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Mini-review

Algorithms for the interpretation of HIV-1 genotypic drug resistance information

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006.

Abstract

Drug resistance testing has proven its use to guide treatment decisions in HIV-1 infected patients. Genotyping is the preferred technique for clinical drug resistance testing. Many factors complicate the interpretation of mutations towards therapy response, such that an interpretation system is necessary to help the clinical virologist. No consensus interpretation exists to date and experts often have quite different opinions. As a result, several algorithms for the interpretation of HIV-1 genotypic drug resistance information have been designed. Clinical evaluation of their genotypic interpretation is not always straightforward. We describe a few publicly available systems and their clinical evaluation. We also stress that in addition to drug resistance, for effective management of HIV infection the clinician needs to take into account all potential causes of treatment failure. Successful therapy heavily relies on the expertise of the clinician.

Keywords: HIV; Drug resistance; Interpretation system; Algorithm; Genotype; Phenotype

Contents

1. Introduction

In countries where therapy is available, administration of a combination of three or more antiretroviral drugs has been associated with a significant improvement of morbidity and mortality in HIV infected patients (Murphy et al., 2001; Vandamme et al., 1998). The 21 FDA approved anti-HIV drugs used in the treatment of HIV infection belong to four classes: Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Nonnucleoside Reverse Transcriptase Inhibitors (NNRTIs), Protease Inhibitors (PIs) and Entry Inhibitors (EI). For a more detailed overview of the current clinically used drugs, see De Clercq (2004, 2005). Even though effective, the initiation of highly active antiretroviral treatment (HAART) in drug-naive HIV type 1 patients prevents viral breakthrough for a median period of approximately 3 years in only 60% of the patients (Van Vaerenbergh et al., 2002). Therapy failure is due to such factors as lack of potency of the combination, insufficient drug adherence, transmission

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of drug resistant virus (Cane, 2005), resulting in incomplete suppression of virus replication. Virus replication under drug selective pressure will invariably lead to increased drug resistance and cross-resistance, limiting further treatment options. Consequently, it is anticipated that drug resistance is and will continue to be a major issue in the effective treatment of HIV infection (Frenkel and Tobin, 2004).

Taking into account antiviral drug resistance, when choosing a therapy, is included in international guidelines (Vandamme et al., 2004; Yeni et al., 2004). Although both resistance phenotyping and genotyping after treatment failure have proven to be predictive for the next therapy response (Cingolani et al., 2002; Cohen et al., 2002; Mazzotta et al., 2003; Meynard et al., 2002; Perez-Elias et al., 2003; Tural et al., 2002; Wegner et al., 2004), genotyping is the preferred test for the routine follow-up of patients because of its faster turn-around time, the less complicated technique, and its lower cost (300–500€ versus 800–1000€). So far, no controlled prospective clinical studies have shown the advantage of using resistance testing in untreated patients although many retrospective studies support resistance testing also in drug naives (Little et al., 2002; Novak et al., 2005) such that guidelines also include resistance testing in particular circumstances for the first regimen.

2. Genotypic drug-resistance interpretation systems

The interpretation of the mutation patterns for the prediction of drug susceptibility and anticipated therapy response is quite complicated. There are several reasons for this. The genetic context of the mutations, which can vary a lot between patients and especially between subtypes, can influence phenotypic effect of previously identified mutations, and can even result in differences in resistance pathways and the selection of new mutations under drug selective pressure (Abecasis et al., 2005; Wainberg, 2004; Wang et al., 2004). Combinations of known resistance mutations can have an antagonistic or synergistic effect. Some of these effects have already been characterized (Sugiura et al., 2002) but new combinations of known mutations are often detected in individual patient samples, and regularly, new antagonistic and synergistic effects are being reported (Deforche et al., 2005a; Svicher et al., 2005). Also, some mutations can induce hypersusceptibility or resensitisation (Masquelier et al., 1999; Shulman et al., 2004; Wolf et al., 2003). Therefore, to accurately know its phenotype, a phenotypic test has to be done for each strain. This is however practically not feasible because of the cost and the specific requirements of phenotypic tests.

The primary goal of resistance testing is to predict therapy response. Although intuitively phenotyping could be considered a better predictor than genotyping, some studies indicates that in some situations genotyping correlates better with therapy response than phenotyping (Meynard et al., 2002), while no study yet found indications for the superiority of phenotyping. This may be related to the fact that genotyping can take into account the genetic barrier towards high level resistance and that genotyping allows the detection of reversal mutations (e.g. 215 A/C/D/S) as ‘signatures’ of past drug resistance. Some mutations can therefore indicate an imminent failure, while not being very predictive of a resistant phenotype (Vandamme et al., 2004). Moreover, genotyping can detect mutations associated with drugs for which a clinically relevant phenotypic cut-off is within the reproducibility range of the assay, such as stavudine (Shulman et al., 2002), which may be a problem of the non-physiological conditions of the assay and not necessarily of the drug (Lennerstrand et al., 2001; Meyer et al., 2000). Although the ultimate goal should be to try to predict therapy response, and not the phenotype, if insufficient data on therapy response are available or if the mutational pattern is too complex, phenotyping or the prediction of the phenotype can be very useful.

The replication capacity in absence of drugs is often compromised by acquired resistance-related mutations (Quinones-Mateu and Arts, 2006). As a consequence, compensatory mutations are selected under drug selective pressure. These have only a limited impact on the level of resistance, but rather improve the replication capacity of the resistant virus (Buckheit, 2004). It is still under discussion how such mutations have to be taken into account and whether reduced replication capacity has a clinical benefit (Geretti, 2005). As a result, whenoff treatment, the wild-type virus with higher replication capacity can re-emerge and may thus mask the mutant virus (Deeks et al., 2001). Guidelines therefore recommend to perform drug-resistance testing on samples obtained from individuals before changing or stopping treatment.

Even though genotyping seems to be the method of choice in most situations, current clinical genotyping can underestimate the degree of resistance because the techniques used are not very sensitive for monitoring resistance mutations present in only a minority of a patient’s variants, while such variants may contribute to therapy failure (Schuurman et al., 2002). However, phenotyping also suffers from underestimating resistance in mixed populations (Van Laethem et al., 1999).

A major drawback for all current interpretation systems is that they evaluate each drug separately, while therapy in general consists of a combination of three or more drugs. The correlation of a mutation to therapy response for the combination is hard to assess. Large datasets will be needed to allow a proper analysis of the relevance of mutations to possible combinations.

These difficulties but also lack of data have resulted in the generation of several different interpretation algorithms, depending on the opinion of the experts involved. They use protease and reverse transcriptase sequences to assess HIV-1 susceptibility or therapy response to each of the available NRTI, NNRTI and PI drugs, with some of them already including predictions to EI based on envelope sequences. The format can vary from mutation tables over specific scoring algorithms to websites and software packages. Some systems are commercial or delivered with a commercial testing kit, while most are publicly available.

Interpretation systems can have a specific scientific basis, a clinical validation or not, different formats of input and output to the user (Schmidt et al., 2002). The most frequently used clinically available systems are listed in Table 1. These systems are based on two different sources of information. On the one hand, there exist rules-based systems which were deduced by

Table 1
Clinically available genotypic drug-resistance interpretation systems

Interpretation system[a]	Source	Clinical evaluation	Levels	Access
Stanford, HIVRT&PrDB v4.1	Experts Rule-based	Yes, retrospectively (De Luca et al., 2003, 2004)	S/PL/LL/IR/HR	http://www.hivdb.stanford.edu/ http://www.hiv-grade.de
Virtual Phenotype	Database (>29000 G/P)	Yes, retrospectively (Cohen et al., 2002; De Gruttola et al., 2000; Torti et al., 2003) and prospectively (Mazzotta et al., 2003; Perez-Elias et al., 2003)	Quantitative. Resistance 'likely' or 'unlikely', for rare patterns	http://www.vircolab.com
Geno2pheno, v3.0	Database (>800 G/P)	Yes, retrospectively (V2.1) (Beerenwinkel et al., 2002; De Luca et al., 2004)	S/R, Quantitative	http://www.genafor.org/ http://www.hiv-grade.de
RetroGram™, v1.6	Experts Rules-based	Yes, retrospectively (V1.4) (De Luca et al., 2003, 2004; Torti et al., 2003) and prospectively (V1.0) (Tural et al., 2002)	A/B/C/D/U	http://www.retrogram.com/
Rega v6.4.0, Belgium	Experts Rules-based	Yes, retrospectively (V4.0) (De Luca et al., 2003), (V5.5) (De Luca et al., 2004; Van Laethem et al., 2002), (V6.0) (Derdelinckx et al., 2003)	S/I/R	http://www.kuleuven.be/rega/cev/links/index.htm http://www.ablsa.com/site/en/product_vs_algos.html http://www.hiv-grade.de http://hivdb.stanford.edu/pages/asi/
CHL v3.2, Luxembourg	Experts Rules-based	Yes, retrospectively (De Luca et al., 2003, 2004)	S/I/R	http://www.ablsa.com/site/en/product_vs_algos.html
ANRS v13, France	Experts Rules-based	Yes, retrospectively (V2003) (De Luca et al., 2003, 2004; Meynard et al., 2002)	S/I/R	http://www.hivfrenchresistance.org/ http://hivdb.stanford.edu/pages/asi/ http://pugliese.club.fr/index.htm http://www.hiv-grade.de
GuideLines 10, TruGene™, VGI/Bayer	Experts Rules-based	Yes, retrospectively (Cingolani et al., 2002; De Luca et al., 2003, 2004; Torti et al., 2003)	S/I/R	http://www.bayer.com http://www.labnews.de/en/products/pr_truso.php
ViroSeq™, ABI/Abbott	Experts Rules-based	No	S/PM/P/HM/H	http://www.celeradiagnostics.com/cdx/ViroSeq
GeneSeqHIV v3.0, Monogram Biosience	Experts and Database (>38000 G/P) Rules-based	No	S/R	http://www.monogramhiv.com/assays/hcp/geneSeqHIV.aspx

[a] These algorithms are regularly updated, please visit the indicated websites. A, B, C and D are a ranking system used for Retrogram, where A indicates no evidence for resistance and D indicates evidence for high level resistance, while U is used to indicate uncertainties. S: susceptible; PL: possible low-level resistance; LL: low-level resistance; IR or I: intermediate resistance; HR: high level resistance; R: resistance; PM: possible multi-NRTI resistance; P: possible resistance; HM: high-level multi-NRTI resistance; H: high-level resistance; G/P: genotype/phenotype.

experts in the field, by making use of literature data on correlations between geno and phenotype as well as correlations with treatment history and clinical response. Herein the expert knowledge is of major importance since it is very difficult to select the relevant information out of the abundant literature on mutation interpretation. On the other hand, bioinformatic techniques are being developed for generation of mathematical models for prediction of phenotypic drug resistance and therapy response from genotype. Most current methods developed 'in silico' are based on information from large databases which encompass pairs of geno- and phenotypes (Schmidt et al., 2002). The first to apply such a method was the company Virco, calling it a 'virtual phenotype' (Virtual Phenotype™, Virco). This algorithm predicts phenotypes from genotypes by comparing the query sequence with all available sequences in such a database and averaging the resistance of the matching samples. Subsequently within the German Arevir project, datamining techniques were used for similar purposes (see Geno2pheno). Other examples of bioinformatic approaches based on analyses of large databases are the artificial neural networks that can predict resistance from complex mutation patterns (Wang and Larder, 2003) and virological response from treatment and resistance history (Larder et al., 2005), or the Bayesian networks that can predict individual in vivo evolution towards resistance under drug selective pressure (Deforche et al., 2005b, 2006). However these last two systems are currently still not available to clinical virologists for use in routine clinical practice. The field is now moving into incorporation of the two types of information (literature and database driven) in a single interpretation system.

The interpretation system must be comprehensive for the clinician or the clinical virologist, cover all available antiretroviral drugs and must be easily accessed, for example through the Internet. The vast amount of literature on mutation interpretation makes it almost impossible for clinicians to stay informed on the effect of all relevant mutations. Clinicians thus rely on the expertise of the clinical virologist who on their turn rely on the expertise of interpretations systems developers. In this rapidly evolving field, regular updates of the interpretation systems (at least once a year) are therefore required, and their clinical evaluation is a must. In addition, the clinician has to take into account many more factors than resistance results only. Interpretation system developers are currently trying to include some of these factors into a therapy response prediction tool. Factors such as treatment history, resistance history, viral load history, CD4 count history or drug levels could be included if proven to improve the predictive power of the systems. In this respect, it is interesting that some systems (such as the Rega algorithm) already have some kind of evaluation of drug levels, through incorporating distinct interpretations for boosted compared to unboosted protease inhibitors. The resistance to newly developed drugs should be interpreted with caution, since their resistance pattern is not yet fully understood. Unfortunately, the clinical data on development of resistance to a drug is mostly not publicly available, and is often limited at the time the drug is first introduced into routine clinical practice.

This mini-review shortly summarizes our current knowledge on genotypic drug-resistance interpretation systems and their evaluation. The most frequently used publicly available systems were selected for a more detailed description.

2.1. The Rega algorithm

The Rega algorithm was the first algorithm for the interpretation of genotypic drug resistance that proved to be predictive for therapy response. It was clinically validated in a cohort of patients on salvage therapy where the three month response was the outcome variable (Van Laethem et al., 2002). It is freely accessible on-line through the Stanford website and the German Arevir HIV-Grade website, and through the company Advanced Biological Laboratories (see Table 1) on which complete sequences or individual mutations can be entered. The scoring tables and the XML file are downloadable via the website of the Rega Institute (see Table 1). The Rega algorithm was established for daily use in clinical practice. In-house data as well as published literature and conference abstracts served as basis for deriving interpretation rules. Initially, the work was done by an individual expert with long-term experience, but currently, a team of experts is updating the rules on a yearly base. Mutations taken into consideration are those for which phenotypic drug resistance or reduced therapy response have been reported. The latest versions also took advantage from information obtained through datamining in the large databases of the group. It is a complex algorithm, taking into account known synergistic and antagonistic effects of combinations of mutations. Three levels of interpretation criteria are considered: criteria to consider an isolate resistant, intermediate resistant and susceptible. Its philosophy is to score imminent therapy failure, through weighing a large set of minor mutations almost equally as a single major mutation. A large set of minor mutations may not reduce the drug susceptibility too much, but its presence greatly reduces the genetic barrier towards high level drug resistance. Also some of the single mutations in the resistant panel of the table do not normally result in high level phenotypic resistance. Instead, they are key mutations in a pathway towards high level resistance or have already proven to be predictive for therapy failure. As a consequence, the algorithm may score better in early failure, and may leave the clinician with insufficient guidance for options in multiresistant patients. On the in-house drug-resistance report this is pointed out by stating that drugs for which the virus has a reduced susceptibility can be partially active in a combination therapy and that drugs for which the virus is resistant can still exhibit a temporary activity when using mega-HAART (>4 antiretroviral drugs). The extensive research of the group in resistance pathways for subtypes other than B has lead to rules which are valid across all subtypes, by including scores based on the genetic background of the virus (Abecasis et al., 2005, in press). The Rega algorithm is now available as version 7.0, it scores resistance to all FDA approved drugs (including T-20, an EI) and drugs in expanded access, for both HIV-1 and HIV-2. Subtype information can be obtained from a separate subtyping tool (de Oliveira et al., 2005), available on the Stanford website (http://dbpartners.stanford.edu/RegaSubtyping/) or through the Rega website (http://www.kuleuven.be/rega/cev/links/index.htm) and several

other websites worldwide. A list of the mirror sites can be found on (http://bioafrica.mrc.ac.za/subtypetool/html/index.html).

2.2. HIVdb – Stanford University Genotypic Resistance Interpretation Algorithm

This algorithm is probably the most widely known. The sequence information is entered in the HIVdb algorithm via the website of the Stanford University or the German Arevir HIV-Grade website (see Table 1) as plain nucleic acid code or via a mutation list. The information is then compared to the consensus subtype B reference sequence, and the differences are used as query parameters for interrogating the HIV Drug Resistance database (Shafer et al., 2000). The HIVdb algorithm assigns a drug penalty score for each drug resistance mutation. Mutation scores are derived from published literature linking mutations and antiretroviral drugs, including correlations between genotype and treatment history, genotype and phenotype, and genotype and clinical outcome. Mutations that cause hypersusceptibility to a drug or are associated with reversion of resistance, have a negative score for that drug. The total score for a drug is derived by adding the scores associated with each mutation. Mutations are divided into those associated with drug resistance ('Resistance Mutations') and those that have not been shown to contribute to drug resistance ('Other Mutations'). The program uses the total drug score to assign one of the following levels of inferred drug resistance to each of the listed drugs: susceptible, potential low-level resistance, low-level resistance, intermediate resistance, and high-level resistance. The output shows the mutation scoring, gives comments on them and provides information about the subtype. The scores are updated based on new published data and based on feedback from users and experts in the field.

2.3. ANRS – Agence Nationale de Recherche sur le SIDA

The French ANRS HIV-1 genotypic drug resistance interpretation algorithm is gaining recognition, because the rules of the current versions are almost exclusively based on data of correlation between drug resistance mutations and virological outcome from a large database of patients failing antiretroviral therapy (Brun-Vezinet et al., 2003). It can be accessed through the Stanford website (see Table 1) or the German Arevir HIV-Grade website on which complete sequences or individual mutations can be entered, or through MPGenotype (see Table 1) where you can submit a list of mutations. The output lists all drugs with the assignment 'susceptible', 'possible resistance' or 'resistant' and the associated mutations. The rules on which the ANRS algorithm is based, can be found on the web (see Table 1) as tables listing mutations conferring genotypic resistance or associated to 'possible resistance' to anti-HIV drugs. Also the references to the according literature are given which mainly are conference abstracts. Often these have not yet been published, yet they are of great value due to the fact that this field is rapidly evolving. The rules are updated on a yearly base.

2.4. Geno2pheno

A number of machine learning approaches to predict phenotype from genotype have been proposed, of which Geno2Pheno (Beerenwinkel et al., 2003) is the only one freely accessible (see Table 1), developed within the German Arevir project. This user friendly web-based prediction tool, currently version 3.0, is based on more than 800 correlated genotype-phenotype pairs. On submitting an HIV-1 pol-gene DNA sequence, a sequence alignment to the reference strain HXB2 is obtained, as well as a list of mutations and different predictions of phenotypic resistance of the respective virus to 17 antiretroviral drugs. Additional information about therapy optimization is given by a new integrated Java applet called 'Theo'. The Geno2pheno system uses techniques as decision trees and support vector machines that have been trained on the dataset of correlated geno- and phenotypes (Beerenwinkel et al., 2003). The decision trees offer the advantage that the knowledge can be extracted as rules by tracing out a path from the root of a tree to a leaf. Furthermore, as support vector machines can deal with quantitative data, the output also includes activity scores rendering predictions comparable between drugs (Beerenwinkel et al., 2005). To date, more than 37 000 predictions have been made on-line since the start in December 2000. Geno2pheno can also be accessed from HIV-Grade.

3. Evaluations of systems

Several genotypic interpretation systems have been developed, but although the systems seem to converge with newer versions, there still exists disagreement on a consensus interpretation of drug resistance. Different interpretation systems often produce different interpretations when applied to the same virus mutations. A study of Ravela et al. revealed that only two-third of the investigated interpretations done by four distinct algorithms (ANRS-3-02, TRUGENE VGI-6, Rega 5.5 and HIVdb-8-02) displayed complete concordance (Ravela et al., 2003). The most discordances were seen for nucleoside reverse trancriptase inhibitors (NRTIs) since these drugs require multiple mutations for the development of resistance. Because insufficient data on clinical outcome was available at that time, this study could not compare the predictive value of the investigated algorithms. Still, Ravela and colleagues emphasized that clinicians should realize that not all genotypic interpretation systems lead to the same resistance interpretation and expert advice is needed to decide on treatment especially for persons with a complex mutational pattern. Snoeck et al. studied several interpretation systems using a large dataset of sequences from non-B subtypes, treatment naïve and experienced. Their study suggested that some of the discordances could be attributed to specific (subtype-dependent) combinations of mutations, though it is not yet known whether therapy response is subtype-dependent (Snoeck et al., 2006). Concomitantly, a study of Vergne et al. (2006) showed different discordances in therapy-naive versus therapy-experienced patients. The dominant discordances in naive patients were observed for predictions of protease inhibitor which were mainly caused by the presence of resistance mutations that were

natural protease polymorphisms especially in non-B subtypes. In treated patients this study confirmed the results of Ravela et al. (2003): most interalgorithm discordances were for predictions of NRTIs.

De Luca and colleagues could show for the first time that discordant interpretation may influence the predictive value of resistance testing over subsequent virological outcomes (De Luca et al., 2003). Multivariate regression that included patient characteristics relevant in terms of salvage treatment decisions, demonstrated that only 3 out of the 11 (ANRS AC11, Rega 4.0 and Guidelines 3.0) studied algorithms showed significant prediction for the 3-month clinical response and only 4 of 11 (Guidelines 3.0, HIVdb, HIVresistanceWeb and Retrogram 1.4) were predictive at 6 months. More recently De Luca et al. also conducted a performance study of 13 interpretation systems in therapy-naive patients in which they confirmed the high variability in the interpretation of genotypic resistance. In their multivariate approach, only two systems (Rega 5.5 and hivresistanceWeb v3) significantly predicted therapy response (De Luca et al., 2004).

However, studies designed for the comparison of interpretation systems have their limitations. System-specific definitions of different levels in resistance have to be translated into uniform, discrete categories which can confound the sensitivity of the algorithm. Furthermore, the drugs get the same weight across the scoring systems, weights that are set up by the authors performing the comparison, without taking drug potency into account. Comparisons are done on different datasets, using different statistical methods, making it difficult to find reasons why some comparisons contradict each other. Moreover some systems may be better for short-term virological outcomes, whereas others perform better on a longer term. A disadvantage of such studies in general is that not all versions of the compared algorithms are contemporaneous. For example in the De Luca study (De Luca et al., 2003), Rega 4.0 and ANRS AC11 were from 2000, while GuideLines 3.0, HIVdb and Retrogram 1.4 were from 2001, yet both Rega and ANRS had new updates dating from 2001. In addition, every comparison is doomed to be published after newer versions have been released, thus always being a step behind what is really needed for clinical virologists. Such retrospective studies are currently performed on datasets in which therapy change was already guided by genotypic resistance data, using one of the available systems. In these studies it is usually not mentioned which system was used, such that the potential bias in favor or against a particular system cannot be estimated. Unfortunately, no prospective studies comparing interpretation systems head to head are currently performed. In addition, the choice of therapy is never guided solely by genotypic resistance interpretation systems, medication adherence, drug interactions and toxicities, and individual pharmacokinetic variability are important factors influencing treatment choice and treatment response.

4. Conclusion

Besides the limitations of the available assays, the major challenge lies in the interpretation of drug-resistance for which no consensus exists among experts. Even though it is hard to generate a large clinical database including therapy response data, attempts should be made in order to further improve and evaluate interpretation systems for their predictive power. Moreover, to better take into account archived resistance mutations, the most realistic approach for the near future may be to built resistance history and therapy history into the interpretation system. But even the best interpretation systems cannot replace the expertise of the clinician. It is only by considering all potential causes of treatment failure, that a treatment can be optimized. As new and more effective drugs are brought into clinical practice, it will be easier to design optimal treatment strategies, however resistance testing will remain an integral part of the management of HIV infection and its use likely will expand to other chronic viral infections.

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