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Update on Genotype-Guided Antiretroviral Therapy

Carmen de Mendoza, Oscar Gallego, Luisa Valer and Juan Gónzalez-Lahoz

Service of Infectious Diseases, Instituto de Salud Carlos III, Madrid, Spain

Abstract

Drug resistance is either the cause or the inevitable consequence of treatment falilure. HIV genotyping is now recommended to select the best antiretroviral therapy in a given patient in selected situations, but the benefit is greatly dependent of adequate interpretation and of the number of drugs for which sensitivity is apparently preserved. Information available support the use of drug resistance testing in subjects not exposed to antiretroviral agents in at least four situations: HIV-infected pregnant women, children born and infected from treated mothers, individuals presenting with the acute retroviral syndrome or having seroconverted within the past year, and in the setting of post-exposure prophylaxis. Among pre-treated persons, drug resistance testing seems to be a particularly useful tool in subjects on early virologic failure. The benefit of drug resistance testing in naive individuals with chronic HIV infection or in heavily pre-treated persons on failure is so far not well established. The reliability of testing subjects with non-B viruses or with low levels of viremia is under discussion. The search for new drug-resistant genotypes and the establishment of large databases pairing geno-phenotypes must be encouraged.

Key words

Drug resistance. Antiretroviral therapy. Viral load. Treatment failure.

Introduction

Despite the availability of 16 FDA-approved drugs for the treatment of HIV infection, many HIV-infected patients experience drug failure[1-3]. In a meta-analysis of 21 treatment groups from randomized clinical studies, only 46% of patients were able to reach the targeted HIV-RNA viral load (<50 copies/ml) by 48 weeks[4]. Although there are many possible explanations for treatment failure, including poor adherence, inadequate potency of the regimen, poor absorption, and drug-drug interactions, drug resistance is either the cause or the inevitable consequence of treatment failure[5]. Information on drug susceptibility might help to improve the response to first-line or sequencing antiretroviral regimens. Genotyping is currently more affordable than phenotyping in the clinical setting, and the benefit of implementing genotype-guided antiretroviral therapy should not be delayed. Herein, we review current epidemiological data on resistance and the relevant clinical trials that have elucidated the role of antiretroviral drug resistance testing in clinical practice.

Guidelines for the use of resistance testing in clinical practice have been issued by several expert panels[6-9]. Table 1 summarizes the recommendations from the experts that support the use of these assays in certain clinical situations. Drug resistance might be examined either in naive individuals (pri-

Correspondence to:
Carmen de Mendoza
Service of Infectious Diseases,
Instituto de Salud Carlos III
Calle Sinesio Delgado 10, 28029 Madrid, Spain
Phone 34 91 4532515
Fax 34 91 7336614
E-mail: cmendoza@teleline.es

Table 1. *Summary of expert panel recommendations for resistance testing.*

Clinical Setting	IAS-USA[6]	DHHS[7]	Spanish guidelines[8]	Euroguidelines[9]
Pregnancy	R	R	R	R
Infected children born	R	R	R	R
Primary/Recent HIV infection	C	C	R	R or C
PEP	R	NR	NR	R
Chronic HIV infection	C	NR or C	NR	C or store specimen
First regimen failure	R	R	R	R
Multiple regimen failure	R	R	R	R

R: Recommended
C: Consider
NR: Not Recommended
*if treatment to be initiated and either high local transmission rate of resistance or transmission suspected from a treated individual.
+ in other situation (or store specimen)

mary resistance) or in those failing therapy (secondary resistance)[10,11]. The information available supports the use of drug resistance testing in subjects not exposed to antiretroviral agents in at least four situations: HIV-infected pregnant women, children (infected) born from treated mothers, subjects presenting primary HIV infection or having had a recent seroconversion (within the past year), and post-exposure prophylaxis (PEP). Among pretreated persons, drug-resistance testing seems to be a useful tool in the setting of early virological failure, as well as for selecting the best options in heavily pretreated subjects. We will discuss separately, in detail, each of these situations. So far, no data are available supporting the use of drug-resistance testing in naive subjects with chronic HIV infection, or in patients having undetectable plasma viremia in response to therapy.

HIV-infected pregnant women

Mother-to-child transmission of HIV infection has declined dramatically since the wide prescription of antiretroviral drugs during pregnancy and at the time of delivery. Amongst other factors, suppression of plasma viral load to undetectable levels at birth seems to be one, if not the most important, variable accounting for this protective effect[12]. Although prospective, randomized trials proving this benefit are mainly limited to zidovudine (ZDV)[13] and nevirapine[14], it is generally believed that the success in preventing vertical HIV transmission is highly dependent on the antiviral effect of any drug during pregnancy and labor. Therefore, if resistance to drug(s) in use develops during pregnancy, it should be presumed that viral rebound could predispose the newborn to be at risk for HIV infection. Likewise, if pre-existing resistant viruses exist in the mother, treatment failure might be more common and increase the risk for perinatal HIV transmission. Taking these considerations into account, drug-resistance testing should be given to all HIV-infected pregnant women with detectable viremia, including those without previous exposure to antiretroviral drugs, i.e. those who became aware of their HIV-positive status during a prenatal exam. As will be discussed later, a rate of primary resistance ranging from 5 to 25% in adults from many developed countries supports this intervention[15].

Children (infected) born to treated mothers

Despite the number of new HIV-infected newborns being currently very low in developed countries, children who acquire HIV infection from mothers exposed to antiretroviral drugs during pregnancy are more likely to be infected with resistant viruses[16-18]. Recent studies have highlighted the high rate of AZT or NVP mutations when these drugs are used as prophylaxis to prevent transmission. In the context of a wide HIV-1 variability, resistance can be one of the factors contributing to mother-child transmission[19,20]. Treatment is often introduced early in HIV-infected children and, therefore, the first combination is particularly crucial for them. Thus, resistance testing should be given to all children born to HIV-infected mothers who become infected, and this recommendation could be extended to children born of mothers never exposed to antiretroviral drugs, since the incidence of primary resistance is growing in many areas[15].

Primary HIV infection or recent seroconversion

The transmission of drug-resistant HIV was first described in 1992 with documentation of reduced susceptibility to ZDV in a subject presenting with primary HIV infection[21]. Since this first report, the sexual transmission of resistant viruses has been well documented and extended to other drugs including lamivudine (3TC), nevirapine, and protease inhibitors[15,22-32]. Moreover, the transmission of multidrug-resistant HIV strains has been reported[33] and is of major concern in terms of public health[34].

Resistances to ZDV and, to a lesser extent, to 3TC are most frequently recognized among naive individuals. In subjects with primary HIV infection or in recent seroconverters, primary resistance reaches rates of 10-20% for ZDV and 5-10% for 3TC in the US and Western Europe (Table 2)[15,22-31]. These drugs have been the most widely used, and show either a low

Table 2. *Rate of primary resistance to antiretroviral drugs in studies performed in recent seroconverters.*

	Yerly[22]	Boden[23]	Brodine[24]	Salomon[25]	Wegner[26]	Briones[31]	Miró[27]	Little[29]	Yerly3[5]	De Mendoza[36]
Country	Switzerland	USA	USA	Canada	USA	Madrid	Barcelona	USA	Switzerland	Madrid
Period	1996-1998	1995-1999	1997-1998	1997-1999	1999	1997-1999	1997-1999	1999-2000	1999	2000-2001
Population	82	80	31	81	114	30	25	108	61	21
Genotypic resistance:										
Any drug	10%	16%	26%	20%	22.1%	26.7%	16%	14%	5%	4.8%
NRTIs	10%	12.5%	10%	18%	4.3%	23.3%	4%	8.2	ND	0%
NNRTIs	2.4%	7.5%	13%	4%	15.8%	3.3%	8%	7.1	ND	0%
PIs	4%	2.5%	16%	7%	9.5%	6.7%	4%	8.2	1.9%	4.8%

NRTIs: nucleoside retrotranscriptase inhibitors
NNRTIs: non nucleoside retrotranscriptase inhibitors
PIs: protease inhibitors
ND: not done

(3TC) or intermediate (ZDV) genetic barrier to resistance. However, recent surveys in Europe have noticed a reduction in the rate of resistance (~5%) among recent seroconverters[35,36]. This unexpected finding seems to reflect the growing number of new infections originating from individuals who have never been exposed to antiretroviral drugs, often because they are not aware of their HIV-positive status. Supporting this hypothesis is the fact that a large proportion of these recent infections came from immigrants from endemic regions and due to non-B viruses[35].

Response to first antiretroviral regimens has been shown to be compromised in subjects carrying primary drug resistance[22,37,38], although the use of drug combinations can preclude its recognition, at least in the short-term[39]. In the ICONA trial[38], a multicentre Italian study, a poor virological response to first-line therapy was correlated with the presence of baseline genotypic drug resistance. In the ACTG 343, an induction maintenance trial, subjects harboring ZDV resistance at baseline tended to suffer viral rebound more frequently at the maintenance phase[40].

Resistance mutations tend to disappear from plasma HIV-RNA if drug pressure is not maintained[41]. In chronically infected subjects failing therapy there is a latent reservoir of wild-type virus (hidden as proviral DNA) that may outgrow mutant virus as soon as drug pressure is removed. The situation may be quite different in recent seroconverters with primary resistance, in whom there may not be any wild-type virus to quickly outgrow mutant virus. In fact, some reports have shown that mutant virus in primary HIV infection may persist for an unusually long time[42]. However, recovery of HIV-specific immune responses may be possible if antiretroviral therapy is initiated during acute infection. Patients presenting with acute infection, and in whom antiretroviral therapy is being initiated, should be considered for resistance testing in order to detect transmission of drug resistance virus and the regimen modified accordingly.

Chronic HIV Infection

Persons infected with HIV for many years, or for an unknown length of time, are less likely to harbor mutant viruses than those recently infected. The current armamentarium of drugs was only introduced onto the market after 1995, and the proportion of patients beginning therapy expanded dramatically only after that time. Persons previously infected were less likely to be exposed to others with antiretroviral drug experience. Moreover, resistance could have been selected only against a few drugs, mainly to ZDV. All these circumstances explain why the rate of primary resistance to antiretroviral drugs is much lower among naive individuals with long-lasting HIV infection than in recent seroconverters. In the Spanish ERASE-1 and -2 studies[43,44], two multicentre trials in which samples collected since 1993 were examined, the prevalence of primary resistance was below 10-15% in naive subjects with prolonged HIV infection. More recently, in the ERASE-3 study[45] conducted in the year 2000, we noticed the same decline in the rate of primary resistance that has been noticed among recent seroconvertors. Therefore, resistance testing seems not to be justified in chronic HIV-infected persons before beginning treatment, in the absence of particular circumstances[8].

Post-exposure prophylaxis (PEP)

Post-exposure prophylaxis (PEP) to prevent HIV infection after occupational exposure to HIV is recommended by the US Public Health Service[46]. The HIV antibody status, antiretroviral treatment history, and plasma HIV-1 RNA level of the source of exposure may assist in constructing a PEP regimen to which the virus is least likely to be resistant[47]. PEP regimens may also be optimized through resistance testing in cases in which a recent sample from the index case is available, although initiation of prophylaxis obviously should not be delayed.

Early virological failure

Both baseline genotyping and phenotyping have been shown to predict the response to salvage therapy in patients experiencing antiretroviral failure[48-53]. At least 8 prospective studies have provided evidence favoring the use of resistance testing for

Table 3. *Virological response to salvage therapy guided by either resistance testing or standard of care in 3 prospective studies.*

Study	Design	Duration (weeks)	No patients (%)	1st PI failure	Change in VL log HIV-RNA	<400 HIV-RNA cop/ml (% of patients)
VIRADAPT[54]	GT vs. SOC	24	108	40	–1.15 vs. –0.67 (p = 0.05)	32 vs. 14* (p = 0.067)
GART[55]	GT vs. SOC‡	12	153	50	–0.94 vs. –0.47 (p = 0.003)	34 vs. 22
VIRA 3001[56]	PT vs. SOC	16	274	100	–1.23 vs. –0.87 (p = 0.004)	45 vs. 34 (p = 0.099)
HAVANNA[57]	GT vs. SOC‡	24	274	23	–1.1 vs. –0.8	57 vs. 42%†
KAISER[59]	PT vs. SOC	16	115	25	–0.25 vs. –0.4 (p = ns)	Not available
NARVAL[58]	GT vs. PT vs. SOC‡	12	541	<30	ND	41 vs. 33 vs. 34* (p = 0.249)
ARGENTA[60]	GT vs. SOC	12 24	174	50	ND	27 vs. 12 (p = 0.02)† 21 vs. 17 (p = ns)†
CCTG 575[61]	PT vs. SOC	24	256	80	–0.71 vs. –0.69 (p = ns)	48 vs. 48†

GT:genotype. PT:phenotype. SOC:standard of care. ND:not done. ns = not significant
*Limit of detection = 200 copies/ml
†Limit of detection = 500 copies/ml
‡Resistance test results included expert interpretation

choosing the best combination of drugs as rescue intervention in persons failing therapy[54-61]. Although, on average, the virological benefit is modest (Table 3), it might have a significant clinical impact. There are important differences between these studies. The NARVAL[58] and the Kaiser[59] trials included patients who were much more pre-treated and no clear advantage of resistance assays in terms of viral load response was observed. By contrast, VIRADAPT[54], GART[55] and VIRA3001[56] demonstrated a benefit in terms of virological outcome: patients whose therapy changes were guided by resistance testing reached better viral load response compared with patients receiving usual care. The HAVANNA[57] trial was an important step forward in our understanding of the relative effects of resistance testing and expert advice. In early prospective trials using genotyping, such as the GART study[55], it was never clear how much of the benefit in virological response was due to resistance testing itself and how much was due to the expert advice that accompanied the results. From the HAVANNA study we know that expert advice and crude HIV genotypes have independent and additional benefits on patient care.

If resistance testing is expected to provide a clear advantage in rescue interventions for patients experiencing failure with their current therapy, it is mainly because it can allow the substitution of only one or two drugs in the failing regimen instead of replacing all the drugs with new ones (Fig. 1). Compounds with low genetic barrier, i.e. lamivudine or nevirapine, are those mainly compromised in early viral rebounds after variable periods of undetectable viremia under therapy[40,62]. It is at this time when a repeatedly confirmed viral rebound can give the opportunity to intensify, if resistance is not recognized[63-65], or to make a selective substitution if just one or two drugs are compromised by resistance (Fig. 1).

The availability of resistance testing at the time of early virological failure permits the design of sequencing therapies with greater chances of success[66]. There are examples for all three classes of antiretrovirals. For nucleosides, failures using ZDV-containing regimens often select viruses with mutations at codon 215, which reduce the response to any rescue intervention using stavudine[48]; conversely, since one third of subjects failing stavudine develop ZDV-like mutations[67,68], a lower response to ZDV-containing regimens is expected in this circumstance. Therefore, alternative nucleosides, such as didanosine, lamivudine or abacavir should be preferred if only a few nucleoside-associated mutations are present. For non-nucleosides, the lack of the K103N mutation seems to allow rescue with efavirenz after failing on nevirapine in a significant proportion of patients[69-71]. Finally, subjects failing on nelfinavir-containing regimens might select initially for one of two different mutations at the protease gene. The D30N mutation reduces the viral fitness and does not affect the susceptibility to other PIs, which therefore can be used as part of the regimens in rescue therapies[72]. In contrast, the L90M mutation, which appears less frequently, produces a high level of cross-resistance to other PIs, which precludes their use as single PIs in salvage interventions[73].

Current methods for genotyping or phenotyping require a minimum level of plasma viremia to provide results, in the range of 1000 HIV-RNA copies/ml[74]. The detection as early as possible of emerging drug-resistant mutant viruses, might provide the opportunity for switching therapy before multiple mutations accumulate. It is well known that further mutations will increase the level of resistance and/or produce cross-resistance to other compounds of the same family[11,75]. Since the current viral-load tests permit the quantification of samples with viral load levels in the range of 20-50 HIV-RNA copies/ml, there is a need for more sensitive resistance tests able to provide results in specimens with low levels of viremia. One limitation, however, is as regards the reliability of the

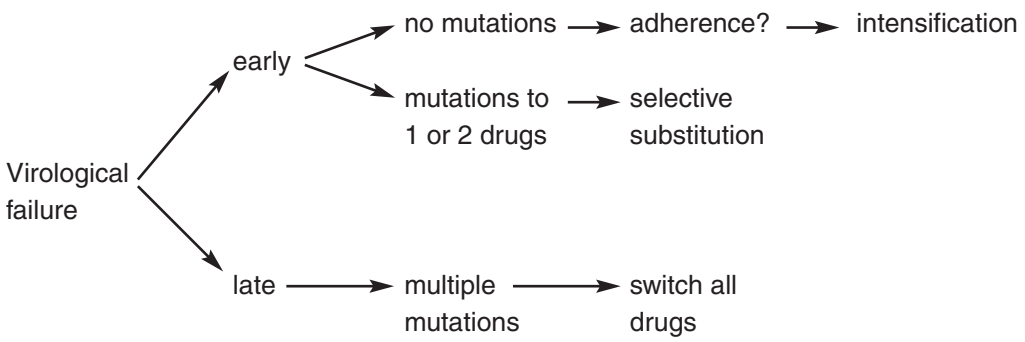

Figure 1. *Rescue interventions as a function of genotyping at the time of virological failure.*

results obtained in these circumstances, since the reproducibility of PCR testing is greatly dependent on the nucleic acid concentration[76]. Moreover, the clinical relevance of resistant mutations in the short- or mid-term in subjects showing undetectable plasma viremia[77] is currently unclear.

Before introducing salvage regimens in multi-treated patients

The management of subjects with virological failure who have been exposed to all drugs available and/or have developed toxicity related to their use, presents limited therapeutic options. In this context, the best care should be focused on the prevention of opportunistic infections. The chances of reaching undetectable viremia in persons with broad exposure to all currently available antiretroviral compounds are very limited[3,78,79]. Multiple-resistance mutations then exist[80,81]. Even in those with low CD4 counts, as long as they remain under therapy, virological failure is seldom associated with the appearance of clinical opportunistic events[82,83]. Therefore, antiretroviral therapy often provides protection against AIDS, even when no complete virus suppression is attainable[82-87]. Resistance testing in this population may, however, have other benefits, for example, eliminating drugs that are unlikely to be beneficial limits unnecessary drug exposure, toxicity, and cost.

In this setting, resistance testing should be considered in a different context. In the past year, several reports have documented that, in heavily pretreated patients, the number of resistance mutations in the RT and/or protease genes have much more importance on predicting the virological success of potent rescue interventions[88]. The recognition that classical AZT mutations can be selected and contribute to causing resistance to d4T, abacavir and ddI[48,67,68,89,90] has lead to redefining these mutations as NAMs (Nucleoside-Associated Mutations). They include a set of 6 changes: 41L, 67N, 70R, 210W, 215Y/F and 219Q/E. Although NAMs arise using almost all nucleoside analogues (except 3TC), the rate at which they appear, and the loss of sensitivity they produce, varies widely for each drug (Table 4)[91]. For PIs, the total number of protease resistance mutations, including primary and secondary or accessory mutations, can predict the response to PI regimens that increase their levels with baby doses of ritonavir[92-95]. A low replicative capacity of escape mutants carrying multiple drug-resistant mutations could partially contribute to explaining this observation[96].

In this context, only drug-related toxicity issues can justify the recommendation of drug holidays[97,98]. However, recent reports have demonstrated that, although primary mutations tend to disappear after cessation of antiretroviral therapy, the persistence of other drug mutations indicates that mutated strains may still replicate efficiently[99,100]. Alternatively, the recycling and concurrent administration of new drugs such as lopinavir/rit or tenofovir[93-95,101,102], or the use of double/triple PI combinations[92,103] could be an option in those patients with multiple drug-resistance genotypes.

Table 4. *Resistance to nucleoside analogues caused by NAMs.*

Genotype	abacavir	ddI	d4T
3 NAMs*	no	no	no
+ 44	no	no	yes
+ 44 + 118	no	no	yes
+ 44 + 118 +184	yes	yes	yes
+ 118 + 69	no	yes	yes
+ 184	yes	no	no
+ 184 + 74	yes	yes	no

*Including at least 41L and/or 215Y/F

Table 5. *Main pharmacokinetic parameters of protease inhibitors and non-nucleoside reverse transcriptase inhibitors.*

			PI		
DRUG	**DOSES (mg/h)**	**C_{min} (mg/ml)**	**Protein Binding (%)**	**EC_{95}**	**C_{min}/EC_{95}**
RITONAVIR	600/12h	3.3	99	1.5	2.2
SAQUINAVIR HGC	600/8h	0.02	>98	0.3	0.06
RTV	400/400/12h	0.5			1.6
SAQUINAVIR SGC	1200/8h	0.25			0.833
RTV	1000/100/24h	0.9			3
INDINAVIR	800/8h	0.15	60	0.05	3.1
RTV	800/100/12h	0.99			20.6
NELFINAVIR	1250/12h	2.2-0.7	>98	0.66	3.3-1.2
LOPINAVIR (ABT/RTV)	400/100 12h	0.6-3.1	98	0.007	8-44
			NNRTI		
NEVIRAPINE	200/12h	3.7	60	0.066	56
EFAVIRENZ	600/24h	1.8	>99.5	0.136	13.3

Additional considerations for an appropriate use of resistance testing

The technology available for measuring drug resistance is still complex, and reproducibility and interpretation of results is far from consistent in different labs, as was shown in the ENVA studies[104,105]. The performance of the current assays when testing non-B subtypes should be explored in detail, since the proportion of new infections caused by these viral variants is increasing in Europe[35,106-108] and, to a lesser extent, in the USA[109]. Cost-effectiveness analyses need to be performed in order to prove the benefit of adding the resistance information to current parameters guiding antiretroviral treatment[110,111]. Drug levels and IC_{90} values must be integrated in new algorithms designed to yield a more reliable interpretation of genotypes and phenotypes[112]. For example, Table 5 records the main pharmacokinetic parameters and their relation to virus sensitivity for currently available drugs. Of note, trough plasma levels and 95% inhibitory concentrations corrected by protein binding (efficient concentrations) need to be assessed for each drug. The appropriate use of this information will help to design more accurately the therapeutic regimen for each patient. Lastly, the search for new genotypes accounting for resistant phenotypes must be pursued, and large databases pairing geno-phenos must be built and offered to all clinicians in a wide fashion[113,114].

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