AIDS Rev. 2012;14:247-55

Interpretation of Resistance Data from Randomized Trials of First-Line Antiretroviral Treatment

Bonaventura Clotet[1], Andrew Hill[2], Yvon van Delft[3], Ravindra Kumar Gupta[4] and Christiane Moecklinghoff[5]
[1]Germans Trias i Pujol Hospital, Barcelona, Spain; [2]MetaVirology Ltd, London, UK; [3]Janssen, Tilburg, The Netherlands; [4]University College Medical School, London, UK; [5]Janssen, Neuss, Germany

Abstract

There are four key differences between HIV clinical trials in the analysis of HIV drug resistance: (i) baseline resistance testing used versus not used for patient inclusion; (ii) using HIV RNA cutoff levels of ≥ 50 versus ≥ 400 copies/ml to define virologic failure; (iii) testing versus not testing drug resistance in patients who discontinue treatment; (iv) analyzing drug resistance based on intent-to-treat analysis versus the subset of patients with samples genotyped.
In this review we illustrate the importance of these issues, using data from 17 clinical trials of first-line nonnucleoside reverse transcriptase inhibitor-based treatment reported in the past 10 years. We also analyzed the data from the efavirenz arm of the SENSE trial, using all the different methods to show the range of results that can be obtained using different methods of analysis.
Detection of treatment-emergent nucleoside/nonnucleoside reverse transcriptase inhibitor resistance differs significantly between clinical trials of the same first-line treatment (two nucleoside reverse transcriptase inhibitors/efavirenz), depending on the methods used for testing and analysis. Several clinical trials may have underestimated the prevalence of treatment-emergent drug resistance, by (i) not testing virologic failures with HIV RNA 50-400 copies/ml or (ii) not testing patients after discontinuation of treatment. **(AIDS Rev. 2012;14:247-55)**
Corresponding author: Andrew Hill, microhaart@aol.com

Key words

HIV drug resistance. Genotyping. Nucleoside analogues. Non nucleosides. Protease inhibitors. HIV RNA.

Introduction

There is a need for antiretroviral treatments with a low risk of treatment-emergent drug resistance to maximize the durability of HIV RNA suppression and to preserve future treatment options. Most randomized clinical trials are statistically powered with the end-point of HIV RNA suppression[1,2]; analysis of HIV drug resistance is a secondary objective. Lower risks of drug resistance have been shown for some treatments within trials: for example, lopinavir/ritonavir versus efavirenz in the ACTG 5142 trial[3], lopinavir/ritonavir versus nelfinavir in the Abbott 613 trial[4], and tenofovir/emtricitabine versus zidovudine/lamivudine in the Gilead 934 trial[5]. In addition, there have been systematic reviews and cohort study analyses, assessing differences in the risk of treatment-emergent HIV drug resistance over time between treatment classes or individual antiretrovirals[6-8]. However, when comparing resistance data between HIV clinical trials or within cohorts, it is important to understand any differences between these studies in the methods of resistance testing and analysis.

There is a complex sequence of events which could lead to a patient being tested for drug resistance during an HIV clinical trial. In more recently conducted trials, all patients are tested for HIV drug resistance at screening, and patients harboring virus conferring

Correspondence to:
Andrew Hill
MetaVirology Ltd
35 Endlesham Road
London SW12 8JX, UK
E-mail: microhaart@aol.com

resistance to study drugs are generally excluded (screen failures). Patients with drug-sensitive virus are then randomized to treatment.

Patients could then achieve full HIV RNA suppression, in which case their samples are not genotyped again. Patients can then fail treatment in different ways. Samples from patients with confirmed rebound in HIV RNA > 400-1000 copies/ml, or who fail to suppress HIV RNA below these levels, are typically genotyped in HIV clinical trials. However, samples from patients with virologic rebound in the range of 50-400 copies/ml are not genotyped in many trials, particularly if these elevations are observed only on single visits. In addition, patients who discontinue trial medication with detectable HIV RNA levels, but no confirmed virologic failure, may not have samples genotyped in all trials.

For the analysis of drug resistance, there is a subset of samples from patients with virologic failure, which are then genotyped. This may not be successful in all cases: there is the "genotyped population", including all patient samples with genotypic data at the time of virologic failure. This is then compared to genotypic data at the screening or baseline visit, to determine whether there are new "treatment-emergent" mutations, which were not present at the baseline visit.

The results from this review of drug resistance were obtained from a detailed MEDLINE search for clinical trials of first-line treatment with nucleoside/nonnucleoside reverse transcriptase inhibitor (NRTI/NNRTI) combinations for at least 48 weeks. Clinical trials needed to include at least 75 patients per treatment arm and have resistance data available at week 48 or later. A list of the 17 clinical trials is shown in table 1; only the NRTI/NNRTI arms of the trials are shown. This table also includes information on the year of publication, the number of patients randomized to each treatment arm, whether drug resistance was an inclusion criterion, and the HIV RNA cutoff level used to select patients for resistance testing during the trial. There are 17 clinical trials included in this review, which randomized a total of 9,789 patients. The most widely used NNRTI was efavirenz (n = 7,031), with nevirapine (n = 1,994), etravirine (n = 78), and rilpivirine (n = 686) also evaluated. The NRTI backbones used were either tenofovir/emtricitabine, abacavir/lamivudine, zidovudine/lamivudine, or stavudine/lamivudine. Some trials allowed investigator-selected nucleoside analogues (for example, the ACTG 5142[3] or SENSE[9] trials).

Testing for HIV drug resistance in treatment-naive patients

International HIV treatment guidelines currently recommend that all treatment-naive patients should be tested for drug resistance[10-12], but this advice was only included in the past five years. As shown in table 1, antiretroviral treatment-naive patients were not tested for drug resistance at baseline in studies published before 2009; most of these trials were designed and conducted between 2000 and 2005, before resistance testing was widely introduced for treatment-naive patients. In the ACTG 5142 trial, resistance testing was performed for patients with recent HIV infection[3].

More detailed analyses of some of these earlier trials have shown higher rates of virologic failure for patients with drug resistance at baseline. Among efavirenz-treated patients in the ACTG 5095 trial[13], the risk of virologic failure was 2.27-times more likely for patients who had NNRTI resistance at baseline ($p = 0.018$)[14]. The same effect was seen in the Gilead 934 trial of tenofovir/emtricitabine/efavirenz or zidovudine/lamivudine/efavirenz[15]. In these trials, baseline drug resistance was tested on stored samples to determine which mutations detected during the trial were preexisting versus treatment-emergent. Patients who have resistance to one antiretroviral in the selected combination treatment at baseline could then develop new mutations to other antiretrovirals at the time of virologic failure.

All the trials published after 2008 have included drug resistance testing at screening, with only drug-sensitive patients being enrolled. The lists of mutations used to exclude patients may differ between studies, but patients with mutations included in the World Health Organization list[16] of transmitted drug resistance (e.g. K103N, M184V) would be excluded from almost all trials. However, some clinical trials conducted in sub-Saharan Africa do not routinely assess drug resistance before randomization[17]. This is generally in line with local clinical practice, where resistance testing is rarely, if ever, performed.

Standard genotyping assays can detect drug resistance mutations if present at high prevalence in patient samples. However, more sensitive, mutation-specific minority assays can detect drug resistance if present in a small percentage of viruses from a patient sample[18]. A recent systematic review has shown that the presence of these low-frequency drug resistance mutations at baseline can lower the efficacy of first-line

Table 1. Methods of HIV drug resistance testing in clinical trials of first-line nonnucleoside reverse transcriptase inhibitor-based antiretroviral treatment

Trial	Year	Study drugs (n)	Baseline genotyping	HIV RNA cutoff for genotyping
Group 1: No baseline resistance testing, HIV RNA > 400 copy cutoff for genotyping				
ACTG5095[13]	2004	ZDV/3TC/EFV (765)	No	2 x > 500 (subset)
2NN[30]	2004	d4T/3TC/EFV (400) d4T/3TC/NVP (607)	No	> 1000 (subset)
CNA3024[31]	2004	ZDV/3TC/EFV (325) ABC/3TC/EFV (324)	No	2 x > 400
Gilead 903[32]	2004	TDF/3TC/EFV (299) d4T/3TC/EFV (301)	No	2 x > 400
EPV2001[33]	2004	ZDV/3TC/EFV (554)	No	2 x > 400
CNA3021[34]	2005	ABC/3TC/EFV (764)	No	2 x > 400
Gilead 934[5]	2006	TDF/FTC/EFV (244) ZDV/3TC/EFV (243)	No	2 x > 400
Group 2: Baseline resistance testing, HIV RNA > 400 copy cutoff for genotyping				
ACTG 5142[3]	2008	2NRTI/EFV (250)	Part	2 x > 500
STARTMRK[35]	2009	TDF/FTC/EFV (282)	Yes	2 x > 400
ALTAIR[36]	2010	TDF/FTC/EFV (114)	Yes	2 x > 400
ASSERT[37]	2010	TDF/FTC/EFV (193) ABC/3TC/EFV (192)	Yes	2 x > 400
MERIT[27]	2010	ZDV/3TC/EFV (361)	Yes	2 x > 500
ACTG 5202[38]	2011	TDF/FTC/EFV (464) ABC/3TC/EFV (465)	Yes	2 x > 200
Group 3: Baseline resistance testing, HIV RNA > 50 copy cutoff for genotyping				
ARTEN[21]	2011	TDF/FTC/NVP (376)	Yes	2 x > 50
VERXVE[20]	2011	TDF/FTC/NVP (506) TDF/FTC/NVPx (505)	Yes	2 x > 50
ECHO/THRIVE[25]	2011	2NRTI/EFV (682) 2NRTI/RPV (686)	Yes	2 x > 50
SENSE[9]	2011	2NRTI/EFV (78) 2NRTI/ETR (79)	Yes	1 x > 50

3TC: lamivudine; ABC: abacavir; d4T: stavudine; EFV: efavirenz; ETR: etravirine; FTC: emtricitabine; NVP: nevirapine; RPV: rilpivirine; ZDV: zidovudine; NRTI: nucleoside reverse transcriptase inhibitor.

NNRTI-based treatment[19]. It is not clear whether there is the same correlation between low-frequency drug resistance at baseline and the efficacy of other antiretroviral drug classes, for example protease inhibitors or integrase inhibitors. The correlation between low-frequency drug resistance and response should be investigated as part of clinical research for new antiretrovirals. However, it may be too complex and expensive to use these assays in routine clinical practice.

HIV RNA cutoff levels for virologic failure and resistance testing

Virologic failure is normally defined as either a rebound in HIV RNA above a threshold level after earlier suppression, failure to reduce the HIV RNA level below the threshold by the end of the trial, or discontinuation for virologic reasons[1]. Table 1 shows the cutoff levels used to define virologic failure and subsequent testing

Table 2. Key recommendations for HIV resistance testing in clinical trials

Issue	Recommendation
Population for analysis	Analyze both the intent-to-treat population and the subset of genotyped patients.
HIV RNA cut-off level	Test all patient samples with HIV RNA > 50 copies/ml after week 24 (rebound or failure to suppress).
Early stage testing	Risk of resistance could be evaluated in clinical trials during initial virologic suppression (i.e. weeks 4-12).
Discontinuations	Genotype sequential patient samples after discontinuation of treatment, while HIV RNA is detectable. Follow-up until re-suppression of HIV RNA on subsequent treatments.

for drug resistance. In earlier studies, patients were only tested for drug resistance if they showed virologic failure with two consecutive HIV RNA levels of at least 400-1000 copies/ml.

In more recent studies[9,20-22], patients have been tested for drug resistance after showing rebound in HIV RNA > 50 copies/ml or failure to suppress below this level (Table 1). The move to testing any patient with HIV RNA > 50 copies/ml, regardless of whether they remain in the study, has increased the number of virologic failures tested for drug resistance. The success of genotypic resistance tests is lower for patients with HIV RNA levels in the range of 50-400 copies/ml, compared to those with HIV RNA > 400 copies/ml[23]. Even so, when amplification of HIV RNA is achieved, drug resistance can be detected in patients with low HIV RNA levels. In the ECHO/THRIVE and SENSE trials, NRTI and NNRTI resistance was detected in patients in the efavirenz and rilpivirine arms with HIV RNA levels of 50-400 copies/ml[9,20].

Antiretroviral treatment and drug resistance testing guidelines indicate a plasma HIV-1 RNA load level of 500-1000 copies/ml as the recommended threshold for drug resistance testing, defined by the detection limits of commercial assays and early clinical experience. However, several laboratories have improved the performance of their resistance testing protocols, thereby increasing the success of amplification and sequencing at viral load levels < 1000 copies/ml[23]. In a large European study of 16,511 genotypic results from treatment experienced patients, 15% were obtained from samples with an HIV RNA level below 1000 copies/ml. The percentage of samples showing resistance to nucleoside analogues rose from 40% for samples with HIV RNA levels of 50 copies/ml, to 86% for samples with HIV RNA levels between 1000 and 10,000 copies/ml[24].

Has the prevalence of drug resistance been underestimated in older clinical trials, which have not evaluated the patients with HIV RNA between 50-1000 copies/ml at virologic failure? This question was addressed in the ARTEMIS trial of first-line boosted protease inhibitors – either lopinavir/ritonavir or darunavir/ritonavir, used with tenofovir/emtricitabine[25]. The first 48-week analysis of drug resistance only included patients with virologic failure > 1000 copies/ml. The analysis was repeated on stored samples, using a cutoff level of 50 copies/ml for virologic failure. There were five additional patients who developed new protease inhibitor or NRTI mutations and had HIV RNA levels < 1000 copies/ml at the time of virologic failure[25]. Similarly, in the MONET trial of darunavir/ritonavir with or without nucleoside analogues, the only genotypic protease inhibitor drug resistance detected was in two patients with HIV RNA levels of 50 and 63 copies/ml, respectively[26]; use of the 400 copy limit as a threshold to perform resistance testing in this trial would not have shown any drug resistance in the MONET trial.

If virologic failure is defined in most HIV trial protocols as increases in HIV RNA > 50 copies/ml, and resistance can be reliably measured in samples with HIV RNA at low levels, then clinical trials should routinely measure for resistance when patients show virologic failure in the range of 50-400 copies/ml. A summary of recommendations for HIV resistance testing in clinical trials is shown in table 2.

It is rare to test for HIV drug resistance in patients whose levels are still falling towards the lower limit of assay quantification. In the SENSE trial protocol[9], there was a planned test for drug resistance in all patients whose HIV RNA level was > 500 copies/ml at week 12. There was one patient in the etravirine arm with an HIV RNA level of 501 copies/ml at week 12, who showed one new IAS-USA NNRTI mutation at this visit, but then had full HIV RNA suppression < 50 copies/ml from the next visit (week 24) to the end of the trial. This issue could be investigated further if a larger number of patients could be tested for drug resistance early in a clinical trial before full HIV RNA suppression. However,

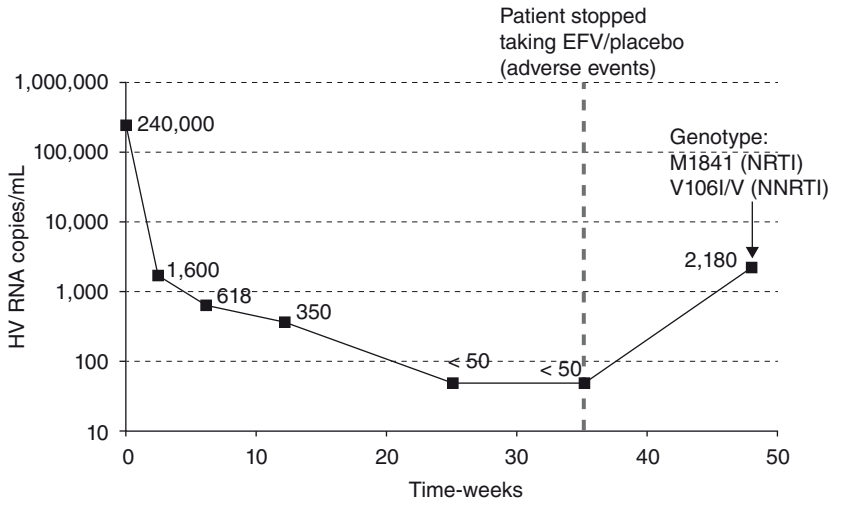


Figure 1. *Patient in the SENSE Trial with HIV RNA rebound after drug discontinuation.*

the clinical implications of these findings remain unclear, given that this patient successfully suppressed HIV RNA at the end of the study.

The newest HIV RNA PCR assays, such as Roche TaqMan® and Abbott RealTime®, have lower detection limits < 50 copies/ml; recent research suggests that patients with viremia detectable but at levels < 50 copies/ml could still show an increased risk for virologic rebound[24]. However, more research is needed to determine whether viremia at this low level is also associated with drug resistance across the different classes of antiretrovirals.

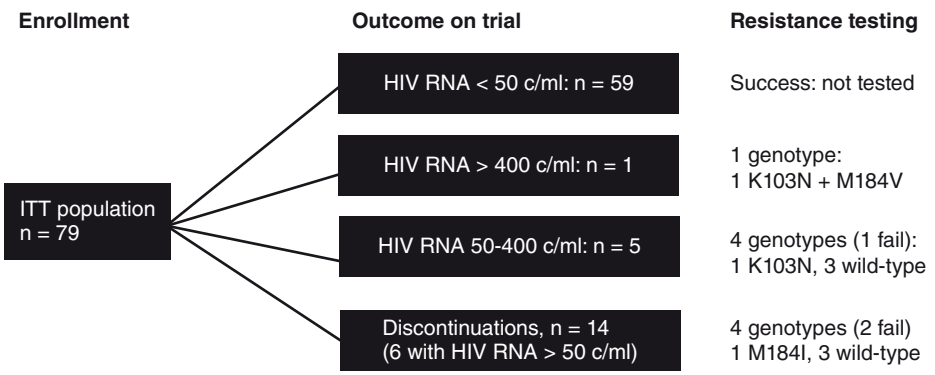
Resistance testing in patients who discontinue trial medication

In earlier studies, patients were only tested for HIV drug resistance at the time of virologic failure. There has been a recent trend in some trials to test all patients who discontinue randomized trial medication with detectable HIV RNA levels, including follow-up visits after drug discontinuation. Some antiretrovirals have long terminal elimination half-lives, and there may be detectable drug levels, for example of the NNRTI efavirenz several weeks after the drug is discontinued; these levels could lead to the development of drug resistance.

Figure 1 shows an example from the SENSE trial[9]. The patient was treated with tenofovir/emtricitabine/efavirenz and showed reductions in HIV RNA from 240,000 copies/ml at baseline to < 50 copies/ml at week 36. The patient then discontinued efavirenz owing to neuropsychiatric adverse events, but continued to take tenofovir and emtricitabine (protocol violator). The patient was classified as a discontinuation, but was still followed up until the end of the study. A sample genotyped at week 48 showed the M184I mutation (lamivudine resistance) and the V106I/V mutation (limited NNRTI resistance). There was a similar effect in the MERIT trial[27]: five patients in the zidovudine/lamivudine/efavirenz group who discontinued therapy because of adverse events developed new efavirenz resistance mutations during follow-up, whereas there was no evidence of drug resistance after follow-up of patients who discontinued in the maraviroc arm[27].

Most trial protocols define treatment failure as HIV RNA > 50 copies/ml on two consecutive visits. If a clinical trial protocol only allows genotyping of patients with HIV RNA levels > 400 copies/ml, then a patient could show protocol-defined failure with HIV RNA in the range of 50-400 copies/ml, discontinue from the trial and never be genotyped.

Patients who remain viremic while on treatment have an increasing risk of drug resistance[28,29]. It is therefore important to continue genotyping patients from the time of their first recorded rebound in HIV RNA until the time that they achieve resuppression on subsequent treatments (Table 2).



Intent to treat analysis: includes all randomized patients
Genotypes analysis: includes only the patients with successful genotypes

Percentage of patients with treatment-emergent primary IAS-USA NRTI or NNRTI resistance mutations in the SENSE trial

Method	Genotypes analysis	ITT analysis
Only include VF > 400 copies/ml	1/1 = 100%	1/79 = 1.3%
Include all VF > 50 copies/ml, not discontinuations	2/5 = 40%	2/79 = 2.5%
All VF > 50 copies/ml and discontinuations	3/9 = 33%	3/79 = 4.0%

ITT: intent-to-treat; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; VF: virologic failure; EFV: efavirenz;

Figure 2. *Patients genotyped up to week 48 in the SENSE trial (two nucleoside reverse transcriptase inhibitors + efavirenz arm).*

Methods of analysis of treatment-emergent drug resistance

Efficacy in clinical trials is normally analyzed using a time to loss of virologic response (TLOVR) or similar algorithm[1]. This method classifies patients as treatment failures if they either have virologic failure, or discontinue randomized treatment for adverse events or other reasons. The number of patients with virologic failure may therefore be a small minority of overall treatment failure. In addition, the percentage of patients with virologic failure who have samples successfully genotyped may not be 100%, owing to the success rates of genotypic assays.

The prevalence of treatment-emergent drug resistance has been calculated either as a percentage of all patients randomized to a given treatment (we will call this the "intent-to-treat [ITT] analysis"), or as a percentage of the patients who were genotyped (we will call this "genotypes analysis"). In more recent studies, the genotypes analysis has been most widely reported, but the results need to be judged in combination with the overall ITT analysis.

The SENSE trial

In the example of the SENSE Trial shown in figure 2, 78 patients were randomized and treated with two nucleoside analogues plus efavirenz[9]. At week 48 there were 20/78 patients with treatment failure by the TLOVR algorithm. Of these 20 patients, six had confirmed virologic failure and were genotyped. Five patients had HIV RNA in the range of 50-400 copies/ml and one had HIV RNA sustained > 400 copies/ml at the time of virologic failure. Fourteen of the 20 patients discontinued treatment for adverse events of other reasons, of whom six had HIV RNA > 50 copies/ml and were genotyped. Of the 12 patients overall with samples genotyped, there were three failures to amplify, leaving nine successful genotypic test results. One patient with virologic failure in the range of 50-400 copies/ml had a sample showing the K103N mutation, conferring resistance to efavirenz.

Table 3. HIV drug resistance testing at week 48 in clinical trials of first-line nonnucleoside reverse transcriptase inhibitor-based antiretroviral treatment

Trial	Study drugs (n - ITT)	Number of patients with		
		Genotype/ Treatment failures (%)	M184I/V ITT (%)	Genotypes (%)
Group 1: No baseline resistance testing, HIV RNA > 400 copy cutoff for genotyping				
CNA3024[31]	ZDV/3TC/EFV (325) ABC/3TC/EFV (324)	6/101 (6%) 10/98 (10%)	4/325 (0.6%) 2/324 (0.6%)	4/6 (67%) 2/10 (20%)
EPV 20001[33]	ZDV/3TC/EFV (554)	44/202 (22%)	14/554 (2.5%)	14/44 (32%)
CNA3021[34]	ABC/3TC/EFV (764)	31/250 (12%)	15/764 (2.0%)	15/31 (48%)
Gilead 934[5]	TDF/FTC/EFV (244) ZDV/3TC/EFV (243)	12/50 (24%) 22/72 (31%)	2/244 (0.8%) 7/243 (2.9%)	2/12 (17%) 7/22 (32%)
Group 2: Baseline resistance testing, HIV RNA > 400 copy cutoff for genotyping				
STARTMRK[35]	TDF/FTC/EFV (282)	5/52 (10%)	0/282 (0%)	0/5 (0%)
ALTAIR[36]	TDF/FTC/EFV (114)	3/17 (18%)	1/114 (0.9%)	1/3 (33%)
ASSERT[37]	TDF/FTC/EFV (93) ABC/3TC/EFV (192)	2/56 (4%) 4/78 (5%)	0/193 (0%) 0/192 (0%)	0/2 (0%) 0/4 (0%)
MERIT[27]	ZDV/3TC/EFV (361)	13/111 (11%)	4/361 (1.1%)	4/13 (31%)
Group 3: Baseline resistance testing, HIV RNA > 50 copy cutoff for genotyping				
ECHO/THRIVE[22]	2NRTI/EFV (682)	28/121 (23%)	7/682 (1.0%)	7/28 (25%)
SENSE[9]	2NRTI/EFV (78)	9/20 (45%)	2/78 (2.6%)	2/9 (22%)

3TC: lamivudine; ABC: abacavir; EFV: efavirenz; FTC: emtricitabine; ZDV: zidovudine; NRTI: nucleoside reverse transcriptase inhibitor.

One patient with virologic failure > 400 copies/ml had a sample with the K103N mutation and M184V (lamivudine resistance). Finally, one patient of the six who discontinued treatment had a sample with the M184I mutation (lamivudine resistance).

If the analysis is conducted including all the above patients with samples tested for genotypic resistance, the prevalence of NRTI or NNRTI resistance is 3/78 randomized patients (3.8%, ITT analysis) or 3/9 successfully genotyped patients (33%, genotypes analysis). However, these results would look different if analyzed according to the methods in other trials. For example, if only the samples from patients with confirmed HIV RNA > 400 copies/ml had been genotyped, only one of the three patients with resistance would have been identified, so the prevalence of resistance would fall to 1/78 (1.3%, ITT analysis). If patients with discontinuation were not tested for resistance, one of the patients with treatment-emergent resistance would have been missed.

Systematic review of first-line nonnucleoside reverse transcriptase inhibitor trials

Table 3 shows a summary of results for other first-line trials of NNRTI-based treatment. For each clinical trial, the table shows the number of patients who were randomized, had treatment failure by a TLOVR analysis or switch equals failure type algorithm, had samples genotyped and showed the M184I/V mutation during the trial. The trials are divided into three categories, according to the use of resistance testing at screening and the HIV RNA level used to define virologic failure and subsequent resistance testing.

The percentage of patients with treatment failure who had samples genotyped differed between the groups: the median in Group 1 was 18%, in Group 2, 10% and in Group 3, 45%. The percentage of failing patients who were genotyped showed a statistically significant difference between the groups ($p < 0.001$). In Group 3, where patients with samples also tested for resistance when

HIV RNA levels were in the range of 50-400 copies/ml, the percentage of treatment failures who had samples genotyped is highest. In the SENSE trial (in Group 3), all patient samples with any HIV RNA rebound > 50 copies/ml were genotyped; in other trials, patient samples were only genotyped if there were two consecutive HIV RNA elevations.

Table 3 shows the prevalence of the M184I/V mutation for each treatment arm of the trials of 2NRTI/efavirenz treatment in Groups 1-3, using either an ITT or genotypes approach. The prevalence of the M184I/V mutation at virologic failure for 2NRTI/efavirenz was 1.8% in Group 1, 0.4% in Group 2, and 1.4% in Group 3 (all ITT analysis), with a significant difference between Group 1 versus 2 ($p = 0.004$). The difference between Groups 1 and 2 may have been driven by the lack of resistance testing at baseline in Group 1; the two groups had the same cutoff level for HIV RNA, > 400-500 copies/ml, for resistance testing. There was a trend for the prevalence of resistance to rise again in Group 3. The clinical trials in this group used a cutoff level for HIV RNA of ≥ 50 copy cutoff for resistance testing; this may have led to the detection of more drug resistance at treatment failure. Also in the SENSE and ECHO/THRIVE trials, the last stored samples were genotyped from all patients who discontinued from the trials[9,20]; in other trials, samples were not always genotyped when patients discontinued treatment with detectable HIV RNA.

Conclusions

Clinical trials should be analyzed using both an ITT and genotypes approach. The ITT analysis shows the absolute risk of developing drug resistance during the trial, including all patients starting randomized treatment. The genotypes analysis shows the prevalence of drug resistance among samples from patients who are tested.

All trial protocols of new or investigational antiretrovirals should include genotypic resistance testing of samples from patients who have confirmed virologic failure ≥ 50 copies/ml, or the quantification limit of the assay used. Previous studies using the 400 copy limit may have underestimated the prevalence of treatment-emergent drug resistance. The prevalence of resistance in patients with low-level viremia should guide decisions on routine genotyping of these patients in clinical practice; if the risk of resistance is low in clinical trials of a new antiretroviral, it may not be cost-effective to evaluate resistance in all cases of viremia in routine practice.

Testing for drug resistance has not generally been performed in HIV clinical trials for patients in the early stages of HIV RNA suppression (for example after 12 weeks of treatment). Testing of a subset of patients may show whether drug resistance emerges during initial HIV RNA suppression, as opposed to at the time of virologic rebound.

Wherever possible, patients who discontinue randomized medication should be followed up to assess long-term risks of developing drug resistance, especially when taking drug classes such as NNRTI, with longer half-lives.

Several clinical trials may have underestimated the risk of treatment-emergent drug resistance by (i) not genotyping samples from patients with HIV RNA 50-400 copies/ml at the time of virologic failure, and (ii) not genotyping samples from patients who discontinued trial medication.

Funding source

This manuscript was produced with support from Janssen, which developed the antiretrovirals darunavir, etravirine, and rilpivirine.

Disclosure statement

Bonaventura Clotet has received consulting fees and speaker fees from Janssen, Roche, ViiV, Gilead, Abbott and Boehringer-Ingelheim. Andrew Hill has received consultancy payments from Janssen. Yvon van Delft and Christiane Moecklinghoff are employees of Janssen. Ravi Gupta has no conflicts of interest.

References

1. US Department of Health and Human Services, Food and Drug Administration and Center for Drug Evaluation and Research. Guidance for industry: antiretroviral drugs using plasma HIV RNA measurements–clinical considerations for accelerated and traditional approval. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070968.pdf
2. Hill A, Sabin S. Designing and interpreting HIV non-inferiority trials in naïve and experienced patients. AIDS. 2008;22:913-21.
3. Riddler S, Haubrich R, DiRienzo A, et al. ACTG Study 5142 Team. Class-sparing regimens for initial treatment of HIV-1 infection. N Engl J Med. 2008;358:2095-106.
4. Walmsley S, Bernstein B, King M, et al. Lopinavir-ritonavir versus nelfinavir-ritonavir for the initial treatment of HIV-1 infection. N Engl J Med. 2002;346:2039-46.
5. Gallant J, DeJesus E, Arribas J, et al. Tenofovir DF, emtricitabine and efavirenz vs zidovudine, lamivudine and efavirenz for HIV. N Engl J Med. 2006;354:251-60.
6. Gupta R, Hill A, Sawyer A, et al. Emergence of drug resistance in HIV type 1-infected patients after receipt of first-line highly active antiretroviral therapy: a systematic review of clinical trials. Clin Infect Dis. 2008;47:712-22.
7. Von Wyl V, Yerly S, Boni S, et al. Emergence of HIV-1 drug resistance in previously untreated patients initiating combination antiretroviral therapy. A comparison of different regimen types. Arch Intern Med. 2007;167:1782-90.

8. The UK Collaborative Group on HIV Drug Resistance and UK CHIC Study Group. Long term probability of detection of HIV-1 drug resistance after starting antiretroviral therapy in routine clinical practice. AIDS. 2005;19:487-94.
9. Gazzard B, Duvivier C, Zagler C, et al. Phase 2 double-blind, randomized trial of etravirine versus efavirenz in treatment-naïve patients: 48-week results. AIDS. 2011;25:2249-58.
10. Thompson M, Aberg J, Cahn P, et al. Antiretroviral treatment of Adult HIV infection. 2010 recommendations of the International AIDS Society-USA panel. JAMA. 2010;304:321-33.
11. US Department for Health and Social Security. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents, January 10th 2011. http://www.aidsinfo.nih.gov/Guidelines/GuidelineDetail.aspx?GuidelineID=7 (Accessed 17th October 2011).
12. European AIDS Clinical Society (EACS). Guidelines for the clinical management of HIV infected adults in Europe. http://www.europeanaidsclinicalsociety.org/index.php?option=com_content&view=article&id=59&Itemid=41 (Accessed 17th October 2011).
13. Gulick R, Ribaudo H, Shikuma C, et al. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. N Engl J Med. 2004;350:1850-61.
14. Kuritzkes D, Lalama C, Ribaudo H, et al. Preexisting resistance to non-nucleoside reverse-transcriptase inhibitors predicts virologic failure of an efavirenz-based regimen in treatment naive HIV-1 infected subjects. J Infect Dis. 2008;197:867-70.
15. Margot N, Enejosa J, Cheng A, et al. Development of HIV-1 drug resistance through 144 weeks in antiretroviral-naive subjects on emtricitabine, tenofovir disoproxil fumarate, and efavirenz compared with lamivudine/zidovudine and efavirenz in study GS-01-934. J Aqcuir Immune Defic Syndr. 2009;52:209-21.
16. Bennett D, Kiuchi M, Rhee S, et al. The World Health Organization 2008 list of mutations for surveillance of transmitted drug resistant HIV strains. 3rd International Workshop on HIV Transmission: Principles of Intervention. 2008, Mexico City. [Abstract 38].
17. Clumeck N, Mwamba C, Kabeya K, et al. The Lubumbashi trial: Week 48 data: comparison between lopinavir/r (LPV/r) versus nevirapine (NVP) based regimens in treatment-naïve patients in a developing country. 13th European AIDS Conference 2011. Belgrade, Serbia [Abstract PS1/3].
18. Buckton A, Prabhu D, Motamed C, et al. Increased detection of the HIV-1 reverse transcriptase M184V mutation using mutation-specific minority assays in a UK surveillance study suggests evidence of unrecognized transmitted drug resistance. HIV Med. 2011;12:250-4.
19. Li J, Paredes R, Ribaudo H, et al. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure. JAMA. 2011;305:1327-35.
20. Gathe J, Andrade-Villanueva J, Santiago S, et al. Efficacy and safety of nevirapine extended-release once daily versus nevirapine immediate release twice-daily in treatment naïve HIV-1 infected patients. Antivir Ther. 2011;16:759-69.
21. Soriano V, Arasteh K, Migrone H, et al. Nevirapine versus atazanavir/ritonavir each combined with tenofovir disoproxil fumarate/emtricitabine, in antiretroviral-naive HIV-1 patients: the ARTEN trial. Antivir Ther. 2011;16:339-48.
22. Rimsky L, Eron J, Clotet C, et al. Characterisation of the resistance profile of TMC278: 48 week analysis of the Phase III studies ECHO and THRIVE. ICAAC 2010. Boston, USA. [Abstract H-1810].
23. Mackie N, Phillips A, Kaye S, et al. Antiretroviral drug resistance in HIV-1-infected patients with low-level viraemia. J Infect Dis. 2010;201:1303-7.
24. Prosperi M, Mackie N, di Giambenedetto S, et al. Detection of drug resistance mutations at low plasma HIV-1 RNA load in a European multicentre cohort study. J Antimicrob Chemother. 2011;66:1886-96.
25. Lathouwers E, DeMeyer S, Dierynck I, et al. Virological characterization of patients failing darunavir/ritonavir or lopinavir/ritonavir treatment in the ARTEMIS study: 96-week analysis. Antivir Ther. 2011;16:99-108.
26. Clumeck N, Rieger A, Banhegyi D, et al. 96 week results from the MONET trial: a randomized comparison of darunavir/ritonavir with versus without nucleoside analogues, for patients with HIV RNA<50 copies/mL at baseline. J Antimicrob Chemother. 2011;6:1878-85.
27. Cooper D, Heera J, Goodrich J, et al. Maraviroc versus efavirenz, both in combination with zidovudine-lamivudine, for the treatment of antiretroviral-naïve subjects with CCR% tropic HIV-1 infection. J Infect Dis. 2010;201:803-13.
28. Cozzi-Lepri A, Paredes R, Phillips A, et al. The rate of accumulation of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance in patients kept on a virologically failing regimen containing an NNRTI. HIV Med. 2012;13:62-72.
29. Taiwo B, Gallien S, Aga E, et al. Antiretroviral drug resistance in HIV-1-infected patients experiencing persistent low-level viremia during first-line therapy. J Inf Dis. 2011;204:515-20.
30. van Leth F, Phanuphak P, Ruxrungtham K, et al. Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. Lancet. 2004;363:1253-63.
31. DeJesus E, Herrera G, Teofilo E, et al. Abacavir versus zidovudine combined with lamivudine and efavirenz, for the treatment of antiretroviral naïve HIV infected adults. Clin Infect Dis. 2004;39:1038-46.
32. Gallant J, Staszewski S, Pozniak A, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA. 2004;292:191-201.
33. De Jesus E, McCarty D, Farthing C, et al. Once-daily versus twice-daily lamivudine, in combination with zidovudine and efavirenz, for the treatment of antiretroviral-naive adults with HIV infection: a randomized equivalence trial. Clin Infect Dis. 2004;39:411-18.
34. Moyle G, DeJesus E, Cahn P et al. Abacavir once or twice daily combined with once-daily lamivudine and efavirenz for the treatment of antiretroviral naive HIV-infected patients. J Acquir Immune Defic Syndr. 2005;38:417-25.
35. Lennox J, DeJesus E, Lazzarin A, et al. Safety and efficacy of raltegravir-based versus efavirenz-based combination therapy in treatment-naïve patients with HIV-1 infection: a multicentre, double-blind randomized controlled trial. Lancet. 2009;374:796-806.
36. Puls R, Srasuebkul P, Petoumenos K, et al. Efavirenz versus boosted atazanavir or zidovudine and abacavir in antiretroviral treatment-naïve, HIV-infected subjects: Week 48 data from the Altair study. Clin Infect Dis. 2010;51:855-64.
37. Post F, Moyle G, Stellbrink H, et al. Randomised comparison of renal effects, efficacy, and safety with once-daily abacavir/lamivudine versus tenofovir/emtricitabine, administered with efavirenz, in antiretroviral-naïve HIV-1 infected adults: 48-week results from the ASSERT study. J Aquir Immune Defic Syndr. 2010;55:49-57.
38. Sax P, Tierney C, Collier A, et al. Abacavir/lamivudine versus tenofovir df/emtricitabine as part of combination regimens for initial treatment of HIV: Final results. J Infect Dis. 2011;204:1191-1201.