, it brought 68% of the naive and 40% of the NRTI-experienced below 50 copies HIV RNA/mL at 16 weeks [45]. In the 003 trial the association with indinavir (whose dosage must be increased by 25%) 73% of the patients having below 50 copies/mL led at 72 weeks [46], while the most publicized results came from the 006 trial [47] that compared a) AZT/3TC/EFV with b) EFV/IDV and c) AZT/3TC/IDV. After 24 weeks at the intent-to treat analysis, 62% of the patients in arm a) went below 50 copies, compared with 50% and 48% in the other two arms. Such an advantage comes mainly from the fact that the drug, beside its potency, is well tolerated and simple to take (once a day).

PRESENT THERAPEUTIC STRATEGIES

On the basis of these data from a couple of years of study there is a strong tendency to adopt therapeutical approaches combining three antiretrovirals as first line therapy, generally involving two nucleosides plus a protease inhibitor or an NNRTI [14]. The goal of this kind of approach is to reduce the viral load possibly below the limit of detection, to limit disease progression, and to delay the occurrence of resistant mutants that would compromise its therapeutic efficacy [48]. Moreover it appears evident that we need to anticipate the beginning of the treatment as early as possible to reduce the structural damage to the immune system caused by HIV and the spontaneous emergence of highly virulent mutant strains [49]. The present guidelines for antiretroviral therapy indeed suggest to start therapy in all asymptomatic patients independently of their baseline $CD4^+$ count (therefore even at more than 500 /mm^3) if there are signs of important viral activity, assessed in terms of viral burden [50, 51]. In particular, the 'International AIDS Society-USA' guidelines assert that therapy should be offered to all those patients who present HIV RNA plasma levels above 5,000–10,000 copies/mL and that starting therapy should be considered for all patients who have detectable plasma viremia.

Even though other combinations are accumulating on the stage, regimens including one protease inhibitor and two reverse transcriptase inhibitors are still nowadays the gold standard of therapy for HIV infection. The weak point in protease inhibitor treatment is the possible development of resistance: the predominant mechanism through which resistance takes place is the emergence of mutations in or close to the active site of the enzyme so that the inhibitor binds less easily. In vitro studies on indinavir have shown that at least three or four point mutations are required to reach significant phenotypic resistance. Unfortunately revealed resistance among protease inhibitors is also found [52-54]. Thus the choice of a compound will have a great impact on future options the in case of therapy failure. For this reason it is likely that genotypic or phenotypic analysis will assist the clinician's choice in the future management of HIV patients [55].

THE LIMITS OF TRIPLE THERAPY

The advent of HAART has suddenly compelled the clinicians to face a series of problems which until now have been unknown: protease inhibitors' pharmacokinetics, complex interactions with other drugs, new and

Table III. Protease inhibitors' AUC modifications in combination with other antivirals.

	Saquinavir (%)	*Indinavir (%)*	*Ritonavir (%)*	*Nelfinavir (%)*
Nevirapine	– 24	– 28	– 11	+ 4
Efavirenz	– 62	– 31	+ 18	+ 20
Delavirdine	+ 500	+ 89	+ 66	+ 92
Indinavir	+ 600	-	0	+ 84
Ritonavir	+ 2000	+ 500	–	+ 250
Saquinavir	–	NR	0	+ 17
Nelfinavir	+ 500	+ 51	0	–
Amprenavir	– 18	– 38	NR	+ 15

NR: not reported.

Table IV. Most frequent adverse reactions observed with PIs.

Indinavir	*Ritonavir*	*Saquinavir-HGC*	*Saquinavir-SGC*	*Nelfinavir*
Nephrolytiasis	gastro-intestinal intolerance	gastro-intestinal intolerance: nausea, diarrhea	gastro-intestinal intolerance: nausea, diarrhea, abdominal discomfort, dyspepsia	diarrhea
gastric intolerance	peripheral and circumoral paraesthesia	elevation of transaminases	elevation of transaminases	hyperglycemia
Increased indirect bilirubinemia	hepatitis	headache	headache	
Mix: headache, fatigue, rash, metallic taste	taste alterations	hyperglycemia	hyperglycemia	
Hyperglycemia	hypertriglyceridemia			
	hyperglycemia			

unexpected adverse events, the wide world of resistance and the dramatic problem of adherence.

Pharmacokinetics

In addition to the problems associated with the saquinavir hard gel bioavailability, indinavir [56] and ritonavir [57] also show wide discrepancies in plasma concentrations after the same oral doses. The drugs were taken under clinical observation and, given their lack of accumulation, compliance cannot be implicated in this phenomenon. High levels correlate significantly with adverse reactions to ritonavir [57], while low plasma concentrations might lead to therapy failure. Pharmacokinetic monitoring of patients may be required in the future allowing for individualized dose adjustments.

Interactions

All the new anti-HIV drugs are mainly metabolized through the P450 cytochrome enzymatic system. Many of these molecules can act as enzymatic inhibitors or inducers; this determines potential interactions with numerous drugs undergoing metabolism at this site. Indeed, this is one of the major problems in managing antiretroviral therapy, as some interactions require the exclusion of certain combinations, others need dose adjustments, and all interacting combinations require intensive follow-up. The inhibitory effect on P450 cytochrome has been employed by many researchers to their advantage, combining antiretrovirals in such a way which allows the dosage to be reduced thus improving compliance and overcoming resistance. Much interest presently surrounds the combinations of two protease inhibitors [58]. *Table III* shows pharmacokinetic interactions between PIs and NNRTIs.

Adverse events

The widespread employment in the clinical setting of new antiretrovirals has shown that: a) adverse events, as reported in clinical trials, occur more frequently (see *table IV*); b) new, formerly unknown and serious adverse events can occur. Concerning this first issue, the reason for the discrepancy between clinical trials and clinical practice depends on a methodological problem. Clinical research 'physiologically' selects through a rigid inclusion criteria a population that does not correspond to the real population. The second aspect is more worrying because its implications. There is a growing number of reports on lipodystrophy, which is an abnormal accumulation of fat without body weight variations in patients treated with HAART [59]. Also quite common are reports of lipid metabolism alter-

ations such as hyper-triglyceridemia and hypercholesterolemia. Moreover in some patients, more serious diseases have occurred, such as diabetes mellitus or premature coronary disease [60].

Although the spectrum of manifestations differs from patient to patient, many researchers believe that all these events are part of the same syndrome associated with an altered metabolism. Carr et al. found two proteins, one a low density lipoprotein receptor-like protein (LRP), the second a region of the cytoplasmic retinoic-acid binding protein type 1 (CRABP-1) that shared a 58–63% homology with 12 amino acids spanning the catalytic site of HIV protease. CRABP-1 presents retinoic acid to the P450 3A liver enzyme system (the same enzyme system which is inhibited by protease inhibitors) for conversion into 9-cis-retinoic acid, which appears to play a role in fat storage, particularly in peripheral areas of the body. LRP is a scavenger of lipids in the liver. The Australian team postulates that a complex interaction of the PI with each of these systems leads to a fat depletion in the periphery, high lipids in the blood and accumulation by default in the viscera [61].

On the contrary, others think that this event is more probably related to HIV itself than to the antiretrovirals [62]. The problem however exists, particularly since due to their efficacy the therapies might be taken by patients for many years and yet we do not know their long-term toxicity. Finally it should be reminded that all PIs can cause liver toxicity (sometimes clinically evident as toxic hepatitis), and that indinavir can cause renal colics and a variety of renal damage [63, 64].

Resistance to antiviral drugs

Resistance is presently a major issue in antiretroviral therapy because it inevitably leads to drug failure. Resistance arises from poor compliance, from inadequate knowledge of the pharmacokinetics of the drugs due to intrinsic characteristics of the drugs or their interactions, or from inadequate viral suppression. Even at very low levels of viremia resistance can occur, beginning from the lymph nodes [65, 66], so that every regimen sooner or later will select for resistance. The time to occurrence of a certain resistance mutation can be approximately foretold, but the single case is really unpredictable, since it seems to respond to a stochastic model [67]. A drug is said to have a low genetic barrier when the virus can overcome its effect by a single point mutation, an event that takes place in two weeks under monotherapy pressure. In contrast, a drug that has a high genetic barrier requires multiple mutations in the virus to show a decrease in its antiviral activity [68]. The main genotypic mutations responsible for resistance in the three main classes of antiretrovirals are reported as follows.

Resistance to nucleosides (NRTIs)

Nucleosides are actually the most various class with regard to the development of resistance. Resistance to zidovudine generally develops with an initial transient mutation at codon 70 [69] and subsequently with the more resistant T215Y/F and, if therapy is prolonged, with an accumulation of point mutations at codons 41, 67, 70, and 219 [70]. High-level resistance to AZT also implies low-level resistance (about two-fold increase in IC50) to didanosine and zalcitabine [71]. Lamivudine rapidly selects for the M184V/I mutation which confers high-level resistance to itself, but low-level resistance to ddI and ddC [72, 73], and reverses resistance to AZT in 215-mutated strains [74]. Didanosine and zalcitabine, in turn, generate the point mutations L74V and T69D [75, 76], that cause low-level resistance to themselves and no cross-resistance to others. Occasionally they select for the K65R mutation which confers high-level resistance to 3TC or for the M184V/I [72, 77]. Stavudine infrequently selects for a V75T mutation moderately cross-reacting with ddI and ddC [78, 79]. More recently, a novel SSS69 mutation has been identified for this drug, conferring resistance to stavudine (d4T) [80]. Abacavir treatment results in the slow accumulation of mutations and the level of cross-reaction with the other NRTIs is still unclear, selecting for K65R, L74V and M184V; it does not appear to cross-react with zidovudine [81]. Particular multi-drug resistant strains have also been reported in a very low percentage of patients (2%) with groups of mutations at codons 151 and 333 [82, 83]. Overall, this class of drug seems to leave a certain space to plan strategies in drawing first and second line therapies.

Resistance to non-nucleoside RT inhibitors

Non-nucleoside RT inhibitors are, with respect to the resistance profile, the most sensitive class of drugs. Almost all of them have a low genetic barrier: loviride, nevirapine, delavirdine, efavirenz and the new and not yet available MKC 442 used in monotherapy, rapidly select for mutations that render the virus highly resistant to the entire class, particularly the K103N mutation [80]. The nevirapine and loviride-induced Y181C/S mutation partially restores sensitiv-

ity to AZT in 215-mutated strains [84] and delavirdine-induced P236L mutation sensitizes RT 10-fold to nevirapine [85]. NNRTIs seem to be a group of drugs where the failure of one leaves very little space for the possibility of employing the others.

Resistance to protease inhibitors

Indinavir is the antiretroviral drug that has the highest genetic barrier. Resistance occurs when at least three primary mutations have arisen [86, 87]. Unfortunately, it has a resistance mutation profile completely overlapping to that of ritonavir [53]. Saquinavir and nelfinavir select for quite different primary mutations and it had been initially hypothesized that they might in some way escape cross-resistance [88, 89]. However, the preliminary data from ACTG 333 and the first data presented on nelfinavir showed that resistance to one PI is extended also to the others presently available, as recently underlined by Mellors [90]. Another aspect of PI resistance is that, as shown by the Taylor Square Institute study, the sooner you change, the better result you obtain [91]. Furthermore, compensatory mutations in the gag cleavage site have been described under PI exposure [92], suggesting that prolonged treatment in conditions that allow the detection of the virus may generate aberrant and biologically resistant strains. New flexible PIs are being tested with the aim to render them adaptable to conformation mutations of the protease binding region or to 'resist resistance'. PIs are therefore characterized by the fact that only fast changes may allow one to escape cross-resistance. Non-peptidic protease inhibitors may yield important advantages in this field.

Adherence to treatment

The advent of HAART has also brought complications for the patients. PIs require that patients take a large number of pills (6 to 12/d), often requiring fixed relationship with food that may contrast with associated NRTIs (i.e., ddI), often in tid regimens and in the case of ritonavir need to be kept in the refrigerator. Overall, no other disease requires such a complicated regimen for life. On the other hand, it has been widely demonstrated that partial adherence to therapy causes the occurrence of drug resistance, and it is important to underline that since mutated strains can be transmitted, resistance is a problem of public health. Studies aimed to increase the adherence to a therapy and to identify the features of non-compliant patients are ongoing [93].

ISSUES FOR THE FUTURE OF ANTIRETROVIRAL THERAPY

Strategies of antiretroviral treatment are constantly evolving and new problems arise every day. In the last few months the scientific debate has touched on many aspects. We report those issues that in our opinion are the most significant in the clinical setting.

Salvage therapy

Although a strategic approach is universally invoked in reality, when a triple combination therapy fails, the choice of new regimens is restricted by cross-resistance profiles or patient's intolerance. In highly pretreated patients, even passing to triple combinations composed of brand new drugs seems to achieve only a very limited benefit, as shown by the Aurora Medical Group in San Diego [94]. On the other hand, data reported by Mellors at the Geneva World AIDS Conference, as well as the analysis of the AVANTI-2 and ACTG 343 studies, have shown that viral load may increase during triple therapy yielding strains that are not genotypically resistant to all the drugs of the combination (i.e., in AZT/3TC/indinavir-treated patients, strains that contain only the 3TC-associated M184V/I mutation). This suggests that a reduction of potency has occurred but not total resistance [90, 95, 96]. The French VIRADAPT study this year assessed the efficiency of changing therapy based on the results of genotypic resistance testing [97]. The patients whose therapy was changed according to the indications of the genotypic profile of their HIV strains, achieved at 6 months of changing therapy, a mean 1.12 $\log_{10}$ viral load decrease with 30% of the patients still below delectability vs. – 0.45 and 17% below delectability in the group that changed therapy according to the clinical guidelines and physicians' judgment. Although the best test (genotypic, phenotypic or both) and the correct interpretation are still to be defined, the study seems to show that genotypic profile-assisted strategies are more efficient.

Maintenance therapy

The idea of 'maintenance' therapy with one or two drugs after an initial 'push' (12 weeks or longer) on triple combination arose at the beginning of the protease era, when it was assumed that HAART could completely suppress viral replication. Unfortunately four important trials on maintenance therapy (Trilege, ACTG 343, ADAM and MIRO) have been prematurely

closed in the course of the year 1998 because of high rates of viral rebound in the simplified arms [98-101] as compared to the patients who continued triple or quadruple therapy. Trilege and ACTG 343 considered the classical AZT/3TC/IDV combination and showed that while rebinding strains from the simplified AZT/3TC arm uniformly had the M184V mutation to 3TC, the AZT/IDV or IDV monotherapy arising strains were wild-type for protease and had no AZT-related mutations. Therefore, in these cases failure seems to be attributable to diminished potency of viral suppression rather than resistance.

Triple combination regimens without PIs

During the course of 1998 two important trials, CNA A/B 3005 by Glaxo-Wellcome and DMP266-006 by DuPont, showed data from interim analysis that suggested that convergent triple combinations with AZT/3TC/abacavir or AZT/3TC/efavirenz are at least as potent and better tolerated than AZT/3TC/indinavir. Though the trials are still ongoing, this result has been presented at all the recent Congresses, from Geneva's World AIDS Congress to Glasgow's International Congress on Drug Therapy in HIV Infection [102, 103]. A further advantage of these regimens, beside a lower number of pills and a bid or qd administration that increase the efficacy at the intent-to-treat analysis, is the fact that acting on a single substrate, HIV reverse transcriptase, they leave the protease naive for a subsequent PI-based triple combination regimen.

New drugs

The chances of making HIV infection a chronic disease seem to depend mainly on the availability of new drugs, active on different targets of the HIV life-cycle replication or non cross-resistant. We conclude this review on antiretroviral therapy with a description of some of the most promising compounds at different stages of development. After efavirenz in October 1998, running for FDA approval are abacavir (Glaxo-Wellcome, NRTI, Ziagen™), adefovir dipivoxil (Gilead Sciences, nucleotide, Preveon™), and amprenavir (Glaxo-Wellcome, PI, Agenerase™).

Abacavir

Carboxcyclic nucleoside with high antiviral potency (1.9 log). In NRTI-experienced patients the shift to combivir (AZT/3TC)+ abacavir has yielded at week 8, a 1.6 log reduction in viremia with 50% of the patients below 50 copies, thus showing limited cross-resistance with the other NRTIs [104]. The main problem with abacavir is a hypersensitivity of 3% that presents a non-specific clinical pattern that may worsen or even be fatal at re-exposure [105].

Adefovir

This nucleotide has a potency of 0.4 log when added to failing ART [106], rising to 0.9 log when the failing therapy is AZT/3TC and the M184V mutation is present [107, 108]. Its activity is enhanced by the association with hydroxyurea. The main adverse events related to adefovir treatment are a decline in body weight and 31.9% reversible proximal renal tubular disease after 48 weeks on therapy [106].

Amprenavir

A novel protease inhibitor with a pattern of resistance slightly different from the other known PIs, although there seems to be a certain degree of overlapping clinical resistance. Trials where amprenavir is associated with AZT/3TC or other NRTIs or abacavir are on going with encouraging results [109-111].

Newer and very promising drugs are behind the corner, in phase I/II studies:

FTC (Triangle Pharmaceuticals)

Similar to 3TC for its molecular structure, allows a once-daily dosing regimen. In a phase I/II dose-escalating trial on man, the higher doses (> 200 mg/d) obtained viral suppression of – 2 log copies/mL [112]. It shares cross-resistance patterns with 3TC.

FddA

Novel NRTI has shown potent anti-HIV activity in vitro and in vivo, a long half-life (20 h), high oral bioavailability and appears to protect $CD4^+$ T-cells in HIV infection, causing a higher $CD4^+$ increase as compared to AZT monotherapy in HIV-infected mice [113].

MKC-442 (Triangle Pharmaceuticals)

Works as an NNRTI although it has a nucleoside structure. It has showed synergy with AZT, 3TC, d4T, ddI, ddC, abacavir, nelfinavir, indinavir, ritonavir, amprenavir and delavirdine [114, 115]. In rats it has showed good penetration of the CNS, having the same concentration curve in the brain (Cmax, AUC, t1/2) as in plasma [116]. Its pharmacokinetics in man seems to allow bid regimens, it is overall well tolerated and in HIV positive volunteers in a phase I/II dose-escalating study has reached a more than 1 log viral load suppression given 750 mg bid [117].

Tipranavir (Pharmacia & Upjohn)

A non-peptidic protease inhibitor and highly active. Synergy has been demonstrated with ritonavir [118], zidovudine and delavirdine [119]. It has shown to be also active on strains resistant to the peptidomimetic protease inhibitors [119]. Clinical trials are currently underway [120].

DMP-450 (Triangle Pharmaceuticals)

PI with more than 3 log anti-HIV potency in vitro. Phase I/II studies on man are starting at the end of 1998 (data from Triangle Pharmaceuticals, Inc.).

BMS-232632 (Bristol-Myers Squibb)

Azapeptide, another PI that has shown in vitro the highest anti-HIV potency among all the known protease inhibitors. BMS-232632 resistance occurs less rapidly than with ritonavir or nelfinavir and resistant strains remain sensitive to saquinavir although show some resistance to nelfinavir, indinavir, ritonavir and amprenavir. Nelfinavir, saquinavir and amprenavir-resistant viruses remain sensitive to BMS-232632 [121].

KNI 272 and KNI 764

New flexible protease inhibitors designed in order to adapt to the protease mutations, active against indinavir-resistant strains in vitro. KNI 272 is synergistic in vitro with indinavir, ritonavir, nelfinavir and delavirdine [122-124].

REFERENCES

1 Pantaleo G, Graziosi C, Demarest J, Butini L, Montroni M, Fox CH, et al. HIV infection is active and progressive in lymphoid tissue during the clinically stage of disease. Nature 1993 ; 362 : 355-8.

2 Ho DD, Neumann AS, Perelson, Chen W, Leonard JM, Markowitz M. Rapid turn over of plasma virions and CD4 lymphocytes in HIV infection. Nature 1995 ; 373 : 123-5.

3 Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, et al. Viral dynamics in human immunodeficiency virus type 1. Nature 1995 ; 373 : 117-22.

4 Saag MS, Holodniy M, Kuritzkes, O Brien W, Coombs R, Poscher ME, et al. HIV viral load markers in clinical practice. Nature Med 1996 ; 6 : 625-9.

5 Mellors JW, Rinaldo CR, Gupta P, White RM, Todd J, Kingskey LA, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996 ; 272 : 1167-70.

6 Mellors JW, Kingsley LA, Rinaldo CR, Todd J, Hoo BS, Kokka RP, et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. Ann Intern Med 1995 ; 112 : 573-9.

7 O'Brien TR, Blattner WA, Waters D, Eystert E, Hilgartner MW, Cohen AR, et al. Serum HIV-1 RNA levels and time to development of AIDS in the Multicenter Hemophilia cohort Study. JAMA 1996 ; 276 : 105-10.

8 Mc Donald CK, Kuritzkes DR. Human immunodeficiency virus type 1 protease inhibitors. Arch Intern Med 1997 ; 157 : 951-9.

9 Moyle G, Gazzard B. Current knowledge and future prospects for the use of HIV Protease inhibitors. Drugs 1996 ; 51 : 701-12.

10 Hammer SM, Katzenstein DA, Hughes MD, Gundacker K, Schooley RT, Haubrich RH, et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 µL to 500 µL. N Engl J Med 1996 ; 335 : 1081-90.

11 Delta Coordinating Committee. Delta: a randomized double-blind trial controlled trial comparing combinations of zidovudine plus didanosine or zalcitabine with zidovudine alone in HIV-infected individuals. Lancet 1996 ; 348 : 283-91.

12 CAESAR Coordinating Committee. Randomized Trial of addition of lamivudine or lamivudine plus loviride to zidovudine containing regimens for patients with HIV-1 infection: the CAESAR trial. Lancet 1997 ; 349 : 1413-21.

13 Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, McMahon D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl Med 1997 ; 337 : 734-9.

14 Carpenter C, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, et al. Antiretroviral therapy for HIV infection in 1996. JAMA 1996 ; 276 : 146-54.

15 Carpenter C, Fischl MA, Hammer SM, Hirsch MS, Jacobsen DM, Katzenstein DA, et al. Antiretroviral therapy for HIV infection in 1997. JAMA 1997 ; 277 : 1962-9.

16 BHIVA Guidelines Co-ordinating Committee. British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. Lancet 1997 ; 349 : 1086-92.

17 Hogg RS, Heath KV, Yip B, Craig KJ, O'Shangnessy MV, Schecter MT, et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. JAMA 1998 ; 279 : 450-4.

18 Detels R, Munoz A, McFarlane G, Kingsley LA, Margolick JB, Giorgi G, et al. Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. JAMA 1998 : 1497-503.

19 Wong JK, Hezareh M, Gunthard H, Havlir D, Ignacio CC, Spina C, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997 ; 278 : 1291-5.

20 Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, et al. Identification of a reservoir of HIV-1 in patients on highly active antiretroviral therapy. Science 1997 ; 278 : 1295-300.

21 Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, et al. The efficacy of azidothimidine in the treatment of patients with AIDS and AIDS-related complex. N Engl J Med 1987 ; 317 : 186-97.

22 Fischl MA, Richman DD, Hansen N, Collier AC, Carey J, Para MF, et al. The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1(HIV) infection. A double-blind, placebo-controlled trial. Ann Intern Med 1990 ; 112 : 727-37.

23 Fischl MA, Parker CB, Pettinelli C, Wulfsohn M, Hirsch MS, Collier AC, et al. A randomized controlled trial of a reduced daily dose of zidovudine in patients with the acquired immunodeficiency syndrome. N Engl J Med 1990 ; 323 : 1009-14.

24 State-of-the-art Conference on Azidothymidine Therapy for Early HIV Infection. Am J Med 1990 ; 89 : 335-44.

25 Concorde Coordinating Committee. Concorde: MRC/ARNS randomized double-blind controlled trial of immediate and

deferred zidovudine in symptom-free HIV infection. Lancet 1994 ; 343 : 871-81.
26 Volberding PA, Lagakos SW, Grimes JM, Stein DS, Rooney J, Meng T, et al. A comparison of immediate with deferred zidovudine therapy for asymptomatic HIV-infected adults with CD4 cell counts of 500 or more per cubic millimeter. N Engl J Med 1995 ; 333 : 401-7.
27 Fischl MA, Stanley K, Collier AC, Arduino J, Stein DS, Feinber JE, et al. Combination and monotherapy with zidovudine and zalcitabine in patients with advanced HIV disease. Ann Intern Med 1995 ; 122 : 24-32.
28 Sande MA, Carpenter CCJ, Cobbs CG, Holmes KK, Sanford JP. Antiretroviral therapy for adult HIV-infected patients: recommendations from a state-of-the-art conference. JAMA 1993 ; 270 : 2583-9.
29 Saravolatz LD, Winslow DL, Collins G, Hodges JS, Pettinelli C, Stein DS, et al. Zidovudine alone or combination with didanosine or zalcitabine in HIV-infected patients with the acquired immunodeficiency syndrome or fewer than 200 CD4 cells per cubic millimeter. N Engl J Med 1996 ; 335 : 1099-100.
30 Flexner C. HIV Protease Inhibitors. N Engl J Med 1998 ; 338 : 1281-92.
31 Collier AC, Coombs RW, Schoenfeld DA, Basset RL, Timpone J, Baruch A, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine and zalcitabine. AIDS Clinical Trials Group. N Engl J Med 1996 ; 334 : 1011-7.
32 Borleffs JC, the CHEESE Study Team. First comparative study of saquinavir soft gel capsules vs. indinavir as part of triple therapy regimen (CHEESE) Abstract 387b. In: Program and abstracts of the 5th Conference on Retroviruses and Opportunistic Infections; 1998 Feb. 1-5; Chicago, USA.
33 Markowitz M, Saag M, Powderly WG, Hurley AM, Hsu A, Valdes JM, et al. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. N Engl J Med 1995 ; 333 : 1534-9.
34 Cameron DW, Heath-Chiozzi M, Danner S, Cohen C, Kraucik S, Maurath C, et al. Randomized placebo-controlled trial of ritonavir in advanced HIV-1 disease. Advanced HIV Disease Ritonavir Study Group. Lancet 1998 ; 351 : 543-9.
35 Cohen C, Revicki DA, Nabulsi A, Sarocco PW, Jiang P. A randomized trial of the effect of ritonavir in maintaining quality of life in advanced HIV disease. Advanced HIV Disease study Group. AIDS 1998 ; 12 : 1495-502.
36 Hammer SM, Squires KE, Hughes MD, Grimes JM, Demetemer LM, Currier JS, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. N Engl J Med 1997 ; 337 : 725-33.
37 Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez J, McMahon D, et al. Simultaneos vs. sequential initiation of therapy with indinavir, zidovudine and lamivudine for HIV-1 infection. 100-week follow-up. JAMA 1998 ; 280 : 35-41.
38 Moyle GJ, Youle M, Higgs C, Monaghan J, prince W, Charman S, et al. Safety, pharmacokinetics, and antiretroviral activity of the potent, specific human immunodeficiency virus protease inhibitor nelfinavir: results of a phase I/II trial and extended follow-up in patients with HIV. J Clin Pharmacol 1998 ; 8 : 736-43.
39 Jarvis B, Faulds D. Nelfinavir. A review of its therapeutic efficacy in HIV infection. Drugs 1998 ; 56 : 147-67.
40 De Clerq E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV infection. Antiviral Res 1998 ; 38 : 153-79.
41 Richman DD. Resistance of clinical isolates of human immunodeficiency virus to antiretroviral agents. Antimicrob Agents Chemoter 1993 ; 37 : 1207-13.
42 D'aquila RT, Hughes MD, Johnson VA, Fischl MA, Sommadossi JP, Liou SH, et al. Nevirapine, zidovudine and didanosine compared with zidovudineand didanosine in patients with HIV-1 infection - a randomized, double blind, placebo controlled trial. Ann Intern Med 1996 ; 124 : 1019-30.
43 Montaner JS, Reiss P, Cooper D, Vella S, Marris M, Conway B, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS trial. Italy, the Netherlands, Canada, Australia Study. JAMA 1998 ; 279 : 930-7.
44 Bellman PC. Clinical experience with adding delavirdine to combination therapy in patients in whom multiple antiretroviral treatment including protease inhibitors has failed. AIDS 1998 ; 12 : 1333-40.
45 Kagan S, Jemsek J, Martin DG, Pierone G, Manion DJ, Lee SR, et al. Initial effectiveness and tolerability of nelfinavir (NFV) in combination with efavirenz (EFV, SUSTIVA, DMP 266) in antiretroviral therapy naive or nucleoside analogue experienced HIV-1 infected patients: characterization in a phase II, open-label, multi-center study at > 36 weeks Abstract I-102. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
46 Havlir D, Hicks C, Kahn J, Riddler S, Lino Q, Ruiz N, et al. Durability of antiviral activity of efavirenz (EFV, DMP 266) in combination with indinavir (IDV) Abstract I-104. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
47 Morales-Ramirez J, Tashima K, Hardy D, Johnson P, Nelson M, Staszewsky S, et al. A phase II multi-center, randomized, open label study to compare the antiretroviral activity and tolerability of efavirenz (EFV) + indinavir (IDV) versus EFV + zidovudine (ZDV) + lamivudine (3TC) versus IDV + ZDV + 3TC at > 36 weeks [DMP 266–006] Abstract I-103. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
48 Coffin JM. HIV population dynamics in vivo: implications for genetic variation, pathogenesis and therapy. Science 1995 ; 267 : 483-9.
49 Ho DD. Time to hit HIV, early and hard. N Engl J Med 1995 ; 333 : 450-1.
50 Carpenter JC, Fischl MA, Hammer SA, Hirsch MS, Jacobsen DM, Katzenstein DA, et al. Antiretroviral therapy for HIV infection 1998. Updated recommendations of the International AIDS Society-USA Panel. JAMA 1998 ; 280 : 78-86.
51 MMWR supplement. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Ann Int Med 1998 ; 128 : 1079-100.
52 Markowitz M, Mo H, Kempf DJ, Norbeck DW, Bhat TN, Erickson JW, et al. Selection and analysis of HIV-1 variants with increased resistance in ABT-538, a novel protease inhibitor. J Virol 1995 ; 69 : 701-6.
53 Molla A, Korneyeva M, Gao Q, Vasanonda S, Schipper PJ, Mo HM, et al. Ordered accumulation of mutations in HIV protease confers resistance to ritonavir. Nature Med 1996 ; 2 : 760-6.
54 Condra JH, Schlelf WA, Blahy OM, Gabryelsky LY, Graham DM, Quintero JC, et al. In vivo emergence oh HIV-1 variants resistant to multiple protease inhibitors. Nature 1995 ; 374 : 569-71.
55 Hirsch MS, Conway B, D'Aquila R, Johnson VA, Brun Vezinet F, Clotet B, et al. Antiretroviral drug resistance testing in adults with HIV infection. Implications for clinical management. JAMA 1998 ; 279 : 1984-91.

56 Gatti G, Di Biagio A, De Pascalis C, Pontali E, Bassetti M, Cruciani M, et al. High variability of indinavir parameters of systemic exposure in HIV patients observed prospectively in everyday clinical practice Abstract A-66. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
57 Gatti G, Di Biagio A, Casazza R, Bassetti M, Cruciani M, Vella S, et al. Pharmacodynamic relationship between ritonavir side effects and its plasma concentrations Abstract A-75. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
58 Lange J. Issues for the future of antiretroviral therapy. Antiviral Therapy 1997 ; 2 (Suppl 4) ; 71-83.
59 Viraben R, Aquilina C. Indinavir-associated lipodystrophy. AIDS 1998 ; 12 : 37-9.
60 Henry K, Melroe H, Huebsch J, Hermundson J, levine C, Swensen L, et al. Severe premature coronary artery disease with protease inhibitors. Lancet 1998 ; 351 : 1328.
61 Carr A, Samaras K, Chisolm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipdaemia, and insulin resistance. Lancet 1998 ; 352 : 1881-3.
62 Kotler D, Heymsfield SB. HIV infection: a model chronic illness for studying wasting diseases. Am J Clin Nutr 1998 ; 68 : 519-20.
63 Arribas JR, Gonzales-Garcia J, Ibanez C, Ruiz B, Pena JM, Esteban C, et al. Acute hepatitis in AIDS patients during ritonavir treatment Abstract 417. In: Program and abstracts of the 5th Conference on Retroviruses and Opportunistic Infections; 1998 Feb.; Chicago, USA.
64 Rietsema WJ. Fever. Erythroderma, abdominal pain, and renal failure following initiation of indinavir therapy. CID 1997 ; 25 : 1268-9.
65 Wong JK, Günthard HF, Havlir DV, Zang ZQ, Haase AT, Ignacio CC, et al. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. Proc Natl Acad Sci USA 1997 ; 94 : 12574-9.
66 Günthard HF, Wong JK, Ignacio CC, Guatelli JC, Riggs NL, Havlir DV, et al. Human immunodeficiency virus replication and genotypic resistance in blood and lymph nodes after a year of potent antiretroviral therapy. J Virol 1998 ; 72 : 2422-8.
67 Nijhuis M, Schipper P, Leitner T, Schuurman R, Boucher C, Albert J. During Development of Ritonavir Resistance, HIV-1 Evolution is Determined by Stochastic Processes Abstract I-80. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
68 Perelson A. Mathematical models for describing drug resistance Abstract S-120. In: Program and abstracts of the 37th Interscience Conference on Antimicrobial agents and Chemotherapy; 1997 Sept.; Toronto, Canada.
69 De Jong MD, Veenstra J, Stilianakis N. Return to baseline values of HIV-1 RNA load during zidovudine treatment is associated with the emergence of K70R mutant virus Abstract 20. In: Program and abstracts of the 4th International HIV-Drug Resistance Workshop; 1995 July; Sardinia, Italy.
70 Larder BA, Kellam P, Kemp SD. Zidovudine resistance predicted by direct detection of mutants in DNA from HIV-infected lymphocytes. AIDS 1991 ; 5 : 137-44.
71 Mayers DL, Japour A.J, Arduino JM, Hammer SM, Reichman R, Wagner KF, et al. Dideoxynucleoside resistance emerges with prolonged zidovudine therapy. Antimicrob Agents Chemother 1994 ; 37 : 1207-3.
72 Gao Q, Gu Z, Parniak M, Cameron J, Cammack N, Boucher C, et al. The same mutation that encodes low-level human immunodeficiency virus type 1 resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine confers high level resistance to the (–) enantiomer of 2',3'-dideoxy,3'-thyacytidine. Antimicrob Agents chemother 1993 ; 37 : 1390-2.
73 Miller V, Stürmer M, Staszewski S, Groschel B, Hertogs K, De Berthune MP, et al. The M184V mutation in HIV-1 reverse transcriptase conferring lamivudine resistance does not result in broad cross-resistance to nucleoside analogue reverse transcriptase inhibitors. AIDS 1998 ; 12 : 705-12.
74 Larder BA. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science 1995 ; 269 : 696-9.
75 Eron JJ, Chow Y K, Caliendo AM, Videler J, Devore KM, Cooley TP, et al. Pol mutations conferring zidovudine and didanosine resistance with different effects in vitro yield multiply resistant human immunodeficiency virus type-1 isolates in vivo. Antimicrob Agents Chemother 1993 ; 37 : 1480-7.
76 Fitzgibbon JE, Howell RM, Haberzettl CA, Sperber SJ, Goche DS, Dubin DT, et al. Human immunodeficiency virus type-1 pol gene mutations which cause decreased susceptibility to 2'3'-dideoxycytidine. Antimicrob Agents Chemother 1992 ; 36 : 153-7.
77 Gu Z, Gao Q, Fang H, Salomon H, Parniak MA, Goldberg E, et al. Identification of a mutation at codon 65 in the IKKK motif of reverse transcriptase that encodes human immunodeficiency virus resistance to 2',3'-dideoxycytidine and 2',3'-dideoxy, 3'-thyacytydine. Antimicrob Agents Chemother 1994 ; 38 : 275-81.
78 Sommadossi J.P. Comparison of metabolism and in vitro antiviral activity of stavudine versus other 2',3' dideoxynucleoside analogues. J Infect Dis 1995 ; 171 : 588.
79 Lacey SF, Larder BA. Novel mutation (V75T) in human immunodeficiency virus type 1 reverse transcriptase confers resistance to 2',3'-didehydro, 2',3'-dideoxythymidine in cell culture. Antimicrob Agents Chemother 1994 ; 38 : 1428-32.
80 Larder BA. Reverse transcriptase inhibitors Abstract PL 3.1. In: Program and abstracts of the Fourth International Congress on Drug Therapy in HIV Infection; 1998 Nov.; Glasgow, Scotland.
81 Lanier ER, Smilaey M, St. Clair MH, Pearce G, Spreen W, Cutrell A, et al. Phenotypic HIV resistance in vitro correlates with viral load response to abacavir (1592, ABC) in vivo Abstract 32289. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.
82 Iversen AK. Multi-drug resistant human immunodeficiency virus type 1 strains resulting from combination antiretroviral therapy. J Virol 1996 ; 70 : 1086-90.
83 Katlama C, Ingrand D, Loveday C, Clumeck N, Mallolas J, Staszewsky S, et al. Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naive patients. A randomized controlled comparison with zidovudine monotherapy. Lamivudine European HIV working group. JAMA 1996 ; 276 : 1140.
84 Arnold E, Ding J, Hughes SH, Hostomsky Z. Structures of DNA and RNA polymerases and their interaction with nucleic acid substrates. Curr Opin Struct Biol 1995 ; 5 : 27-38.
85 Dueweke TJ, Pushkarskays T, Poppe SM, Swaney SM, Zao JQ, Chen IS, et al. A mutation in reverse transcriptase of bis (heteroaryl)piperazine-resistant human immunodeficiency virus type 1 that confers increased sensitivity to other nonnucleoside inhibitors Proc Natl Acad Sci USA 1993 ; 90 : 4713-7.
86 Condra JH, Holder DJ, Schleif WA, Blahi OM, Danovich RM, Gabryelsky LY, et al. Genetic correlates to in vivo viral resistance to Indinavir, a human immunodeficiency virus type 1 protease inhibitor. J Virol 1996 ; 70 : 8270-6.
87 Richman DD. New strategies to combat drug resistance. Hosp Pract 1996 ; 15 : 47-58.

88 Jacobsen H, Hänggl M, Ott M, Duncan IB, Owen S, Andreoni M, et al. In vivo resistance to a human immunodeficiency virus type 1 protease inhibitor: mutations, kinetics and frequencies. J Infect Dis 1996 ; 173 : 1379-87.
89 Patick AK. Genotypic analysis of HIV-1 variants isolated from patients treated with the protease inhibitor Nelfinavir alone or in combination with d4T or AZT and 3TC Abstract 10. In: Program and abstracts of the International Workshop on HIV Drug Resistance, Treatment Strategies and Eradication; 1997; St. Petersburg, Florida.
90 Mellors J. Patterns of phenotypic and genotypic cross-resistance among protease inhibitors in over 1.000 clinical isolates. In : Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.
91 Bodsworth NJ, Slade M, Ewan J, McNally L. Ritonavir and Indinavir Therapy at 28 weeks after Saquinavir therapy. Influence of HIV-1 protease mutations Abstract P-396. In: Program and abstracts of the 5th Congress on Retroviruses and Opportunistic Infections; 1998 Sept.; Chicago, USA.
92 Carrillo A, Sham H, Norbeck D, Kempf D, Kohlbrenner W, Plattner J, et al. Mutation in the proteolytic sites is required for the growth of mutant HIV selected by ABT-378, a novel protease inhibitor Abstract 209. In: Program and abstracts of the 4th conference on Retroviruses and Opportunistic Infections; 1997 Jan.; Washington D.C., USA.
93 Altice FL, Friedland GH. The era of adherence to HIV therapy. Ann Intern Med 1998 ; 129 : 503-5.
94 Gilson I, Busalacchi M. Abacavir/efavirenz/adefovir salvage regimen for refractory HIV disease Abstract I-200. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
95 Hill A, Gazzard B. Low frequency of genotypic mutations associated with resistance to AZT and 3TC after combination treatment with indinavir. Abs. 42197. In: Program and abstracts of the 12th World AIDS Conference; 1998 June 28–July 3; Geneva, Switzerland.
96 Deeks SG. Retroviral therapy: issues in salvage therapy Abstract S-29. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
97 Durant J, Clevenbergh P, Halfon P, Delgiudice P, Porsin S, Simonet P, et al. : Can HIV genotype determination can be useful for individualized adaptation of antiretroviral therapy? The VIRADAPT French Study. [Abs. OP 7.1] In: Program and abstracts of the Fourth International Congress on Drug Therapy in HIV Infection; 1998 Nov.; Glasgow, Scotland.
98 Reijers MHE, Weverling GJ, Jurriaans S, Wit FW, Weigel HM, Ten Kate RW, et al. : Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study. Lancet 1998 ; 352 : 185-90.
99 Havlir DV, Marschener IC, Hirsch MS, Collier AC, Tebas P, Bassett RL, et al. Maintenance antiretroviral therapies in HIV-infected subjects with undetectable plasma HIV RNA after triple-drug therapy. N Engl J Med 1998 ; 339 : 1261-8.
100 Pialoux P, Raffi F, Brun-Vezinet F, Meiffredy U, Flandre P, Gastaut JA, et al. A randomized trial of three maintenance regimens given after three months of induction therapy with zidovudine, lamivudine and indinavir in previously untreated HIV-1-infected patients. N Engl J Med 1998 ; 339 : 1269-76.
101 Clumeck N, Mundere J, Kabeya K, Konopnicki D, Sommerejns B, O'Doherty E, et al. MIRO: a randomized trial of three maintenance therapy with or without protease inhibitors after 4 months of triple or quadruple induction therapy Abstract OP 2.2. In: Program and abstracts of the Fourth International Congress on Drug Therapy in HIV Infection; 1998 Nov.; Glasgow, Scotland.
102 Hammer SM. Using Nucleoside and Nucleotide Reverse Transcriptase Inhibitors Abs. PL7.3. In: Program and abstracts of the Fourth International Congress on Drug Therapy in HIV Infection; 1998 Nov.; Glasgow, Scotland.
103 Montaner JSG, Harrigan PR, Jahnke N, Yip B, Hogg R, Montressori V, et al. Integrating Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) into Clinical Practice Abstract PL 7.2. In: Program and abstracts of the Fourth International Congress on Drug Therapy in HIV Infection; 1998 Nov.; Glasgow, Scotland.
104 Henry K, Shaefer MS, Bellman P, Norris D, Wallace R, Sawyer G, et al. TARGET : preliminary data of effective switch/intensification treatment with Combivir™ and Ziagen™ in therapy-experienced adults Abstract I-98. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
105 Hetherington S, Steel HM, Lafon S, Spreen W, Pearce G. Safety and tolerance of Abacavir (1592, ABC) alone and in combination therapy of HIV infection Abstract 12353. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.
106 Kahn J, Lagakos S, Weng D, Winslow D, Hardy D, Beall G, et al. A multicenter, randomized, double-blind, placebo-controlled study of the efficacy and safety of Adefovir Dipivoxil (ADV) when added to standard antiretroviral therapy (ART) Abstract I-108. In: Program and abstracts of the 38th Interscience Congress on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
107 Cherrington JM, Mulato AS, Lamy PD, Margot NA, Anton KE, Miller MD, et al. Adefovir Dipivoxil (bis-POM PMEA) therapy significantly decreases HIV RNA in patients with high-level AZT/3TC resistant HIV Abs. I-84. In: Program and abstracts of the 38th Interscience Congress on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
108 Miller MD, Margot NA, Anton KE, Mulato AS, Lamy PD, Cherrinton JM. HIV-1 expressing the 3TC-associated M184V mutation in reverse transcriptase shows increased susceptibility to Adefovir and PMPA independent of changes in Ki for the mutant RT Abstract I-123. In: Program and abstracts of the 38th Interscience Congress on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
109 Cassano P, DeWitt S, Hermans P, Kabeya K, Gerard M, Sommerijns B, et al. 141W94-AZT-3TC association: a novel protease inhibitor (PI) added to long-standing AZT-3TC or combined with new reverse transcriptase inhibitors (NRTIs) Abstract I-101. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.
110 Haubrich R. Phase 2 study of amprenavir, a novel protease inhibitor, in combination with zidovudine/3TC Abstract 12321. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.
111 Bart PA, Rizzardi GP, Gallant S, Meylan P, Spreen W, McDade H, et al. Combination abacavir (1592, ABC)/amprenavir (141W94) in HIV-1 infected antiretroviral naive subjects with CD4+ counts > 400 cells/(l and viral load > 5,000 copies/mL Abstract 286*/12204. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.
112 Rousseau F, Wang LH, Delehanty J, Hulett L, McCreedy B, Sommadossi JP, et al. Intracellular FTC-triphosphate levels correlate with the clinical antiviral activity of FTC Abstract OP 7.5. In: Program and abstracts of the Fourth International Congress on Drug Therapy in HIV Infection; 1998 Nov.; Glasgow, Scotland.

113 Ruxrungtham K, Boone E, Ford H Jr, Driscoll JS, Davey RT, Lane HL. Potent activity of 2'-β-fluoro-2',3'-dideoxyadenosine against human immunodeficiency virus type 1 infection in PBL-SCID mice. Antimicrob Agents Chemother 1996 ; 40 : 2369-74.

114 Brennan TM, Taylor DL, Bridges CG, Leyda JP, Tyms AS. The inhibition of human immunodeficiency virus type 1 in vitro by a non-nucleoside reverse transcriptase inhibitor MKC-442, alone or in combination with other anti-HIV compounds. Antivir Res 1995 ; 26 : 173-87.

115 Hill E, Taylor N, Borroto-Esoda K. In vitro synergy studies with MKC 442, a non-nucleoside reverse transcriptase inhibitor Poster 49. In: Program and abstracts of the 11th International Conference on Antiviral Research; 1998 April; San Diego, USA.

116 Szczech GM, Furman P, Painter GR. Safety assessment, in vitro and in vivo, and pharmacokinteics of MKC-442, a potent and selective non-nucleoside reverse transcriptase inhibitor of HIV-1. Antimicrob Agents Chemother, In press.

117 Moxham CP, Szczech GM, Blum MR, Barry DW. Pharmacokinetics (PK) and safety of single escalating doses of MKC-442 in HIV-infected volunteers. [Poster 573]. In: Program and abstracts of the 4th Conference on Retroviruses and Opportunistic Infections; 1997 Jan.; Washington DC, USA.

118 Chong KT, Pagano PJ. In vitro combination of PNU-140690 a human immunodeficiency virus type 1 protease inhibitor, with ritonavir against ritonavir-sensitive and -resistant clinical isolates. Antimicrob Agents Chemother 1997 ; 41 : 2367-73.

119 Poppe SM, Slade DE, Chong KT, Kinshaw RR, Pagano PJ, Markowitz M, et al. Antiviral activity of the dihydropyrone PNU-140690, a new nonpeptidic human immunodeficiency virus protease inhibitor. Antimicrob Agents Chemother 1997 ; 41 : 1058-63.

120 Turner SR, Strohbach JW, Tommasi RA, Aristoff PA, Johnson PD, Skulnick HI, et al. Tipranavir (PNU-140690): a potent, orally bioavailable non peptidic HIV protease inhibitor of the 5,6 dihydro-4-hydroxy-2-pyrone sulfonamide class. J Med Chem 1998 ; 41 : 3467-76.

121 Gong YF, Robinson B, Rose R, Deminie C, Spicer T, Markowitz M, et al. Antiviral activity and resistance profile of an HIV-1 protease inhibitor, BMS-232632 Abstract I-79. In: Program and abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy; 1998 Sept.; San Diego, USA.

122 Kato R, Yusa K, Gulnik SV, Bhat T, Hayashi H, Mitsuya H, et al. Importance of molecular flexibility for the resistance of HIV-1 protease inhibitors Abstract 12277. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.

123 Sato H, Shintani MS, Mimoto TM, Terashima KT, Hayashi H, Mitsuya H. In vitro antiviral activity and pharmacokinetic (PK) profiles of KNI 272 when combined with other protease inhibitors Abstract 12339. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.

124 Ueno T, Mimoto T, Shintani M, Sato H, Yusa K, Mitsuya H, et al. Anti-HIV-1 activity of and HIV-1 resistance profiles against JE-2147 and (KNI-764), a novel inhibitor of HIV-1 protease. Abs. 12270. In: Program and abstracts of the 12th World AIDS Conference; 1998 July; Geneva, Switzerland.