Interpreting Resistance Data for HIV-1 Therapy Management – Know the Limitations

Kristel Van Laethem and Anne-Mieke Vandamme

Clinical and Epidemiological Virology, Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

Abstract

Antiviral drug resistance can be one of the causes for HIV-1 therapy failure. Several studies have shown some beneficial effect of antiviral resistance testing on response to the following therapy regimen. The technical performances of genotypic and phenotypic assays have been considerably improved with time. However, both are still limited in their power to fully map antiviral resistance as cause of therapy failure. Nevertheless, the results of genotypic and phenotypic assays can often deliver complementary information. The greatest challenge still remains in the accurate interpretation of in vitro *resistance results, whether genotypic or phenotypic, into information that can be implemented into clinical practice. For phenotypic assays, bioinformatics analyses linking fold-resistance values and clinical response data have been performed, or are currently ongoing, to assign clinical cut-offs for all drugs. Genotypic drug-resistance interpretation systems have been developed and are continuously being updated to solve the same problem. Retrospective and prospective studies have compared systems in their performance to predict phenotype or in their ability to predict therapy outcome. All these analysis are of value, but still display some weak points. However, due to the fast evolving know-how in the field, a prospective trial in which different systems are compared head-to-head will be difficult to design.* (AIDS Reviews 2006;8:37-43)

Key words

HIV-1. Therapy. Resistance. Genotyping. Phenotyping. Interpretation. Algorithm.

Introduction to HAART and antiviral resistance

Combinations of antiviral drugs are used for the treatment of HIV-1 infection. They are called highly active antiretroviral therapy (HAART) and generally comprise three drugs. The aim of HAART is to prevent clinical progression. The introduction of HAART in the mid-1990s resulted in a decrease of the morbidity and mortality in the HIV-1 patient population[1]. Nevertheless, therapy failure still occurs. The markers for therapy failure, ultimately resulting in clinical progression, are decreasing CD4 cell count and rising viral load[2]. As changes in viral load mostly precede changes in CD4 count, the determination of viral load is the most relevant laboratory procedure to monitor the short-term *in vivo* activity of a therapy given to an individual patient. Incomplete adherence to therapy, suboptimal therapy potency, suboptimal pharmacokinetics, and pre-existing drug resistance can be factors that result in rising viral load and therapy failure.

The evolution of resistance against HIV-1 drugs within a patient depends on the generation of genetic variation and on the selection of drug-resistant variants during antiretroviral therapy. The high HIV-1 genetic variability is caused by the error-prone nature of HIV-1 reverse transcriptase, the absence of any enzymatic proofreading activity, and the huge rate of HIV-1 replication *in vivo*. Some of the genetic variants created will result in alterations to the structure and function of the

Correspondence to:
Anne-Mieke Vandamme
Rega Institute for Medical Research
Clinical and Epidemiological Virology
Minderbroedersstraat 10
3000 Leuven
Belgium
E-mail: annemie.vandamme@uz.kuleuven.ac.be

molecules targeted by the antiviral drugs. These alterations could confer changes in susceptibility to one or more of these drugs. In the presence of therapy, the variants with some level of resistance will gradually overcome the wild-type variants. Under the continuous selective pressure of drugs and with the presence of residual replication due to insufficient potency of the treatment, additional mutations will accumulate[3].

Thus, the main goal of HAART is to reduce the viral load as much as possible to prevent further clinical progression. However, its immediate goal is to reduce the viral load to prevent the formation of variants that result in antiviral resistance to the current therapy and that, because of the phenomenon of cross-resistance within drug classes, ultimately might lead to a limitation or even lack of future treatment options.

Resistance assays and their position within clinical practice

Assessment of antiviral resistance can be done by phenotypic and genotypic assays. In the early days, resistance assays were performed solely to diagnose antiviral resistance upon therapy failure. Gradually the idea arose to use this information to guide subsequent therapy changes. A number of prospective studies showed a short-term, modest, beneficial effect of antiviral resistance testing on response to the subsequent regimen[4-9]. These data resulted in the recommendation of resistance testing in the management of HIV-1 therapy[10,11].

However, when implementing the results of resistance assays into clinical decision making, one has to be sure that the obtained information is accurate and also be aware of the limitations of these assays.

Both phenotypic and genotypic assays rely on the amplification of the target gene by polymerase chain reaction (PCR), which makes the assays highly susceptible to contamination from its own product. However, the incorporation of good laboratory practices can overcome this problem[12].

HIV-1 displays an enormous genetic diversity that is reflected at the population level by the presence of nine subtypes, 20 circulating recombinant forms (CRF) and a multitude of unique recombinant forms (URF)[13] and at the individual level by the presence of an enormous number of quasispecies within one patient. Quasispecies differ from each other by substitutions, deletions and insertions. Both assays rely on the hybridization between primers and template for the generation of amplification product and sequences. Minor differences between primers and target sequence might lead to negative results or unrepresentative selection of particular quasispecies from the total viral population. The amplification of quasispecies with insertions and/or deletions of different length results in shifts within the fluorograms when performing population sequencing and generates non-interpretable data. In particular the envelope, the target for enfuvirtide and CCR5 antagonists, challenges researchers in their goal to develop assays that display a good performance for all patient isolates[14].

To understand the observed therapy failure, it is highly important to monitor the resistance pattern of the active replicating viral population in plasma samples. Results of resistance assays represent only the genotypic or phenotypic profile of the majority of quasispecies present *in vivo*. They have difficulty detecting minor variants that reflect less than 25% of the total viral population[12,15]. If the sample is collected without the presence of selective pressure, the chances are high that wild-type will be detected instead of the mutant[16] (Venturi, et al. 2002). Mutant viruses often display a reduced replication capacity in comparison to wild-type and therefore they are rapidly overcome by the wild-type. However, they remain as minor variants and can rapidly reemerge in a subsequent therapy causing therapy failure. When no selective pressure is present, reversal of resistance can also occur. It occurs however at a much lower frequency and much slower rate than the reemergence of wild-type. Therefore, resistance testing has to be performed before starting, stopping, or changing therapy. Resistance assays are most accurate in determining resistance to the current therapy combination. Not detecting resistance to any previously used drug does not guarantee complete drug susceptibility. If a previous isolate from a patient has been scored as resistant to a particular drug, these resistant variants can still exist as minor variants, or can be archived in latently infected cells, and the response might be limited when that drug is reused. So resistance testing is no substitute for good clinical judgment based on combined therapy and viral load histories.

Interpretation of resistance assays

Phenotypic assays measure the *in vitro* viral replication in the presence of different drug concentrations and determine the concentration of drug required to inhibit the replication of the patient's virus by 50% (IC_{50}). Although the IC_{90} resembles better the requested *in vivo* effect, the IC_{50} is a more reliable indicator as it is measured at the steepest part of the dose-re-

sponse curve. A small change in response has no dramatic impact on the IC_{50} value, whereas it does when the 90% measurement is used. The final result of a phenotypic assay is a factor that compares the IC_{50} of the patient's virus to the IC_{50} measured for a wild-type reference strain (fold-resistance). It is a continuous variable and it is in agreement with the drug resistance continuum *in vivo* as resistance is not an all-or-nothing phenomenon.

However, a clinician has to decide whether or not to include a certain drug and therefore this variable is currently categorized into a more workable final output, e.g. sensitive/reduced susceptibility (PhenoSense™, Monogram Biosciences, San Francisco, USA) or within/above the normal susceptibility range (Antivirogram®, Virco, Mechelen, Belgium). Initially, arbitrary cut-offs were used, reflecting the reproducibility range of the test. This was recognized to be inadequate and therefore biological cut-offs were determined[17]. These cut-offs reflect the range in susceptibility to each individual drug observed in therapy-naive patients. Subsequently, clinical cut-offs have been determined for some drugs. They are based on the distinction between fold-resistance levels for which a reduced virologic response is seen and fold-resistance levels for which no or almost no virologic response is observed[18]. The clinical cut-offs for the two commercial assays can be found at their respective websites – www.monogramhiv.com and www.vircolab.com. Bearing in mind the drug resistance continuum, it is hypothesized that a continuous resistance score might even improve the prediction of therapy outcome. A recent retrospective study showed that incremental phenotypic drug susceptibility scores (PSS) more accurately predicted the virologic and immunologic outcome in patients starting salvage therapy as compared to dichotomous PSS. Continuous PSS were also better than dichotomous PSS, but not in comparison to incremental PSS[19].

Genotypic assays determine the nucleotide sequence of the viral gene that is targeted by the drugs within the therapy combination. The nucleotide sequence is subsequently translated into an amino acid sequence and compared to a reference wild-type strain to generate a mutation list. These mutations are then compared with a list of "resistance-associated" mutations. Mutations are defined as resistance-associated (a) when they are known to be selected *in vitro* or *in vivo* in the presence of a certain drug, (b) when they have been associated with a reduction in phenotypic susceptibility, or (c) when they have been associated with a reduced clinical response[20]. This implies

that the result of a genotypic assay always depends on pre-existing knowledge.

Patients are currently treated with combination therapies that change in time and that result in complex patterns of mutations.

Some mutations that are developed under the selective pressure of a particular drug can cause cross-resistance towards other drugs from the same class without any previous experience. Already early on it was realized that mutations selected by the nonnucleoside reverse transcriptase inhibitor (NNRTI) nevirapine rendered viruses highly resistant to all NNRTI. However, viruses with a limited number of certain mutations retained some susceptibility to efavirenz[21]. The rare multi-nucleoside resistance pathway A62V, S68G, V75I, F77L, F116Y, Q151M was first observed in 1993[22]. This resistance pattern was observed in patients receiving sequential monotherapy or bi-therapy with nucleoside reverse transcriptase inhibitors (NRTI). It confers intermediate to high-level resistance to many NRTI[23]. The reverse transcriptase mutations M41L, D67N, K70R, T210W, T215YF, and K219QE were first ascribed to the selective pressure of zidovudine[24]. Afterwards it was shown that stavudine could also select for this resistance pathway and therefore this particular set of mutations was assigned as thymidine-associated mutations (TAM). TAMs, whether or not associated with an insertion at position 69 in the RT gene, are associated with a reduced phenotypic susceptibility and a reduced clinical response to many NRTI. A study based upon a subset of patients of EuroSIDA confirmed the presence of two evolutionary pathways, i.e. TAM1 and TAM2 profiles characterized by M41L, L210W, T215Y and D67N, K70R, K219QE, respectively. Stavudine appeared to retain a greater viral activity than zidovudine in patients carrying viruses displaying the TAM2 profile[25]. In patients exposed to abacavir and part of the NARVAL trial, the strongest association between the decrease in viral load and the number of mutations was observed with a set of six mutations, of which four were at TAM positions (41, 67, 210 and 215) and two at other RT positions (74 and 184)[26]. Retrospective analysis of the trials GS-99-907 and GS-98-902 showed that response to tenofovir was reduced among patients with ≥ 3 TAM inclusive of either M41L or L210W. Slightly increased therapy responses were observed when M184V was present[27]. The Jaguar trial was used to define a genetic score for didanosine. Eight mutations were associated with a reduced response to didanosine (M41L, D67N, T69D, L74V, V118I, L210W, T215YF and K219QE) and two mutations with

a better response (K70R and M184VI)[28]. Despite the association of particular signature mutations to each protease inhibitor (PI), a broad level of cross-resistance exists within the class of PI. In general, the level of phenotypic resistance or the level of reduced clinical response correlates to the number of PI mutations[29].

Resistance antagonism is defined as the resensitization to one drug through the selection of a particular mutation by another drug within an existing resistance pattern that originally rendered the virus resistant to the first drug. A well-known example of this resistance mechanism is the M184V/I mutation that confers high-level resistance to lamivudine and emtricitabine and that, in a background of a limited number of TAM, reverses the phenotypic resistance to zidovudine, stavudine and tenofovir[30]. Similar findings have been observed *in vitro* for the interactions between respectively K65R, L74V, L100I or Y181C and TAMs[31]. The clinical relevance of the latter re-silencing patterns is still unclear.

Hyper-susceptibility to NNRTI has been observed for samples displaying multiple NRTI mutations and isolated from patients naive for NNRTI. The hyper-susceptibility was associated with an improved clinical outcome to efavirenz-based therapy[32]. A similar phenomenon was observed with the I50L mutation within protease. Viruses displaying this mutation were selected in naive patients starting atazanavir-containing therapy and they had increased susceptibility to other PI[33].

Genotypic resistance assays are more widely used than phenotypic assays. They can be more easily implemented in regular molecular biology facilities, which is not the case for phenotypic assays. Therefore, genotyping is mostly performed locally, whereas phenotyping is commonly put out to commercial companies (Monogram Biosciences and Virco). Nevertheless, these and other companies can also supply genotypic results and interpretations. The virologists that are responsible for the testing should convert the generated mutation list into advice for the clinician. However, due to the complex nature of resistance patterns, it is not always possible to be kept informed of the latest discoveries. Therefore, most of them rely on genotypic drug-resistance interpretation systems that are updated at regular intervals[34]. The commercial genotypic assays are concerted with an interpretation system (TRUGENE® HIV-1 Genotyping System, Bayer Health-Care LLC Diagnostic Division, NY, USA; and ViroSeq™ HIV-1 Genotyping System, Celera Diagnostics, CA, USA). However, sequences can also be submitted to commercial interpretation systems (virco®TYPE, Virco; and ViroScore, ABL, Luxembourg) or publicly available

systems (geno2pheno[35]; ANRS, HIVdb[36]; Rega[37]). Their goal is to translate complex patterns of resistance-associated mutations into a definite (e.g. susceptible and resistant) or continuous (fold-change) variable that can be more easily implemented in clinical practice. These systems can be based on Bayesian rules (e.g. ANRS and Rega), on mutation tables with additive and/or subtracting scoring (e.g. HIVdb), on bio-informatics' analysis (e.g. geno2pheno), or combinations thereof (virco®TYPE HIV-1, GeneSeqHIV™ and AntiRetroScan)[38].

The complementary nature of genotypic and phenotypic resistance assays

Phenotypic resistance assays give a direct measurement of susceptibility towards the tested inhibitors that includes the effect of all mutations and their interactions. In this manner, they are ideal assays for testing the susceptibility towards new antiviral drugs for which hardly any genotypic data is yet available (e.g. tipranavir and TMC114), for testing samples from patients who failed multiple therapies, and for testing samples with atypical (e.g. V106M) or less prevalent (e.g. K103S/T/H) mutations[39,40]. Before the publication of these manuscripts, these mutations were not considered as resistance-associated in genotypic assays.

Before the mechanism clarifying the antagonistic effect of M184V/I within the background of TAMs was known, it was already measured by phenotypic assays[41]. This reversal in phenotypic resistance is reflected by a partial virologic response[42]. The interaction between TAMs and K103N results in the reversal of phenotypic resistance to efavirenz. However, it is not reflected by a change in virologic response. Patients displaying this pattern of mutations still fail efavirenz-containing therapy[32]. Genotypic assays can make a distinction based upon the detection of the mutations whereas phenotypic assays cannot.

The current phenotypic assays have difficulty in detecting resistance to some NRTI, especially when associated with TAMs. In a comparative study, PhenoSense™ was more precise than Antivirogram® and was more likely to detect resistance to abacavir, didanosine and stavudine in patient strains displaying M41L, M184V and T215Y (± L210W)[43]. NRTI compete with the natural deoxynucleotide-triphosphates (dNTP) for binding to the reverse transcriptase and incorporation into the growing DNA chain. The phenotypic assays use activated cells with high intracellular dNTP pools, which leads to the generation of dead-end complexes at low NRTI concen-

trations[44]. The fold-resistance values often fall within the reproducibility range of the assay. However, these *in vitro* conditions do not always reflect *in vivo* cell conditions, and therefore such resistance patterns are associated with a relevant *in vivo* reduced clinical response despite the low *in vitro* resistance levels.

Sequencing has the advantage of detecting substitutions that are considered to be associated with a lower genetic barrier to resistance development, i.e. mutations that are considered as markers for developing resistance patterns (e.g. K70R for the TAM, or Q151M for the multi-nucleoside resistance pathway) or reversal mutations (T215A/C/D/S)[45]. Variants displaying these substitutions require less additional nucleic acid changes than wild-type to develop a phenotypic resistant mutant or to obtain a reduced clinical response. Although these mutations are associated with reduced clinical response, they are not always linked with reduced phenotypic susceptibility.

Comparisons of genotypic drug-resistance interpretation systems

Many studies have reported on the discordances between genotypic drug-resistance interpretation systems[46,47]. Ravela, et al. investigated the level of concordance among four interpretation systems (ANRS, HIVdb, Rega and TRUGENE) when applied to 2045 patient strains. For comparison purposes, the original five levels of resistance from HIVdb were converted into three levels. By doing so, HIVdb might partly loose its original strength. Of the interpretations, 4.4% were completely discordant, with at least one system assigning a susceptible score and another system a resistant score. In 29.2% of the cases, at least one system assigned an intermediate score and another a susceptible or resistant score (partial discordances). Few discordances were observed for NNRTI, and they resulted from a small number of individual resistance-associated mutations. Discordances between the interpretations for NRTI resulted from frequently occurring patterns with a limited number of mutations, whereas the discordances between PI interpretations resulted from a larger number of more complex mutation patterns. This might reflect the uncertainty among experts about the weight of individual NRTI and PI mutations within series of mutations and about their interactions on the effective susceptibility to particular drugs. Recently, the effect of HIV-1 subtype on resistance interpretation was evaluated. Apparently the discordances between the systems are also dependent on the genetic subtype[48] (Vergne, et al. 2006).

The prediction of phenotypic susceptibilities by genotypic drug-resistance interpretation systems has been investigated in a few studies[49-51]. The linear model developed by Wang, et al. in 2004 outperformed existing genotypic drug resistance interpretation systems (HIVdb, TRUGENE, ANRS, Rega and geno2pheno) in their prediction of the phenotype. Ross, et al. investigated the agreement between phenotypic results and the interpretation according to seven interpretation systems (CHL, HIVdb, ANRS, Rega, DMC, GAV and TRUGENE) for two subsets of patient populations[50,51]. In the first analysis, 206 samples from PI-experienced patients were analyzed. Good or excellent agreement ($\kappa \geq 0.40$) was observed for ritonavir, indinavir, saquinavir and nelfinavir. Poor agreement ($\kappa < 0.40$) was obtained with four of the seven systems for amprenavir and with four or three of the seven for lopinavir, when applying the biological cut-off of 2.5-fold or the clinical cut-off of 10-fold, respectively. In the second analysis, 70 therapy experienced patients were investigated. The overall percentage concordance was above 80% for all of the interpretation systems, except for GAV. When evaluating individual drugs, concordance was very good (above 80%) for lamivudine, zidovudine, stavudine, nelfinavir, ritonavir, amprenavir, indinavir, saquinavir, efavirenz and nevirapine. There was less concordance for abacavir, didanosine and zalcitabine.

The variability in prediction of therapy outcome by different systems was investigated in 261 therapy-failing patients who subsequently were changed to a new regimen[52]. The association of the genotypic susceptibility score (GSS) of the new regimen, calculated according to 11 interpretation systems, with the virologic outcome for three and six months was examined. Only three systems (ANRS, Rega and TRUGENE) showed significant prediction of the three-month response. The six-month response was predicted by four systems (HIVdb, TRUGENE, Retrogram and HIVresistanceWeb). The same investigators performed a similar analysis in 415 recently infected, therapy-naive patients. Of the patients, 10% had at least one major resistance-associated mutation due to transmitted drug resistance. In multivariate analysis, the GSS from two systems significantly predicted the time to virologic success (Rega and HIVresistanceWeb) and three systems showed a trend towards a significant prediction of success (Retrogram, Menéndez and HIVdb).

The RADAR study, in which two interpretation systems were prospectively compared, could not demonstrate major differences in the virologic outcome between patients for whom there was access to the rule-based in-

terpretation system TRUGENE or to VirtualPhenotype[53]. However, in this study, the final interpretation was not performed by the treating physicians, but by experts, and the authors hypothesized that possible differences in drug prescription based upon both systems might have been minimized due to the expert advice as resistance mutations were also available on the Virtual-Phenotype report.

Conclusions

A number of studies have shown a short-term, modest, beneficial effect of antiviral resistance testing on the response to a subsequent regimen. The contribution of resistance testing to HIV-1 therapy management could be improved by increasing the assay's sensitivity for the detection of minor resistant variants. However, the major challenge in gaining predictive power remains the correct interpretation of resistance. Retrospective comparisons may guide in defining the most relevant cut-offs and the most predictive mutational patterns for each drug. However, it will be difficult to draw any final conclusions from these studies as the original outputs of the interpretation systems were often re-interpreted into new variables for comparison purposes and different end-points and statistical analyses were used. Additionally, due to differences in prevalence of certain mutations and the complexity of mutational patterns and subscribed drug combinations, it will not be possible to validate all drug rules. Current retrospective comparisons will always suffer from the fact that genotypic resistance guided the choice of therapy change, possibly even by one of the systems analyzed. Prospective studies would theoretically not suffer from this bias. However, as clinicians get more and more acquainted with resistance mutations, it might be difficult to measure the independent value of interpretation systems. Additionally, as HIV research is a fast evolving field where interpretation systems constantly are upgraded as soon as new information and new drugs become available, it will be virtually impossible to design a prospective randomized trial in which different systems will be compared head-to-head.

References

1. Porter K, Babiker A, Bhaskaran K, et al. CASCADE Collaboration. Determinants of survival following HIV-1 seroconversion after the introduction of HAART. Lancet 2003;362:1267-74.
2. Mellors J, Munoz A, Giorgi J, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. 1997; 126:946-54.
3. Vandamme A-M, Van Vaerenbergh K, De Clercq E. Anti-HIV drug combination strategies. Antivir Chem Chemother 1998;9:187-203.
4. Durant J, Clevenbergh P, Halfon P, et al. Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomized controlled trial. Lancet 1999;353:2195-9.
5. Baxter J, Mayers D, Wentworth D, et al. A randomized study of antiretroviral management based on plasma genotypic resistance testing in patients failing therapy. AIDS 2000;14:F83-F93.
6. Meynard J, Vray M, Morand-Joubert L, et al. Narval Trial Group. Phenotypic or genotypic resistance testing for choosing antiretroviral therapy after treatment failure: a randomized trial. AIDS 2002;16:727-36.
7. Cohen C, Hunt S, Sension M, et al. VIRA3001 Study Team. A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy. AIDS 2002;16:579-88.
8. Tural C, Ruiz L, Holtzer C, et al. Havana Study Group. Clinical utility of HIV genotyping and expert advice: the Havana trial. AIDS 2002;16:209-18.
9. Cingolani A, Antinori A, Rizzo M, et al. Usefulness of monitoring HIV drug resistance and adherence in individuals failing HAART: a randomized trial (ARGENTA). AIDS 2002;16:369-79.
10. Vandamme A-M, Sonnerborg A, Ait-Khaled M, et al. Updated European recommendations for the clinical use of HIV drug resistance testing. Antivir Ther 2004;9:829-48.
11. Yeni P, Hammer S, Hirsch M, et al. Treatment for adult HIV infection: 2004 recommendations of the International AIDS Society-USA Panel. JAMA 2004;292:251-65.
12. Schuurman R, Demeter L, Reichelderfer P, Tijnagel J, de Groot T, Boucher C. Worldwide evaluation of DNA sequencing approaches for identification of drug resistance mutations in the HIV-1 reverse transcriptase. J Clin Microbiol 1999;37:2291-6.
13. Thomson M, Nájera R. Molecular epidemiology of HIV-1 variants in the global AIDS pandemic: an update. AIDS Rev 2005;7:210-24.
14. Van Laethem K, Schrooten Y, Lemey P, et al. A genotypic resistance assay for the detection of drug resistance in the HIV-1 envelope gene. J Vir Methods 2005;123:25-34.
15. Van Laethem K, Van Vaerenbergh K, Schmit J-C, et al. Phenotypic assays and sequencing are less sensitive than point mutation assays for the detection of resistance in mixed HIV-1 genotypic populations. J Acquir Immune Defic Syndr and Human Retrovirology 1999;22:107-18.
16. Devereux H, Youle M, Johnson M, Loveday C. Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy. AIDS 1999;13:F123-F127.
17. Harrigan P, Montaner J, Wegner S, et al. Worldwide variation in HIV-1 phenotypic susceptibility in untreated individuals: biologically relevant values for resistance testing. AIDS 2001;15:1671-7.
18. Bachelor L, Winters B, Harrigan R, et al. Estimation of phenotypic clinical cut-offs for vircoTYPE HIV-1 through meta-analyses of clinical trial and cohort data. 44th ICAAC, Washington DC, October 30–November 2, 2004.
19. De Luca A, Weidler J, Di Giambenedetto S, et al. Incremental and continuous phenotypic drug susceptibility scores more accurately predict virologic and immunologic treatment outcomes in HIV+ patients starting salvage therapy: Finding from the Argenta trial. 13th CROI, Denver, February 5-8 2006, abstract 650.
20. Johnson V, Brun-Vezinet F, Clotet B, et al. Update of the drug resistance mutations in HIV-1: Fall 2005. Top HIV Med 2005;13:125-31.
21. Casado J, Hertogs K, Ruiz L, et al. NNRTI resistance among patients failing a nevirapine plus protease inhibitor-containing regimen. AIDS 2000;14:F1-F7.
22. Shirasaka T, Yarchoan R, O'Brien M, et al. Changes in drug sensitivity of HIV-1 during therapy with azidothymidine, dideoxycytidine, and dideaxyinosine: an *in vitro* comparative study. Proceedings of the National Academic of Sciences of the USA 1993;90:562-6.
23. Schmit J-C, Van Laethem K, Ruiz L, et al. Multiple dideoxynucleoside analogue-resistant (MddNR) HIV-1 strains isolated from patients from different European countries. AIDS 1998;12:2005-15.
24. Boucher C, O'Sullivan E, Mulder J, et al. Ordered appearance of zidovudine resistance mutations during treatment of 18 HIV-positive subjects. J Infect Dis 1992;165:105-10.
25. Cozzi-Lepri A, Ruiz L, Loveday C, et al. for the EuroSIDA Study Group. Thymidine analogue mutation profiles: factors associated

with acquiring specific profiles and their impact on the virologic response to therapy. Antivir Ther 2005;10:791-802.

26. Brun-Vézinet F, Descamps D, Ruffault A, et al. Narval (ANRS 088) Study group. Clinically relevant interpretation of genotype for resistance to abacavir. AIDS 2003;17:1795-1802.

27. Miller M, Margot N, Lu M, et al. Genotypic and phenotypic predictors of the magnitude of response to tenofovir disoproxil fumarate treatment in antiretroviral-experienced patients. J Infect Dis 2004;189:837-46.

28. Marcelin A-G, Flandre P, Pavie J, et al. AI454-176 Jaguar Study Team. Clinically relevant genotype interpretation of resistance to didanosine. Antimicrob Agents Chemother 2005;49:1739-44.

29. Turner D, Schapiro J, Brenner B, Wainberg M. The influence of protease inhibitor resistance profiles on selection of HIV therapy in treatment-naive patients. Antivir Ther 2004;9:301-14.

30. Whitcomb J, Parkin N, Chappey C, Hellmann N, Petropoulos C. Broad NRTI cross-resistance in HIV-1 isolates. J Infect Dis 2003;188:992-1000.

31. Miranda L, Götte M, Liang F, Kuritzkes D. The L74V mutation in HIV-1 reverse transcriptase counteracts enhanced excision of zidovudine monophosphate associated with thymidine analog resistance mutations. Antimicrob Agents Chemother 2005;49:2648-56.

32. Shulman N, Zolopa A, Passaro D, et al. Phenotypic hypersusceptibility to NNRTIs in treatment-experienced HIV-infected patients: impact on virologic response to efavirenz-based therapy. AIDS 2001;15:1125-32.

33. Colonno R, Rose R, McLaren C, Thiry A, Parkin N, Friborg J. Identification of I50L as the signature atazanavir (ATV)-resistance mutation in treatment-naive HIV-1-infected patients receiving ATV-containing regimens. J Infect Dis 2004;189:1802-10.

34. De Luca A, Antinori A, Di Giambenedetto S, et al. Interpretation systems for genotypic drug resistance of HIV-1. Scand J Infect Dis 2003;106:29-34.

35. Beerenwinkel N, Däumer M, Oette M, et al. Geno2pheno: estimating phenotypic drug resistance from HIV-1 genotypes. Nucleic Acids Res 2003;31:3850-5.

36. Rhee S-Y, Gonzales M, Kantor R, Betts B, Ravela J, Shafer R. HIV reverse transcriptase and protease sequence database. Nucleic Acids Res 2003;31:298-303.

37. Van Laethem K, De Luca A, Antinori A, Cingolani A, Perno C-F, Vandamme A-M. A genotypic drug resistance interpretation algorithm that significantly predicts therapy response in HIV-1-infected patients. Antivir Ther 2002;7:123-9.

38. Zazzi M, Romano L, Venturi G, et al. Comparative evaluation of three computerized algorithms for prediction of antiretroviral susceptibility from HIV-1 genotype. J Antimicrob Chemother 2004;53:356-60.

39. Brenner B, Turner D, Oliveira M, et al. A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to NNRTI. AIDS 2003;17:F1-5.

40. Harrigan P, Mo T, Wynhoven B, et al. Rare mutations at codon 103 of HIV-1 reverse transcriptase can confer resistance to NNRTIs. AIDS 2005;19:549-54.

41. Schmit J-C, Martinez-Picado J, Ruiz L, et al. Evolution of HIV drug resistance in zidovudine/zalcitabine- and zidovudine/didanosine-experienced patients receiving lamivudine-containing combination therapy. Antivir Ther 1998;3:81-8.

42. Masquelier B, Descamps D, Carriere I, et al. Zidovudine resensitization and dual HIV-1 resistance to zidovudine and lamivudine in the delta lamivudine roll-over study. Antivir Ther 1999;4:69-77.

43. Zhang J, Rhee S, Taylor J, Shafer R. Comparison of the precision and sensitivity of the Antivirogram and PhenoSense HIV drug susceptibility assays. J Acquir Immune Defic Syndr 2005;38:439-44.

44. Meyer P, Matsuura S, Schinazi R, So A, Scott W. Differential removal of thymidine nucleotide analogues from blocked DNA chains by HIV reverse transcriptase in the presence of physiological concentrations of 2'-deoxynucleoside triphosphates. Antimicrob Agents Chemother 2000;44:3465-72.

45. de Ronde A, van Dooren M, van Der Hoek L, et al. Establishment of new transmissible and drug-sensitive HIV-1 wild types due to transmission of nucleoside analogue-resistant virus. J Virol 2001;75:595-602.

46. Ravela J, Betts B, Brun-Vézinet F, et al. HIV-1 protease and reverse transcriptase mutation patterns responsible for discordances between genotypic drug resistance interpretation algorithms. J Acquir Immune Defic Syndr 2003;33:8-14.

47. Stürmer M, Doerr H, Staszewski S, Preiser W. Comparison of nine resistance interpretation systems for HIV-1 genotyping. Antivir Ther 2003;8:55-60.

48. Snoeck J, Kantor R, Shafer R, et al. Discordances between interpretation algorithms for genotypic resistance to protease and reverse transcriptase inhibitors of HIV are subtype dependent. Antimicrob Agents Chemother. 2006;50:694-701

49. Wang K, Jenwitheesuk E, Samudrala R, Mittler J. Simple linear model provides highly accurate genotypic predictions of HIV-1 drug resistance. Antivir Ther 2004;9:343-52.

50. Ross L, Boulmé R, Fisher R, et al. A direct comparison of drug susceptibility to HIV-1 from antiretroviral experienced subjects as assessed by the Antivirogram and PhenoSense assays and by seven resistance algorithms. Aids Res Hum Retroviruses 2005;21:933-9.

51. Ross L, Boulmé R, Fusco G, Scrasella A, Florance A. Comparison of HIV-1 protease inhibitor susceptibility results in viral samples analyzed by phenotypic drug resistance assays and by six resistance algorithms: an analysis of a subpopulation of the CHORUS cohort. Aids Res Hum Retroviruses 2005;21:696-701.

52. De Luca A, Cingolani A, Di Giambenedetto S, et al. Variable prediction of antiretroviral treatment outcome by different systems for interpreting genotypic HIV-1 drug resistance. J Infect Dis 2003;187:1934-43.

53. Torti C, Quiros-Roldan E, Regazzi M, et al. RADAR-MASTER Study Group. A randomized controlled trial to evaluate antiretroviral salvage therapy guided by rules-based or phenotype-driven HIV-1 genotypic drug-resistance interpretation with or without concentration-controlled intervention: the resistance and dosage adapted regimens (RADAR) study. Clin Infect Dis 2005;40:1828-36.

De Luca A, Cozzi-Lepri A, Perno CF, et al. for the ICoNA drug resistance study group and the ICoNA study group. Variability in the interpretation of transmitted genotypic HIV-1 drug resistance and prediction of virologic outcomes of the initial HAART by distinct systems. Antivir Ther 2004;9:745-54.

Haubrich R, Kemper C, Hellmann N, et al. and California Collaborative Treatment Group. A randomized, prospective study of phenotype susceptibility testing versus standard of care to manage antiretroviral therapy: CCTG 575. AIDS 2005;19:295-302.

Schmit J-C, Cogniaux J, Hermans P, et al. Multiple drug resistance to nucleoside analogues and NNRTIs in an efficiently replicating HIV-1 patient strain. J Infect Dis 1996;174:962-8.

Wegner S, Wallace M, Aronson N, et al. and RV-125 Centers for Education and Research on Therapeutics Study Team. Long-term clinical efficacy of routine access to antiretroviral-resistance testing in HIV-1-infected patients: results of the Clinical Efficacy of Resistance Testing trial. Clin Infect Dis 2004;38:723-30.

43