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HIV Drug Resistance and Insufficient Drug Plasma Levels as Factors Determining Antiretroviral Treatment Failure

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Abstract

The present aim of antiretroviral therapy is to suppress HIV replication as much and as long as possible in order to avoid development of AIDS. Although an increasing number of drugs is available to treat HIV-infected patients, virological treatment failure is still frequent in everyday clinical practice. Many factors have been recognized to explain this failure and are related to the virus, the host or the drugs themselves. HIV drug resistance is a major factor of failure. Retrospective studies have established a link between baseline resistance and evolution of viral load in pretreated patients.Two prospective studies have shown promising results of resistance testing in the choice of salvage regimen. The clinical utility of resistance testing becomes more and more obvious although some difficulties due to the interpretation of the test or to technical limitations still arise.
A correlation between protease inhibitor plasma levels and HIV RNA course has been established. Protease inhibitors seem to be good candidate for therapeutic drug monitoring. As for resistance testing, there are a lot of unresolved issues, but the combination of resistance testing and therapeutic drug monitoring is the first step toward a global approach of HIV treatment taking into account the right drug at the appropriate concentration.

Key words

Drug resistance. Drug levels. Pharmacokinetics. Salvage therapy. Antiretroviral therapy. Therapeutic drug monitoring (TDM).

Introduction

Potent antiretroviral treatment combination has dramatically reduced the rate of HIV and AIDS-related morbidity and mortality[1]. As viral eradication does not seem attainable with current strategies[2-4], the ultimate goal of present therapy is to suppress HIV replication as much and as long as possible. Maintaining plasma HIV-RNA as low as possible would prevent the progression to AIDS, minimize the risk of emergence of HIV variants resistant to the drugs used[5,6] and prolong the efficacy of the prescribed regimen.

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Definition and frequency of treatment failure

In the past, treatment failure was defined as the development of clinical endpoints, such as AIDS-defining events and death. In 1996, HIV-RNA plasma concentration, referred to as viral load,

Table 1. *Potential causes of incomplete viral suppression.*

Viral	Treatment	Host
Drug resistant virus:	Low adherence	Low CTL response
*preexisting	Drug interactions	Low CD8 suppression
*selected	Lack of absorption	Chemokine receptors
*transmitted	Low intrinsic efficacy	Chemokine production
SI phenotype	Decreased intracellular phosphorylation	Abnormal CD4 cell function
High baseline HIV-RNA	Sanctuaries	
	Low macrophage penetration	
	Cytochrome P 450 induction	

(Adapted from J Mellors).

emerged as a surrogate marker[7] for drug efficacy as HIV-RNA was shown to be a good predictor of clinical course and as patients treated with potent drugs rarely reached clinical endpoints. However, definition of failure using HIV-RNA quantification is a matter of debate depending on the HIV-RNA assay used for quantification and the chosen cut-off for HIV-RNA decrease to classify patients as responders or not. Some authors would also take into account trends in CD4 cell counts[8]. As the actual goal of treatment is to maximally reduce HIV replication in order to avoid development of resistance mutations, treatment failure could be defined as the inability to reach or maintain HIV-RNA below detection limit: 20 copies/mL (Roche Amplicor HIV assay) or 50 copies/mL (Quantiplex HIV-RNA; Chiron). An ultrasensitive assay developed by a Swiss group has a 5 copies/mL lower limit of detection (L Perrin, personnal communication). Whether all the patients should be driven to that level is not yet clear.

The appearance of resistance mutations even at very low plasma viral load is probably the leading event allowing the virus to further escape treatment. Resistance mutations to combination therapy progressively appear in a stepwise fashion: The virus first becomes resistant to the drug with the lowest genetic barrier[9]. In the near future, definition of failure will probably even be refined by the detection of resistance mutations at very low HIV-RNA levels presently not yet considered as failure[10]. Short term treatment escape in the presence of such resistance mutations is likely to occurs, and appareance of those mutations could itself be defined as failure. Possible therapeutic interventions targeting the drug to which the virus became resistant could be developed (change, intensification).

Whatever the definition used, treatment failure is a frequent phenomenon. In the Swiss cohort[11], only 40% of the patients had a decrease in viral load (VL) below level of quantification (BLQ) associated with a rise in their CD4 cell count; 40% of the patients exhibited a transient suppression in viral load BLQ followed by a rebound, while their CD4 counts remained stable; 5% of the patients had a decrease in viral load BLQ, while their CD4 cells did not increase, and the remaining 15% of the patients exhibited an increase in their viral load associated with a decrease in their CD4 cells. In a retrospective study analysing an unselected HIV-infected population, virological failure (VL decline < 1 log) under a protease inhibitor (PI) containing regimen was as high as 44%[12]. In a cohort of patients receiving a PI containing regimen for the first time, 79% obtained a VL BLQ (500 copies/mL) at month 6. But among these patients, there was an estimated 53% probability of VL rebound over 500 copies/mL by 52 weeks[13]. In a prospective study of patients starting on a PI for the first time, only 53% of the patients obtained a VL BLQ (< 400 copies mL) at 24 weeks, and 25% of those patients experienced a rebound in VL at month 9[14]. Effectiveness of a salvage regimen is even smaller. Only 22% of patients receiving a second line PI-containing regimen obtained a VL BLQ (< 500 copies/mL) at 24 weeks[15].

Potential factors to explain failure

Many factors have already been recognized as potentially responsible for treatment failure. These factors are related to the host, the virus or the antiretroviral drugs (Table 1). Herein, we will mainly focus on virological and pharmacological parameters associated with treatment failure.

Predictive factors of virological failure to therapy: Clinical studies

Several studies have analysed the predictive factors of virological failure (Table 2)[12-20]. Almost all the studies find the same factors as predictors of failure: high baseline viral load, low baseline CD4 cells, previous use of antiretroviral drugs, no change in the nucleoside analogue backbone in combination upon initiation of PI, and the use of saquinavir hard gels (HG). Analysing these parameters in the light of Table 1 can be translated into: Presence of drug resistant HIV variants in treatment experienced patients, high baseline VL, outgrowth of mutant HIV as a consequence of incomplete viral suppression[21,22], and use of suboptimal drug concentrations exemplified by saquinavir HG. The durability of viral suppression appears to be related to the viral load nadir reached[23,24]. This is probably due to the risk reduction of emergence of resistance[25].

Virological factors

Incomplete viral suppression in the context of selective drug pressure inevitably leads to the appearance of HIV variants resistant to the drugs. Mutations at specific sites in the reverse transcriptase (RT) or in the protease (P) genes associated with reduced sensitivity to antiretroviral drugs have been well described[26]. Also, a good correlation has been found between resistance mutations and a decreased sensitivity in phenotypic assays.

Evidences in pretreated patients

Many retrospective studies have established a link between the baseline resistance profile and the change in HIV-RNA in drug experienced patients. Some of them are reported in (Table 2)[27-39]. Most studies demonstrate a strong relationship between HIV drug resistance at baseline and the probability to respond to therapy. This is true using either phenotypic or genotypic resistance assays. The absolute number of resistance mutations rather than the location of these mutations matters for the virologic evolution under a particular therapy. This has been shown for protease inhibitors (PI) as well as for reverse transcriptase inhibitors (RTI).

However, some studies have not been able to identify such a relationship between the baseline resistance profile and virological course. In a small number of patients failing nelfinavir, the short-term response to salvage therapy with saquinavir-ritonavir was not influenced by the presence of baseline mutations[40]. In the preliminary analysis of zidovudine-experienced, 3TC and indinavir-naïve patients, given 3TC with or without indinavir, no correlation was found between baseline HIV protease or RT resistance mutations and week 24 virological response[41]. In 98 PI-naïve patients starting a PI-containing regimen, no relationship between baseline genotype and virological response to HAART was found[42].

Evidences in naïve patients

Resistant viruses can be selected by a failing regimen or acquired horizontally or vertically. Never-treated patients can harbour a virus which has already been exposed to drugs so that the term «antiretroviral naïve» should be applied to the virus itself instead to the patient. The sexual transmission of AZT-resistant strains has been reported[43,44]. Sexual transmission of a strain resistant to multiple drugs has recently been reported[45]. The prevalence of drug-resistant HIV is variable according to time and location of acquisition, and the definition used to characterize drug resistance. In 114 recently infected drug-naïve patients, genotypic or phenotypic evidence of resistance was found in 1% for nucleoside reverse transcriptase inhibitors (NRTI); 5-7.7% for non nucleoside reverse transcriptase inhibitors (NNRTI), and 1% for PI; prevalence of resistance to 2 and 3 classes was between 2 and 3%[46]. In another series of 69 recent seroconverters, the prevalence of drug-resistant HIV was even higher: NRTI: 3%, NNRTI: 17%, PI: 13%[47]. The prevalence of primary drug resistance was assessed in 252 Spanish patients among whom 52 were recent seroconverters. The prevalence of primary mutations increased in recent seroconverters *versus* patients with chronic infection[48]. Treatment efficacy could be compromised in a treatment-naïve patient already harbouring a resistant virus. Patients harbouring the RT gene M184V or P gene A71V mutations did worse when starting triple-drug therapy than patients with a wild type strain or with other mutations[49]. Indeed, a patient newly infected by a multidrug-resistant strain and given AZT, 3TC, and indinavir as initial treatment showed poor response to therapy[50]. The high rate of drug-resistant HIV in naïve recently infected patients suggests that resistance testing prior to starting therapy could be useful in order to optimize initial therapy. In contrast, the presence of drug resistance-associated mutations at the time of primary HIV infection was not predictive of consecutive treatment failure in a French series. In that series the prevalence of resistance mutations was 18% for the RT gene and 4% for the protease gene[51].

The materno-foetal transmission of AZT resistant HIV[52] but also of multiple drug-resistant strains has been documented[53]. The prevalence of genotypic mutations associated with AZT resistance was 19% in AZT-treated pregnant women belonging to the PACTS cohort[54]. These highly prevalent AZT resistant strains can be transmitted to the babies with a potential impact on drug efficacy in the child. HIV-infected babies whose mothers had received AZT prophylaxis fared less well than HIV-infected babies whose mothers did not receive any prophylaxis. This is possibly due to the fact that the strains transmitted from the treated mothers to their babies were resistant to AZT[55], a drug commonly used to treat children. HIV resistant to multiple drugs was found in a child whose mother was previously treated with various NRTI and PI with poor adherence and incomplete viral suppression[53]. The child's treatment failed as HIV-RNA was detectable from the first available sample while the baby was on AZT, to which the virus was resistant.

Importance of pharmacological parameters

Not all virological failure can be attributed to the developement of resistant virus (Table 1). Drug-related factors which can explain treatment failure are: poor adherence, malabsorption, insufficient dosage, drug-drug interaction, impaired intracellular metabolism, hyperactivity of the cytochrome P450, and overexpression of the P glycoprotein. Measuring the plasmatic fraction of the 3 antiretroviral drug classes is feasible, but offers unequal advantages. Reverse transcriptase nucleoside inhibitors need intracellular phosphorylation to be active[56]; so that their plasmatic levels badly reflect their activity. Measure of the triphosphorylate moiety is only available in research laboratories. Non nu-

Table 2. *Retrospective studies linking baseline resistance profile and virological outcome.*

Author	Patick[27]	Zolopa[28]	Lanier[29]	Harrigan[30]	Walmsley[31]	Harrigan[32]	Hammer[33]	Katsenstein[34]	Lorenzi[35]	Skowron[36]	Piketty[37]	Pérez-Elías[38]	Shulman[39]
No. patients	65	51	> 200	84	56	59	94	246	62	132	32	24	33
Salvage	yes	yes	partial	no	yes	yes	yes	no	yes	no	yes	variable	yes
Drug used	nelf	sqv/rtv	abc	sqv/rtv	nelf	mega HAART	abc/NRTI efv/adf nelf/plcb	nelf and/or efv	nelf	3TC	rtv/sqv/efv + 2 NRTI	diverse	Efv/adf
Definition of virological success													
nadir reduction	< 500 cp ≥ .5 log	< 500 cp	< 200 cp	< 500 cp	< 500 cp	/	< 500 cp W 16	< 500 cp 16 W < 500 cp 24 W	> 1 log 4 W–12 W	> 1 log W 4	< 500 cp W 36	> 1 log W6	< 500 cp W 12
Rate of success	51%	37%	/	52%	33%	/	24-45%	/	32%	/	67%	76% (3SD) 45%(≤ 2SD)	60% NNRTI naïve 8% NNRTI exp
Predictive factors of failure													
BL CD4	no	yes	/	yes	no	/	/	/	no	/	/	/	yes
BL VL	no	yes	/	yes	no	/	/	yes (early)	no	/	/	/	yes
CDC stage	/	yes	/	/	/	/	/	/	no	/	/	/	/
Pre TT	no	yes	/	/	/	/	/	yes (early)	no	/	/	/	yes NNRTI
Resistance													
RT mutations	/	/	≥ 3	/	/	/	/	yes (late)	yes	/	/	/	yes: K103N
Protease mutations	≥ 2	≥ 3	/	multiple	≥ 1	yes	yes	/	yes	/	no	/	/
PhenoR	yes	/	≥ 2 NRTI	yes	/	yes	yes	/	/	yes	yes	yes	/

salvage: previous use of PI; BL: baseline; VL: viral load; pre TT: pretreatment; RT: reverse transcriptase; pheno R: phenotypically resistant; nelf: nelfinavir; sqv: saquinavir; rtv: ritonavir; abc: abacavir; adef: adefovir; plcb: placebo; w: week; / not defined; NNRTI: non nucleoside inhibitor of reverse transcriptase.

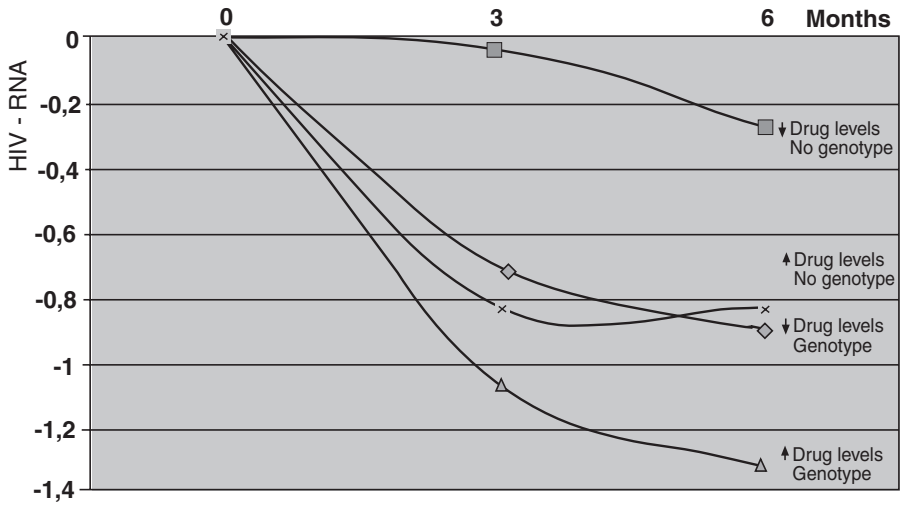

Fig. 1. *Viradapt: Influence of genotyping information and appropriate drug levels on virological response to treatment.*

cleoside reverse transcriptase inhibitors have long half lives and attain high steady-state concentrations[57-59]. These concentrations are several fold higher than the IC_{50} of a wild type virus and it is unlikely to find low plasma concentrations to explain failure. These drugs have a low genetic barrier and failure is almost always due to resistance. Protease inhibitors seem to be the most appropriate candidates for drug monitoring as AUC and trough concentrations appear to be the best predictors of response. A wide inter-patient variability has been shown, while a relationship between concentration and effect has been demonstrated. Finally, intra-patient variability can be wide and this is probably due in part to non adherence.

PI plasma concentration and treatment efficacy

Numerous studies show a correlation between PI plasma levels and HIV RNA. In 40 patients receiving either 3600 or 7200 mg of saquinavir daily dose, a more pronounced and persistent response in plasma HIV-RNA and CD4 cells was observed with higher doses of saquinavir compared with the published results of the standard dose (1800 mg/day)[60]. In another series, linear regression analysis showed a significant correlation between saquinavir plasma concentration and decline in HIV RNA at 12, 36 and 48 weeks[61]. In a cohort of 65 patients given indinavir, a low PI plasma level was a major risk factor for virological failure in addition to high baseline VL and previous PI use[62]. In 23 patients receiving indinavir 800 mg *tid* as their first PI containing regimen, a significant intersubject variability was found. The 8-hour area under the curve for indinavir was significantly higher in patients with a VL BLQ than in patients with detectable HIV RNA[63].

Of the 108 patients included in the Viradapt study, the 87 patients from both arms participating at the Nice center were included in the pharmacological substudy[64]. Serial PI plasma trough levels were determined in these patients during the 12-month study period. Plasma PI concentrations were measured by HPLC. Linear regression analysis showed a statistically significant correlation between plasma concentration and HIV-RNA for each PI. Higher drug concentrations correlated with lower HIV-RNA levels for the 4 PIs. Patients were divided into 2 groups: Suboptimal concentration for patients with a PI plasma concentration below the defined threshold of 2 x IC_{95} at least twice during the study period; Optimal concentrations for patients having had no more than one PI level below 2 times the IC_{95}. According to our efficacy threshold, 32% of the patients had suboptimal concentration and 68% had optimal concentration. HIV-RNA decreased 1.2 logs at month 12 in patients in the optimal concentration group, *versus* 0.36 logs at month 12 for patients in the suboptimal concentration group. Patients were categorized based on randomization arm and drug levels. The smallest reduction in HIV-RNA was obtained in patients with standard of care and suboptimal PI concentration, while the greatest reduction in HIV-RNA was obtained in patients with optimal PI concentration and genotypic guided treatment. Genotypic guided therapy, drug concentrations and the presence of primary protease mutations were all factors which independently affected the response to therapy in experienced patients (Fig. 1). The use of different cut-offs for optimal drug levels and different definitions for primary protease mutations may have influenced these results. In the ACTG 343 trial on induction-maintenance antiretroviral therapy, no indinavir phenotypic or genotypic resistance was found in 19 patients failing indinavir maintenance or triple drug therapy[65]. Rebound with a sensitive virus was attributed to a fitness advantage of the wild type compared to the resistant virus. This could also be due to suboptimal drug levels. In the Trilège study[9], only mutations to 3TC were observed in patients failing therapy. In contrast, a substantial proportion of the patients had indinavir concentrations below efficacy levels or even below detection limit.

Compliance

One major reason for low PI plasma levels is non-compliance. In the Trilège trial[9], poor adherence to indinavir was documented in all patients failing triple therapy maintenance and in most patients in the zidovudine/indinavir maintenance arm. A link between viral load response and treatment adherence has been found in some studies. In a series of 84 subjects using MEMS caps, a highly significant association was found between adherence and virologic suppression. Overall, 81% of subjects with > 95% adherence had complete viral suppression, compared to 64% with 90-95% adherence, 50% with 80-90% adherence, 25% with 70-80% adherence and 6% with < 70% adherence[66]. In another series of 32 patients, 60-70% of the variation in concurrent VL over a 8-week period was explained by the rate of adherence, while genotypic resistance could not predict concurrent VL in subjects whose virus was still sensitive to at least one drug in their regimen[67]. Patients reporting < 80% adherence at 6 months showed an increase in their VL and a loss in CD4, while patients with 100% adherence obtained a 1.1 log decrease in VL and a gain in CD4[68]. In patients failing multiple therapy and given mega-HAART, a U-shape curve between the virological response and the number of active drugs prescribed was observed. Patients given a higher number of drugs to which their virus was still sensitive were probably non compliant to the previously failing regimens and even more non compliant to the mega-HAART given as «salvage» intervention[69].

Bioavailability

Another possible explanation for drug-related treatment failure is lack of absorption or poor bioavailability. In studies analyzing the predictive factors of virological failure (Table 3), treatment with saquinavir hard gels was frequently pointed out. The poor bioavailability of the drug[70] is the probable explanation for its lack of efficacy. In 66 subjects on stable treatment with saquinavir HG, a marked interindividual variability in saquinavir trough levels was found, 33% of the patients having trough concentrations below the IC_{95}[71].

Other pharmacological factors include drug-drug interactions, expression of multidrug resistant genes, and the presence of sanctuaries into which the drugs do not penetrate.

Interventions using drug resistance testing or therapeutic drug monitoring

Prospective use of resistance profile in the choice of a salvage regimen in antiretroviral experienced patients

Two studies have analysed the value of prospective resistance testing in the choice of salvage therapy. In the Viradapt study[72,73], 108 heavily pretreated patients failing therapy were randomized into 2 arms: Standard of care (n = 43), or treatment according to the resistance mutations in protease and reverse transcriptase genes (n = 65). The major endpoint was the change in HIV-RNA. Decisions concerning therapeutic changes in the genotypic group were guided by correlations linking specific mutations with decreased activity of specific drug(s). When these specific mutations were found, corresponding drugs were no longer considered for treatment. After 6 months, a reduction of 1.15 log copies of HIV-RNA versus 0.67 log was seen in the genotypic arm compared with the control arm, with 32.3% *versus* 14% below 200 copies/mL (p = 0.048), in the genotypic arm and the control arm, respectively. The difference in viral load reduction combined at 3 and 6-month was statistically significant (p = 0.015). After the 6 months interim analysis, we decided to conduct genotyping on all patients. Patients in both arms received treatment based on genotyping results which were performed every three months in an open label fashion. In the genotyping arm, the reduction in viral load was maintained throughout the 12-month study with a mean drop in HIV-RNA of -1.15 log. In the control arm, at completion of the randomized study, viral load had dropped 0.67 log. During the following 6-month open label genotyping phase, there was an additional drop to 0.98. However, since this phase was not controlled, no conclusions can be drawn regarding the cause(s) of this additional reduction. In the genotypic arm, the percentage of patients with HIV-RNA below detection limit remained stable around 30% throughout the 12-month follow-up period. In the control arm, the proportion of patients with HIV-RNA below detection limit rose from 14% at month 6 to 30.5% at month 12. We performed additional analyses to determine predictive factors affecting HIV-RNA responses. The presence or absence of primary protease gene mutations at baseline was correlated with reduction in viral load at 3 and 6 months. The greatest reduction was seen in patients who did not have primary protease mutations and received genotypic guided treatment with a drop of 1.5 log. The poorest response was seen in those in whom primary protease mutations were present and received standard of care. Intermediate results were seen in the groups in whom protease mutations were absent and received standard of care, or in patients in whom primary protease mutations where present and received genotypic guided treatment. Multivariate analysis showed that the presence of primary protease mutations and performance of genotypic guided treatment, both independently, affected the virological response. In the Genotypic Guided Antiretroviral Treatment study (GART)[74], 153 patients failing therapy were assigned either to a group treated with standard of care or to a group benefiting from virological advice through interpretation of their genotypic resistance profile. The drop in HIV-RNA was higher for patients given virological advice than for patients in the control group. This difference narrows at week 12. A clear correlation was found between HIV-RNA changes and number of active drugs pre-

Table 3. *Clinical studies examining the predictive factors for treatment failure.*

References	Fätkenheuer[12]	Casado[16]	Mocroft[14]	Wit[17]	Staszewski[13]	Deeks[15]	Deeks[83]	Easterbrook[18]	Zimmerli[19]	Temesgen[20]
Number patients	198	400	243	271	901	337	99	847	274	54
ARV naïve	17%	9%	74%	22%	34%	14%	0%	/	37%	partial
PI naïve	77%	100%	100%	100%	100%	80% *	0%	100%	100%	100%
Virological definiton of failure	< 1 log_{10} reduction from BL at W 24	> 200 cp at 52 W	> 400 cp at 24 W	> 1000 cp at any time or rebound over LLQ	> 500 cp at 24 W	> 500 cp at 48 W	> 500 cp at 24 W	VL > LLQ at 16 W	> 500 cp at > 24 W	< 1 log dec at 12 W or > 500 cp thererafter
Follow-up	24 W	52 W	32 W	48 W	52 W	48 W	24 W	24 W	24 W	48 W
Proportion of virological failure	44%	55%	47%	40%	21%	50%	88%	31%	45% at any time	6% at 24 W 31% at 48 W
Proportion of rebound	/	/	25% after 24 W	24% at any time	53%	/	/	44%	32%	/
Immunological definition of failure	/	< 100 cells increase	/	/	/	/	/	/	/	/
Risk factors associated with Failure:										
Baseline CD4	yes	no	no	yes	yes	yes	yes	/	no	no
Baseline VL	no	yes	yes	yes	yes	yes	no	/	yes	no
Pretreatment	yes	yes	no	no	yes	yes	/	yes	yes	yes
Introduction of new drugs	/	/	yes	no borderline	yes	yes	NNRTI[1]	yes	yes	/
Use of saquinavir HG	yes	yes	no	yes	yes	/	/	yes	yes	/
Adherence	/	/	/	/	/	/	/	/	yes	no

[1] introduction of a NNRTI; ARV: antiretroviral; PI: protease inhibitor; VL: viral load; BL: baseline; cp: copies/ mL, W : week; LLQ: lower limit of quantification; / : not defined.

scribed, i.e. 0.1 log decrease with < 1 active drug, 0.59 log with 2 active drugs, 1.04 log with 3 active drugs, and 1.25 log with 4 active drugs received. Patients in the genotypic guided treatment group were more likely than control patients to receive more active drugs. In this multicenter study, virological advice was diversely followed by the physicians. The closer the advice was followed, the better was the virological response in the genotypic guided treatment group *versus* the control group. In centers not following the advice, there was quite no difference between the «GART» group and the control group regarding the VL reduction. The fact that many physicians did not follow the virological advice based on genotypic resistance testing probably blunted the difference between the study arm and the control arm.

At this time, no study has been published using genotypic guided treatment naïve patients. An international study designed to assess the relevance of resistance testing in the source patient to guide post-exposure prophylaxis in the exposed subject is to begin soon.

Major drawbacks in the use of resistance assays to guide treatment

The use of resistance assays to guide salvage treatment is quite difficult as many aspects of resistance are not yet understood, and also because of technological limitations. At this time, no standarized technique has been registered and wide discrepancies among different technologies and different laboratories frequently arise[75]. Standarized kits will be available in the near future. Present assays cannot detect resistance at HIV RNA levels below 1000 copies/mL, a cut-off that could be considered to high regarding failure definition. Assays detecting resistance mutations at very low plasma HIV-RNA concentration are currently being developed.

Some patients do not have mutations to explain their treatment failure. In the GART study[74], 73% of the patients had mutations on both protease and reverse transcriptase genes, and 25% of the patients had no mutations on the protease gene. In Viradapt[73], the overall prevalence of primary mutations for the reverse transcriptase gene was 90%. The overall prevalence of primary mutations in the protease gene was 48%. The absence of resistance mutations could be due to non-compliance, lack of absorption, poor drug metabolism, release of virions from sanctuaries, or possibly clinically significant minor variants[76,77]. Only the major variants are analyzed by the existing genotyping technology and variants representing less than 25% of the quasispecies cannot usually be detected. Finally, interpretation of the mutation pattern is quite difficult, and guidelines for the interpretation of resistance mutations are needed. The field of resistance mutations is rapidly evolving and new mutations have recently been described explaining failure to drugs such as d4T, abacavir or nelfinavir. Updated data for newly discovered mutations or mutational patterns of newly released drugs are needed. The interpretation of genotypic resistance must also take into account that some mutations are only found in archival HIV-DNA, and that mutations arising with combination therapy could be different from those arising with monotherapy, on the basis of which resistance mutation tables are constructed. Moreover, cross-resistance, drug resensitivation due to the combination of mutations and/or loss of viral fitness due to mutations further increase the complexity of using genotypic resistance testing.

Therapeutic drug monitoring

Many studies highlight the association of drug levels and therapeutic response, but the prospective use of drug measurement to optimize therapy, referred to as therapeutic drug monitoring (TDM), is still controversial[78,79]. However, there are several drawbacks in the use of TDM for PI[78]. Indeed, the significance of a single measurement is weak as intrapatient variability is high. This could be due to various factors such as food interactions, menstrual phase, amount of alpha-1 acid glycoprotein and plasma albumin to which the PI are bound, and finally sample timing. Of utmost importance to interpret the plasma concentration is the definition of an efficacy threshold. This threshold is difficult to define: IC_{50} is probably a weak predictor of efficacy as only 50% of viral replication is inhibited. IC_{90} or IC_{95} could be more appropriate. As they are protein-bound (60% for indinavir to > 98% for saquinavir, nelfinavir and ritonavir) a substantially diminished activity is found for PI in the presence of 50% human serum supernatant in cell cultures. So, published IC_{50} measured with only 10% fetal calf serum seriously overestimate the potency of the PI[80]. The efficacy threshold probably needs to be adjusted for each patient to his particular strain as the mutated virus is less sensitive to the drugs than is the wild type. The phenotypic determination of the IC_{95} for each particular patient could be of interest.

At this time, no clinically relevant efficacy thresholds have been defined for the various PIs and the use of TDM is still elusive. Prospective clinical trials should demonstrate that monitoring PI in order to obtain a desired plasma concentration provides better virologic response and is associated with low toxicity rates.

Use of resistance and pharmacological data

A tentative algorithm for the rational use of these tests can be drawn (Fig. 2) based on retrospective studies analysing the importance of HIV drug resistance profile and PI plasma levels. Few prospective studies using these tools have been reported but the results of many ongoing trials will soon become available. In the proposed algorithm, several unanswered issues are left open for which ongoing trials will probably provide some clues.

Fig 2. *Tentative algorithm for using resistance testing and therapeutic drug monitoring?: unresolved issues.*

Treatment failure: HIV-RNA > lower limit of quantification:
----->Perfrom trough and peak PI concentrations measurements according to the drug (once?):

1) Inadequate PI plasma levels (consider changes < 30% from normal values ?)
- Perform/compliance survey and treatment counselling
- Look for side effects
- Rule out malabsorption
- Review treatment to exclude underdosage or drug-drug interactions
- Withdraw drug responsible of interaction
- Increase drug dosage (?) or introduce drugs inhibiting the CYP 450
- Change to drug easier to take
- Seek expression of the MDR gene (?) and Use P-glycoprotein blockers (?)

2) Adequate PI plasma levels or regimen without PI -----> Perform genotypic or (?) phenotypic analysis for resistant virus
- Exclude drugs to which the virus is genotypically considered resistant; include drugs to which the virus is phenotypically considered sensitive
- Use the highest number of active drugs
- Avoid drugs with low genetic barrier and always combine them with other drugs
- Consider intensification (?)
- Consider the performance of ultrasensitive assays for resistance mutations (?)
- Perform resistance assay in drug naïve (first shoot equal best shoot)
- Monitor intracellular drug levels for nucleoside triphosphate and PI (?)

Conclusions

An HIV drug resistance profile is now available within days (genotype) or weeks (phenotype), and plasma PI levels are easily determined. Thank to these new techniques, we are now in a position not only to understand the various reasons for treatment failure but even to prospectively use this information to optimize therapeutic choice. Major difficulties are still present in the use of these techniques that will be overcome with improved understanding of these tools. This is a first step toward a global approach of the antiretroviral treatment looking at the viral sensitivity and the appropriate drug level to obtain. The fully comprehensive approach to efficiently treat HIV needs to take into account many different factors, either viral, related to the host or to the drugs involved in the treatment succes or failure. Drug resistance testing will soon become part of the standard of care[81] as the clinical situations in which to use it become clearer[82]. Therapeutic drug monitoring is still in his infancy, but ongoing trials will help to define how to use it at best.

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