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Genotypic Drug Resistance Interpretation Systems – The Cutting Edge of Antiretroviral Therapy

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Abstract

The technical quality of genotypic and phenotypic drug resistance testing has considerably improved, and therefore the major challenge now lies in the interpretation of drug resistance. This is due to several facts: (i) in times of combination therapy, the effect of drug resistance-associated mutations cannot be considered independently, (ii) many additive and subtractive interactions between mutations exist, and resistant strains may exhibit varying degrees of cross-resistance, (iii) the phenotype cannot adequately determine slight, but clinically relevant, differences for those drugs with a narrow range of resistance, and (iv) pharmacokinetic interactions may shift relevant levels of drug resistance. Genotypic drug resistance interpretation systems are designed to solve these problems. Rule-based systems incorporate current knowledge about correlations between genotype, phenotype and clinical response. Database-driven systems use the information provided by paired geno- and phenotypic data, applying database matching search or bioinformatic approaches. For detailed comparison, 11 interpretation systems were selected which present a comprehensive system for most of the available drugs, can easily be accessed via the Internet and are regularly updated. The systems were characterized for the source data, access, input, output, and availability of clinical studies. For further comparison, existing clinical databases should be merged into one large database to allow competition between the systems. This may also solve the burning problem of clinically relevant cut-offs. Head-to-head comparisons of interpretation systems require large prospective randomized trials in which only the interpretation system is different between groups, before a consensus can be achieved for the best antiretroviral therapy of the individual patient.

Key words

HIV. Drug resistance. Interpretation system. Bioinformatic technique. Algorithm.

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Drug resistance testing is becoming increasingly accepted as a valuable tool in the management of antiretroviral therapy in HIV-1 infected patients. A number of retrospective and prospective studies[1-7] have shown a beneficial effect if antiretroviral treatment was changed according to the results of drug resistance testing. Consequently,

drug resistance testing was implemented into US and European guidelines. Performing a resistance test is recommended in cases of first or multiple regimen failure, for HIV-1 infected pregnant women and newborns. Furthermore, drug resistance testing may be considered for cases of primary and recent HIV infection without previous antiretroviral therapy[8,9]. The story could end here if all technical demands were solved and all experts agreed on a consensus interpretation of drug resistance tests. Indeed, the technical quality of geno- and phenotypic drug-resistance testing has considerably improved, which has been shown in national and international quality control trials[10-12]. Still, issues such as reliable detection of minority species remain to be solved. The major challenge, however, lies in the interpretation of drug resistance, which varies largely between different laboratories.

If drug resistance-associated mutations as well as phenotypic drug resistance can be determined reliably, why is interpretation a problem?

(i) An unequivocal correlation of a single mutation to drug resistance like for M184V and resistance to lamivudine is the exception rather than the rule. And in times of combination therapy, the correlation with clinical failure or success is difficult to assess even in those cases.

(ii) Additionally, different types of interactions must be considered, such as resensitization of zidovudine resistance by M184V[13], L74V and Y181C[14], induction of hypersusceptibility to amprenavir by N88S[15], and hypersusceptibility towards NNRTIs after multiple NRTI failure[16]. To further complicate the situation, combination therapy may result in the development of other mutations that counteract these reversions and restore resistance (e.g. R211K, E333D)[17,18]. Concomitantly, cross-resistance has been ascribed to many antiretroviral compounds in clinical development[19].

(iii) Not to forget that the phenotypic assays may not be able to adequately determine slight, but clinically relevant, differences for those drugs with a narrow range of resistance, e.g. dideoxynucleoside analogues[20]. This was one of the reasons why genotypic but not phenotypic resistance testing proved to be superior to standard-of-care in certain subpopulations of the NARVAL study[21].

(iv) Finally, the increasing use of boosted protease inhibitors may require a different interpretation of drug resistance results compared to un-boosted protease inhibitors[22].

Genotypic drug resistance interpretation systems try to solve these problems. Since one to two new drugs are annually approved, the systems have to be updated continuously. Regular updates are facilitated by web-based presentations, which can be altered easily and avoid the ongoing use of older versions. Thus, this review can only be a snapshot of current drug resistance interpretation systems, and the reader is encouraged to contact the websites personally.

Human vs artificial intelligence

Genotypic drug resistance interpretation systems differ in their sources of information: on the one hand, there are the rule-based systems which incorporate different types of information such as correlations between geno- and phenotype as well as correlations with treatment history and clinical response. Much of this information is already published[23]. However, the task to condense the vast amount of information from different sources and of different quality into rules to predict treatment response requires a lot of expertise, based on long-standing clinical and laboratory experience[24]. Therefore, human intelligence in the form of expert knowledge is a major constituent of rule-based interpretation systems. The fact that there is a substantial overlap in the experts behind different interpretation systems may be one of the reasons why the rules and algorithms are often quite similar. Rules are the simplest form of presenting knowledge ("M184V causes lamivudine resistance"). Algorithms are a bunch of rules ("High resistance to lamivudine is conferred by M184V, intermediate resistance by E44D and/or V118I in the presence of zidovudine mutations"). They frequently incorporate phrases such as "in the presence of", "in the absence of", "more/less than x mutations of (list of mutations)", which increase the complexity. Genotypic drug resistance interpretation systems consist of algorithms for currently available drugs.

In contrast to this, current database-driven systems use one specific type of information, namely that provided by correlated pairs of geno- and phenotypes. Information can, on the one hand, be extracted from the database by comparing the query sequence with all available sequences in the database and subsequently averaging the resistance of the matching samples (Virtual Phenotype™). This approach will only work if the database is large enough to provide a sufficient amount of matching samples. Similarly, the database should be divergent enough to cover all possible combinations of drug resistance-associated mutations. On the other hand, there are computer-based systems using machine-learning techniques for the prediction of resistance. One of the first approaches combined cluster analysis, recursive partitioning, and linear discriminant analysis to generate models for indinavir and saquinavir resistance within a clinical study[25]. Recursive partitioning repeatedly divides the dataset into subsets using a defined split criterion. It has also been applied for the recently presented decision tree model by using the statistical significance of each sequence position for drug resistance, the so-called mutual information, as split criterion[26]. Two other bioinformatic ap-

proaches are the support vector machines, which use multidimensional vector spaces to correlate the phenotype with certain amino acids at certain positions[27], and the neuronal networks, which have recently been applied for the prediction of resistance to a number of antiretroviral drugs[28-30]. They usually incorporate a substantially larger number of amino acid positions than the decision trees. All mathematical models have the advantage that each sequence position can be considered equally, irrespective of published data, which helps to identify new positions associated with drug resistance. However, it may be valuable to incorporate existing knowledge about drug resistance-associated mutations into the system, because rare mutations may not be adequately represented in the database and thus prediction may not be reliable (e.g. Q151M[31] in the decision tree model).

Cut-off vs continuum

An important consideration for genotypic drug resistance interpretation systems is how to present results to the user. The simplest way is to form just two categories for each drug, susceptible and resistant. However, almost all interpretation systems incorporate at least a third category (intermediate) and some use four or five categories (Table 1). The most complex output is a "virtual resistance factor" as a continuous variable. In the end, all systems are dependent on the information as to which resistance factors are clinically relevant.

Information about such clinically relevant cut-offs is still rare. Most of the available data have been produced by pharmaceutical companies for the FDA approval. These data are in part derived from patients receiving one additional drug on top of a failing regimen ("add-on studies"). These allow one to conclude which resistance level is associated with a reduced or abandoned therapy response. Since the numbers in the categories of failing patients can be very small, more data of this kind are desirable.

For abacavir, the virological response (defined as decrease in viral load of more than 0.5 log and/or below 400 copies/ml) has shown to be reduced from 71-74% to 50% and 14% for a resistance factor of 4.5-6.5 and >6.5, respectively[32]. For lopinavir, studies for the FDA approval demonstrated a significantly reduced virological response for a 10-fold reduced susceptibility to lopinavir, when PI-experienced patients were treated with lopinavir/ritonavir, efavirenz and NRTI for 24 weeks. Since the patients were *naïve* for NNRTI, it cannot be excluded that the treatment response was influenced by a second drug. For tenofovir, antiretroviral-experienced patients having ≥4-fold reduced susceptibility to this drug at baseline had a significantly lower reduction of viral load after 24 weeks of treatment with tenofovir in combination with other antiretroviral drugs[33]. More recent data, however, suggest that the level of clinically relevant resistance may be lower than a 4-fold reduced susceptibility[34]. A concern for all these data is that no standardized criteria for therapy success or failure were used, which may influence the results.

For the PIs indinavir, saquinavir, ritonavir and nelfinavir, a resistance factor above inter-assay variability has been shown to be clinically relevant in several retrospective studies[35-39]. A resistance factor below two has shown to be predictive for resistance to D4T[40]. For NNRTI, hypersusceptibility has been detected in the presence of NRTI resistance, which improved clinical response to NNRTI treatment in this group of patients[16]. The virological response to a certain drug may thus be functionally related to the IC_{50} of the virus: the lower the IC_{50} is, the higher the drug pressure. Vice versa, an increased drug pressure may overcome moderate resistance (e.g. by boosting protease inhibitors with a baby dose of ritonavir). This has been described for boosted lopinavir, but may equally be valid for other PI[41-44]. Therefore, it has to be discussed whether we should rather talk about a resistance continuum than defined cut-offs. The idea that pharmacodynamics may influence viral drug resistance is reflected by the concept of the so-called "virtual inhibitory quotient", which divides the trough level of a certain drug through the predicted phenotype and the serum-adjusted EC_{50} for wild-type HIV[45].

Genotypic drug resistance interpretation systems

Meanwhile, more than 25 interpretation systems have been developed, which vary greatly in scientific basis, clinical validation, required input and output to the user[46]. Some of these are lists of drug resistance-associated mutations that are available as look-up tables[8,11] or public websites such as the page of the International AIDS Society-USA (http://www.iasusa.org/), the Stanford HIV RT and protease sequence database (http://hivdb.stanford.edu/), and the Los Alamos HIV database (http://hiv-web.lanl.gov/content/index). Furthermore, the number of algorithms for one or several drugs is increasing[22,30,47-52]. One of the first algorithms to be used in a prospective clinical study was the Viradapt algorithm[2].

For the following detailed description and comparison, only such genotypic drug resistance interpretation systems were selected which present comprehensive systems for most of the available antiretroviral drugs, can be easily accessed via the Internet, and are regularly updated. In this rapidly evolving field, most of the information has not yet been published, but is at best accessible as conference abstracts. Therefore, the following list of interpretation systems does not claim to be complete nor does it imply that interpretation systems not included here are inferior to those presented. In the text as well as in the table, the interpretation systems are listed according to their

Table 1. *Web-based genotypic drug resistance interpretation systems*

Genotypic drug resistance interpretation system	Source data	Sequence input	Basis for interpretation (algorithm)	Categories of interpretation	Clinical studies	Link	Commercial	Access
– Stanford ß-test version	Rule-based	Nucleotide sequence, mutation list	Evident from the mutation scoring report	S/R (potential low-level/low-level/ intermediate/high-level)°	Yes[53]	http://hiv-4.stanford.edu /cgi-bin/hivtestweb.pl	No	Free
– Geno2pheno, v. 2.1	Database-driven (>600 geno-/phenotype pairs)	Nucleotide sequence	Published for decision trees[25], n.a. for support vector machines	S/R& Quant.	Yes[53]	http://cartan.gmd.de/g2p -bin/geno2pheno.pl	No	Free
– RetroGram™ v. 1.6	Rule-based	HIV substitutions*	Not given+	A/B/C/D/U§	Yes[6,53,60]	http://www.retrogram.com	Yes	Free after registration
– Rega algorithm, v. 5.5, Belgium	Rule-based	Nucleotide sequence	Published[54]	S/I/R/ND^	Yes[54]	http://www.ablnetworks. com/online/online1.html	Yes	Free for academic use after registration
– CHL v. 3.2, Luxembourg	Rule-based	Nucleotide sequence	n.a.	S/I/R/ND	n.a.	http://www.ablnetworks. com/online/online1.html	Yes	Free for academic use after registration
– ANRS AC11, v. 2000, France	Rule-based	Nucleotide sequence	[http://www.sante.gouv.fr /htm/actu/36_vih_2.htm]	S/I/R/ND	Yes[21]	http://www.ablnetworks. com/online/online1.html	Yes	Free for academic use after registration
– Detroit Medical Center, USA	Rule-based	Nucleotide sequence	n.a.	S/I/R/ND	n.a.	http://www.ablnetworks. com/online/online1.html	Yes	Free for academic use after registration
– Grupo de Aconselhamento Virológico, Brazil	Rule-based	Nucleotide sequence	n.a.	S/I/R/ND	n.a.	http://www.ablnetworks. com/online/online1.html	Yes	Free for academic use after registration
– TruGene™ kit interpretation system	Rule-based	Nucleotide sequence	Evident from the resistance report$	No evidence of resistance/possible resistance/resistance/ insufficient evidence~	Yes[53,59,60]	http://www.trugene.com	Yes	Available in conjunction with the genotyping kit
– ViroSeq™ HIV-1 Genotyping System	Rule-based	Nucleotide sequence	Evident from the resistance report	None/poss-m /poss/high-m/high	n.a.	http://home. appliedbiosystems.com/	Yes	Available in conjunction with the genotyping kit
– Virtual Phenotype™	Database-driven (>120,000 samples), rules-based for <10 matches in the database	Nucleotide sequence	n.a.	Quant.#	Yes[60-62,66,67]	http://www.tibotec-virco. com/web/home.asp	Yes	Available as commercial service for submitted sequences
– GeneSeq™ HIV	Mixture of rule-based and database-driven (>12,000 pairs of geno- and phenotypes)	n.a.	n.a.	S/R	n.a.	http://www.virologic.com/	Yes	Only available in conjunction with genotyping at Virologic

quant. = quantitative prediction of resistance, v. = version, S = susceptible, I = intermediately resistant, R = resistant, n.a. = not available

°susceptible (0-9) - virus isolates of this type have not shown reduced susceptibility to the drug; potential low-level resistance (10-14) - virus isolates of this type have mutations which by themselves may not cause drug resistance, yet indicate the possibility of previous drug selection; low-level resistance (15-29) - virus isolates of this type have reduced *in vitro* susceptibility to the drug and/or patients with viruses of this genotype may have a suboptimal virological response to treatment; intermediate resistance (30-59) - the genotype suggests a degree of drug resistance greater than low-level resistance but lower than high-level resistance; high-level resistance (>60) - the genotype is similar to that of isolates with the highest levels of *in vitro* drug resistance and/or patients infected with isolates having similar genotypes generally have little or no virological response to treatment with the drug

&arbitrarily chosen cut-offs for normal susceptible range: zidovudine (8.5), zalcitabine (2.5), didanosine (2.5), stavudine (2.5), lamivudine (8.5), abacavir (2.5), tenofovir DF (2.5), nevirapine (8.5), delavirdine (8.5), efavirenz (8.5), indinavir (3.5), saquinavir (3.5), ritonavir (3.5), nelfinavir (3.5), amprenavir (3.5), lopinavir (3.5), atazanavir (3.5)

*entered as number from 1-99 (protease) and 1-599 (RT), representing the codon position, followed by one or more amino acid codes

+references which support the ranking of drugs for the individual sample can be viewed

§ A = "can be used', B = "consider use if no class A drug not available", C = "consider use if no class A or B drug available", D = "consider use if no class A, B or C drug available ", U = "unranked, insufficient data available, phenotypic data would contribute here"

^susceptible - resulting in no restraint on the use of a particular drug; intermediate - advise against a particular drug when other options are still available; resistant - resistant or in the process of developing resistance resulting in the advice against a particular drug, ND = not defined

$The rules themselves are available on the website of Visible Genetics Inc.

~"no evidence of resistance" (mutations not associated with reduced susceptibility), "possible resistance" (mutations associated with diminished virological response in some, but not all patients or with intermediate decrease in antiretroviral susceptibility in viral isolates), "resistance" (mutations associated with a maximum reduction in susceptibility), and "insufficient evidence".

"none" (insufficient evidence for resistance), "poss-m" (possibility of multi-NRTI associated viral resistance), "poss" (possibility of viral resistance), "high-m" (a high level of genetic evidence for multi-NRTI associated viral resistance), "high" (high level of genetic evidence for viral resistance)

cut-off for normal susceptible range [55]: zidovudine (4.0), zalcitabine (2.0), didanosine (2.0), stavudine (1.8), lamivudine (4.5), abacavir (3.0), tenofovir DF (3.0), nevirapine (8.0), delavirdine (10.0), efavirenz (6.0), indinavir (3.0), saquinavir (2.5), ritonavir (3.5), nelfinavir (4.0), amprenavir (2.0), lopinavir (2.5); if rule-based interpretation is used, two categories of interpretation are given ("resistance likely" or "resistance unlikely")

accessibility, which does neither imply restraints of quality nor frequency of use. The table contains items which can be answered for all interpretation systems, whereas special features of each interpretation system are presented in the text.

– *Stanford ß-test version*

The rules for the ß-test version of the Stanford University are based on published literature about correlations between genotype and treatment history, genotype and phenotype, and genotype and clinical outcome. The sequence information can be entered as plain nucleic acid code or via a mutation list. The output lists those mutations as "resistance mutations" that have been shown to contribute to drug resistance, and "other mutations". Each resistance mutation receives a score for each drug according to the degree of resistance which is attributed to this mutation. Mutations associated with hypersusceptibility, or reversion of resistance to a certain drug, receive a negative score (e.g. M184V for resistance to zidovudine and stavudine). The sum of the scores for each drug predicts five categories of resistance (Table 1). All information about the mutation scoring for the respective sample is evident from the drug resistance output. The scoring is irrespective of whether a mutation is present in pure form or as a mixture. Comments on each resistance mutation are provided as well as information about the HIV-1 subtype.

– *Geno2pheno*

This is one of the bioinformatic approaches which was designed to predict phenotypic resistance from genotypic data. The current version 2.1 is based on more than 600 correlated pairs, which were analyzed by decision trees and support vector machines. Most of the positions and mutations identified in the decision trees have been described before. 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. The data processing is not evident for the support vector machines, however the performance is slightly better. Furthermore, support vector machines can deal with quantitative data, which allows a prediction as fold changes in IC_{50}. Geno- and phenotypic source data can be freely accessed via the Stanford website (http://hivdb.stanford.edu/). Although the system was originally designed to predict phenotypic from genotypic data, the performance for the prediction of clinical success or failure has also been recently demonstrated[53].

– *RetroGram™*

This software uses rules which are based on published correlations between genotype, phenotype and clinical response. As input, all discrepancies from the NL4-3 reference sequence have to be entered for protease and reverse transcriptase by giving the position of the mutation followed by the amino acid code. For mixed populations, more than one substitution at each position can be introduced. The drug resistance report categorizes the amino acid substitutions as relevant (appears in a drug resistance rule), natural (has been detected more than once in untreated patients), and unreported. The drugs are ranked in five categories (Table 1), which are different from other systems, as they do not predict susceptibility or resistance, but a drug's suitability for use. This decision support is particularly interesting for the situation of multidrug resistance. Version 1.4 contains rules for the use of boosted protease inhibitors (indinavir, saquinavir, amprenavir, lopinavir). The resensitizing effect of M184V on zidovudine resistance is indicated in a way that the continuous use of lamivudine is recommended to retain this mutation for synergy. HIV-1 genotyping interpreted by an earlier version of the Retrogram™ software has been shown to improve the virological outcome in a prospective trial, when it was added to the clinical information as a basis for decisions on changing antiretroviral therapy[6]. Although the RetroGram™ software is placed on the website of a commercial company, free access is possible after registration.

– *The HIV ViroScorer™*

The Rega algorithm as well as four other genotypic drug resistance interpretation systems (Centre Hospitalier Luxembourg v.3.2, Luxembourg; Agence Nationale de Recherche sur le SIDA v. 2000, France; Detroit Medical Center, USA; Grupo de Aconselhamento Virológico, Brazil) are available via the website of a commercial company. All algorithms can be accessed freely for academic use after registration. The rules of the interpretation systems are not evident from the resistance report; however, the French consensus algorithm is published in the national guidelines for the antiretroviral treatment of HIV-1 infected patients (http://www.sante.gouv.fr/htm/actu/36_vih_2.htm). The Luxembourg algorithm is continuously updated with the background of an open clinical database, containing genotypic, phenotypic and clinical data of more than 500 patients. The amino acid differences from the reference sequence NL4-3 as well as reported resistance mutations appear on the data report. "Not defined" is used as output for drugs whose resistance is not sufficiently validated by the respective algorithm or for samples without sufficient sequence information. An HIV-1 subtype determination is also provided.

The Rega algorithm was one of the first to use algorithms for the interpretation of genotypic drug resistance. From the beginning, the rules were designed to predict clinical response. Recently, it was shown that the number of active drugs determined with this interpretation system was a significant independent predictor of therapy response at three months in a cohort of patients on salvage therapy[54]. The algorithm differentiates between primary and secondary/accessory mutations: one primary mutation is usually sufficient to define resistance to a respective drug, whereas

combinations of several secondary or accessory mutations are necessary to achieve this criterion. The resensitizing effect of M184V on zidovudine resistance is included in several rules with different combinations of zidovudine mutations. The Rega algorithm predicts three categories of resistance (Table 1). It is advised not to use drugs with intermediate resistance when other options are still available, but it is pointed out that patients carrying viruses with intermediate resistance to some drugs may temporarily respond to these drugs in a HAART combination. The Rega algorithm is now available as version 5.5; a sixth version is currently designed which will contain rules for boosted protease inhibitors. The rules of the version 5.5 algorithm are published[54].

– *TruGene™ kit interpretation system*

The TruGene™ HIV-1 Guidelines™ Rules, which were approved by the FDA in 2001, are based on *in vitro* phenotypic data and *in vivo* virological response. They were developed and are semiannually updated by an independent international expert panel. The software is available in conjunction with the TruGene™ genotyping kit. The resistance report contains a list of all mutations, which are classified as "resistance mutations", "silent mutations at all positions", "polymorphisms: coding changes not at resistant sites", and "unexpected mutations of resistant sites". The relevant mutations in the protease and reverse transcriptase are incorporated into rules which are evident from the resistance report. These rules are ranked according to the evidence for the individual mutation or combination of mutations to confer drug resistance. The final report discriminates between three definitions of the resistance effect (Table 1) and insufficient evidence to determine drug resistance or susceptibility.

– *Viroseq™ HIV-1 Genotyping System*

The software of this interpretation system is available in conjunction with the Viroseq™ genotyping kit. The output consists of "reported mutations" (which are found in the Los Alamos HIV-1 resistance database) and "novel variants" (which are discrepant from the reference sequence, but do not appear in the Los Alamos database). Reported mutations are categorized in single mutations conferring resistance, single mutations conferring possible resistance, mutations conferring possible resistance in the presence of at least one other mutation, and mutations detracting from the viral resistance conferred by one or more mutations. This ranking is evident from the drug resistance report, which finally gives five levels for evidences of resistance (Table 1). Two warnings may be included, one that the detection of at least one mutation shown for this drug has not been verified by this test, the other that the utility of at least one mutation shown for this drug in the resistance interpretation has not been verified.

– *Virtual phenotype™*

It is based on a private relational database of geno- and phenotypic results from more than 120,000 samples. The database contains about 5,000 samples without any drug resistance-associated mutation in the protease, as well as about 8,500 and 15,000 samples without any mutation for resistance to nucleoside and non-nucleoside inhibitors of the reverse transcriptase, respectively. Usually, several dozens to hundreds of matching samples are identified. If less than 10 matches are detected in the database, rule based interpretation is used instead, which is the case for about 10% of predictions. This may happen for sequences containing rare mutations at positions that are used for interpretation or unusual combinations of drug resistance-associated mutations. Here, resistance is predicted as "likely" or "unlikely". The report indicates the drug resistance-associated mutations, the number of matches in the database, and the fold changes in IC_{50}, given with respect to the cut-offs for the normal susceptible range for each drug. This is based on phenotypic resistance tests on 1,000 untreated HIV-positive individuals and for several thousand samples of genetically wild-type virus[55]. The percentage of samples within normal or above susceptible range is indicated. For tenofovir and lopinavir, but not for abacavir, the percentage of samples is indicated that are above normal susceptible range, but below clinical cut-off.

– GeneSeq™ HIV

It is a proprietary consensus algorithm, which is periodically updated to reflect newly reported and novel drug resistance-associated mutations. The rules are derived from public resistance data and a private database containing more than 12,000 pairs of genotypes and phenotypes. The results are interpreted as "susceptible" or "resistant". If the phenotype is additionally determined, the resistance report includes a parallel interpretation of genotype and phenotype (PhenoSense GT™), which is based on the idea that both approaches provide complementary information[56]. Up to now, the interpretation is only available in conjunction with genotyping at ViroLogic, but sequences may be accepted in the future (N. Parkin, personal communication).

Consensus *vs* competition

How can genotypic drug resistance interpretation systems be compared reliably? One first attempt is to compare the performance of interpretation systems to predict phenotypic resistance from genotypic data[57,58]. However, most of the systems are not designed to predict phenotype but clinical response - which has to be given priority. In this respect, some studies have recently been presented which performed retrospective analyses in clinical trials[59,60]. Head-to-head comparisons indicate that the differences between the

interpretation systems may not be major, ranging from 67.2-73.6% for therapy failure and from 65.7-82.9% for therapy success[53]. However, we are still far from a final conclusion, and we will need large prospective randomized clinical trials in which only the interpretation system is different between groups. First data of this kind have been presented recently[60-62].

It may be possible that the best predictive results for all drugs are obtained by combining different rules from different interpretation systems. Therefore, existing clinical databases should be merged into one large database to allow competition between the systems. This may also solve the burning problem of clinically relevant cut-offs. In the meantime, one recent approach may be helpful: for nine different interpretation systems, the cut-offs were determined which showed the lowest error rates for the prediction of phenotypic from genotypic data in a database[63]. If an algorithm performs well for the prediction of virological success or failure, the cut-off determined by this method should be clinically relevant. If information about clinically relevant cut-offs is available, the algorithm that fits best to this cut-off can be chosen by this method.

The incorporation of clinical data into the databases requires further efforts for analysis and interpretation. Most recently, two new promising bioinformatic approaches have been presented: a non-parametric approach in the context of the new collaborative HIV resistance-response database initiative[64], and the fuzzy rules system, which allows vagueness and/or uncertainty instead of restriction to categories[65]. These systems may result in a more adequate interpretation of clinical data, which may finally lead to a consensus recommendation of the best antiretroviral therapy for the individual patient.

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