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Predictors of Virologic Response to Ritonavir-Boosted Protease Inhibitors

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Abstract

The primary mechanism of resistance to protease inhibitors involves the stepwise accumulation of mutations that alter and block the substrate binding site of HIV protease. The large degree of cross-resistance among the different protease inhibitors is a source of considerable concern for the management of patients after treatment failure. Although the output of HIV-resistance tests has been based on therapeutically arbitrary criteria, there is now an ongoing move towards correlating test interpretation with virologic outcomes on treatment. This approach is undeniably superior, in principle, for tests intended to guide drug choices. However, the predictive accuracy of a given stratagem that links genotype or phenotype to drug response is strongly influenced by the study design, data capture and the analytical methodology used to derive it. There is no definitively superior methodology for generating a genotype-response association for use in interpreting a resistance test, and the various approaches used to date all have their strengths and weaknesses. Combining the information of therapeutic drug monitoring and resistance tests is likely to be of greatest clinical utility in antiretroviral-experienced patients harboring HIV strains with reduced susceptibility. The combination of pharmacologic and virologic parameters as a predictor of the virologic response has been merged into the parameter known as "inhibitory quotient". This article discusses the potential interest of the use of inhibitory quotients as an approach for enhancing the potency and durability of boosted protease inhibitors against protease inhibitor-resistant viruses. **(AIDS Reviews 2005;7:225-32)**

Key words

Protease inhibitor. Resistance. Pharmacokinetics. Ritonavir.

Introduction

A substantial proportion of HIV-infected individuals receiving highly active antiretroviral therapy (HAART) experience a virologic failure. The development of HIV drug resistance during antiretroviral therapy (ART) can compromise the efficacy of subsequent regimens following virologic failure. The use of HIV drug-resistance testing has been shown to provide virologic benefits in randomized controlled trials[1,2]. Several studies have shown that changes in baseline viral genotype, compared to that of wild-type virus, adversely affect the virologic response of antiretroviral (ARV)-experienced subjects to a subsequent regimen[3-5]. However, the efficacy of ART can be impaired by several factors, including poor adherence to treatment regimens, suboptimal antiviral potency and/or drug concentrations, and of course selection of ARV-resistant HIV quasispecies. A significant relationship between plasma drug concentrations and virologic response has been described for several agents[6,7]. More information on the effect of these parameters should be beneficial for optimizing the use of protease inhibitors (PI) in salvage therapy.

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The inhibitory quotient (IQ), mainly used for the PIs, has been proposed as a way to integrate drug exposure and viral susceptibility. Defined as the ratio between the trough concentration (C_{trough}) of a drug in a patient and the susceptibility of the virus in the patient to that drug, the IQ has been associated with virologic response to PI-based ART in several studies. The susceptibility of the virus has been initially expressed as the plasma protein-corrected *in vitro* IC_{50}, as determined by a phenotypic assay, or alternately by the virtual phenotype[8,9]. However, genotypic scores (based on the number of baseline mutations out of a cumulative number of mutations that were found to be associated with lowered rates of virologic response) are other ways to evaluate the magnitude of PI resistance[10]. Recently, in several studies, the genotypic inhibitory quotient (GIQ, ratio of PI C_{trough} to number of baseline PI mutations) was shown to be a significant predictor of the virologic response to a PI-containing regimen[11-15]. This article discusses the potential interest of the use of GIQ as an approach for enhancing the potency and the durability of boosted PI against PI-resistant virus.

Resistance to protease inhibitors

Antiretroviral resistance due to viral gene mutations accounts for a large portion of treatment failures. The emergence of these genetic changes in HIV is fostered by ongoing viral replication in the presence of sub-inhibitory concentrations of ARV. This, in turn, may allow for selection of preexisting (archived) drug-resistant mutants. The primary mechanism for PI-resistance involves the stepwise accumulation of mutations that alter and block the substrate binding site of HIV protease. The critical problem in the clinical setting is that a mutant selected for by a failing regimen may have some degree of cross-resistance to other drugs in the same class that have not yet been prescribed to that patient. The development of this cross-resistance may lead to a reduced virologic or immunologic response to subsequent regimens. As scientists develop new agents active against resistant virus, clinical medicine is also implementing diagnostic strategies designed to detect ARV resistance and individualize subsequent regimens. Identification of the presence of drug resistance by means of genotypic or phenotypic resistance assays can help a healthcare provider select a combination of ARV that is likely to suppress HIV-1 replication (i.e. “active drugs” to which that patient’s virus population is not cross-resistant). To maximize the therapeutic benefit and minimize toxicity, information collected from the viral genotype or phenotype must be used in conjunction with the patient’s ART history, response to past regimens, immunologic status, pharmacologic data, and the clinician’s own knowledge of ARV drugs.

The large degree of cross-resistance among the different PIs is a source of considerable concern for the management of patients after first-line treatment failure. Numerous studies have documented associations between baseline PI resistance and virologic response in PI-experienced patients[3,10,16]. A few key studies are summarized here.

Two cohort-based studies examined the response to ritonavir/saquinavir in PI-experienced patients. Harrigan, et al. found that baseline genotype and phenotype predicted poor response in a population of 76 patients, despite the confounding effects of other ARV received[17]. Even as small as a fourfold decrease in susceptibility to either saquinavir or ritonavir was sufficient to compromise response. Zolopa, et al. examined response to ritonavir/saquinavir-based HAART in 54 patients with previous PI treatment failure[5]. They evaluated seven major PI mutations (30, 46, 48, 54, 82, 84, and 90) as predictors of response and found that all except position 84 significantly predicted response. Minor mutations at codons 10, 19, and 71 were also associated with response. According to a detailed analysis of all possible variables influencing response (clinical and treatment history as well as baseline genotype), baseline genotype (including major and minor PI mutations) explained 66% of the variation in outcome. When combined with clinical information, baseline genotype remained the strongest predictor of response, whereas clinical information did not contribute significantly to the prediction. Interestingly, in this study, the presence of D30N was positively associated with response, whereas all other mutations were negatively associated with response, as would be expected.

Kempf, et al., studied the response to lopinavir/ritonavir in 57 PI-experienced patients[18]. In univariate analysis, virologic response was associated with baseline phenotype and genotype (lopinavir mutation score: L10F/I/R/V, K20M/R, L24I, M46I/L, F53L, I54L/T/V, L63P, A71I/L/T/V, V82A/F/T, I84V and L90M). Virologic response was observed in 91, 71 and 33% of subjects with baseline lopinavir mutation score of 0-5, 6-7 and ≥ 8, respectively. However, other factors were predictors of virologic response such as the number of years since HIV diagnosis, nucleoside reverse transcriptase inhibitor (NRTI) phenotypic sensitivity score, number of new NRTI, and the baseline viral load. In a stepwise logistic regression (excluding baseline lopinavir phenotype, since the phenotype and the mutation score are highly correlated, and thus the two parameters could not coexist in the model), the lopinavir mutation score was a significant predictor to all time points (24, 48, and 72 weeks).

The impact of PI resistance at baseline on a salvage regimen depends, in part, on the potential activity of the new regimen. If the new regimen includes a drug class to which the patient is naive, the impact of PI mutations may not be as strong. This was shown in a study by Deeks, et al., in which patients with indinavir or ritonavir failure were randomized to receive nelfinavir, saquinavir, and abacavir plus either nevirapine or another NRTI[19]. In this case, addition of a nonnucleoside reverse transcriptase inhibitor (NNRTI), to which all patients were naive, produced significantly greater virologic suppression at 24 weeks ($p = 0.04$) than the addition of another NRTI. This suggests that studies evaluating the determinants associated to the virologic response to a given drug should avoid patients receiving, in addition to the drug studied, a new ARV class in their treatment.

Because of the low response rate after PI-treatment failure, several investigators have turned to the option of combining more than the standard three or four drugs. Some investigators have used combinations of up to nine individual drugs (often referred to as mega- or giga-HAART) in patients with multiple previous treatment failures[20,21]. Montaner, et al. reported on the largest set of patients (n = 250) treated with six or more drug combinations in three different cohorts of heavily pretreated patients[22]. The overall response rate was 30-50% (defined as viral load < 400 copies/ml) and response was associated with baseline resistance. In a subanalysis of 59 patients, these investigators reported that virologic response was related to the number of effective agents taken, with a greater viral load drop for each additional effective drug (up to three). Interestingly, patients with five to seven effective agents did not respond as well as those with only one to three effective drugs. This suggests that the virus population in these patients was not exposed to sufficiently high levels of these drugs for resistance selection to occur. Miller, et al.[23] reported on the impact of resistance and response to mega-HAART in 50 heavily pretreated patients. Most patients received six drugs, but 10% of patients received eight or more. All patients received a minimum of two PIs, including ritonavir. In this study, the total phenotypic sensitivity score as well as the plasma viral load were significant independent predictors for virologic failure (> 400 copies/ml at week 24). Resistance to PIs was a stronger predictor for virologic failure than NRTI resistance.

These selected studies show that baseline resistance and cross-resistance among PIs produce a significant impact on treatment response in second-line or salvage regimens. Other factors, such as baseline viral load, treatment history, and number of new drugs (or drug classes) may also play an important role, but in many studies, resistance proved to be a strong independent predictor. The fact that both genotype (or phenotype) as well as baseline viral load proved to be the strongest predictors in a set of standardized analyses, implies that continuing treatment after virologic failure has set in diminishes the chances of a salvage regimen being successful.

Methods to determine the virologic parameters involved in the response to PIs

Now that virologic benefit has been established for selecting antiviral drugs based on baseline resistance mutations, attention is being focused on ways of determining clinically relevant interpretation rules or algorithms[24,25]. The difficulty in building these rules includes the presence of undetected/archived/minor species, the complexity and variability of resistant variants, interactions between mutations, and the lack of standardized statistical methods. A major concern in this field is the choice of statistical methodology. Different statistical methods have been used including non-parametric tests, regression modeling, discrimination analysis, recursive partitioning, and artificial neural networks. A simple approach, however, that has been widely used consists of defining a genotypic score as a simple sum of resistance mutations[26,27]. The combination of mutations defining the score is selected (by a nonparametric test) as the one providing the strongest association with virologic response. Combinations of mutations are investigated from a list of mutations selected by a univariate approach, comparing patients with and without the corresponding mutation. The two key components of such analyses are the nonparametric test used and the procedure of selection. The Kruskal-Wallis nonparametric test is widely used, but is not well adapted to this setting because the groups or samples determined by the number of mutations should be treated as an ordered variable, and the Jonckheere-Tepstra (JT) test, also known as the Jonckheere test, should be used in preference[28]. Indeed, we expect better virologic response in patients with no mutation than in patients with one, who are expected to have a better virologic response than patients with two mutations, and so on. The JT test is appropriate as it is specifically designed to test the null hypothesis against an ordered alternative.

Type of study

There are two types of studies that are commonly used to investigate predictors of virologic response to boosted PIs and also to the antiviral regimen in general. The first is a controlled clinical trial, double-blind, blind, or open, that involves randomization of a relatively large number of patients into two or more groups of ARV regimens. The trial is either an add-on study where a single drug is added to a stable failing regimen, or a study implying a complete regimen change, more commonly employed as reflecting usual clinical practice[29]. The search for predictive factors of virologic response focused on the specific drug used in ARV regimens, baseline drug resistance, adherence to treatment regimen, baseline CD4, and baseline viral load.

Observational cohort studies are also employed to investigate predictors of virologic response[30]. Data from observational studies are more complex as treatment management and individual patient's assessments are not as structured. In particular, investigation of the treatment regimen as predictor of virologic response may be misleading when a new regimen is initiated after treatment failure. The larger number of patients, however, allows investigation of other potentially predictive factors such as gender, age, race, and transmission groups.

Methods used to measure virologic response

Once again two types of virologic responses are used in cohort studies or clinical trials. This choice is very important, not only because different virologic endpoints may correspond to slightly different objectives, but also because statistical methods used to analyze the datasets are driven by the choice of the endpoint[31]. The first type is continuous and measured by the HIV-RNA change from baseline to a pre-specified primary follow-up time, usually the end of the study. This implies that a 2 $\log_{10}$ decrease is considered as a better virologic response than 0.5 $\log_{10}$ decreases, but also than a 1.9 $\log_{10}$ decrease. The difficulty is that the existence of a lower limit of quantification leads to partial observation of the reduction or censoring[32,33]. For example, if we consider a patient having an HIV-RNA measurement of 5000 copies/ml at baseline, and a measurement below the limit of quantification of 500 copies/ml at the primary follow-up time, then for this patient, the exact reduction in HIV-RNA level is unknown; however, it is at least 1 $\log_{10}$ copies/ml. Several papers deal with the analysis of such an endpoint suggesting methods used in the analysis of censored survival data[32-34].

The second type is discrete, such as virologic response or virologic failure, and may be time-dependent or not. Time-dependent events are either purely virologic endpoints or combined endpoints[35]. A purely virologic endpoint is time from randomization, or entry in the study, to virologic failure defined as a confirmed rise in HIV-RNA above a threshold of 500 copies/ml[35]. Virologic failure may also include lack of initial virologic response or early virologic relapse. A regimen termination endpoint is defined as time from randomization to earliest event of virologic failure, permanent study treatment discontinuation, AIDS-defining event, and death. A complete discussion on the advantages and shortcomings of each endpoint has been recently published[35]. Endpoints based on suppression of HIV-RNA levels below the limit of quantification are not time-dependent. These endpoints have become increasingly popular in light of recommendations that the goal of ART is to suppress the virus to unquantifiable levels. A discrete virologic response may also be defined as a drop of 1 $\log_{10}$ or more at a pre-specified follow-up time[36].

HIV-RNA reduction

As mentioned above, survival methods are generally used to analyze censored HIV-RNA reduction. When the censoring is moderate, the Kaplan-Meier estimator may be successively used to estimate median HIV-RNA reduction. However, the assumption of non-informative censoring should be evaluated before the use of many survival methods. In the setting of HIV-RNA reduction, the hypothesis corresponds to the assumption that the magnitude of reduction is not related to the baseline level. Although it seems that the lower the baseline level is, the higher the probability to be censored, and the non-informative assumption was found in many clinical trials[32,33]. When the censoring is "informative", parametric models can be used instead. When the percent of censoring is important, it has been shown that the Kaplan-Meier leads to overestimate the HIV-RNA reduction, and that method for interval-censored data should be used[34].

In certain cases, however, the percent of patients censored at the lower limit of quantification of the assay is low and the naive method can be used. The naive method is to define all HIV-RNA levels below the limit of quantification to be equal to the limit of quantification. In this situation, nonparametric tests (Kruskal-Wallis) are often used to investigate the association between some categorical variables and the viral load reduction.

Time-dependent virologic response

For both purely virologic and regimen termination endpoints, survival methods are employed, including Kaplan-Meier estimate, log-rank test and Cox proportional hazards regression model. Kaplan-Meier and log-rank are distribution-free methods used in clinical trials, while Cox regression models, allowing the inclusion many factors, are specifically appropriate for multivariate analyses. Although these methods are not complex, they generally require the validation of different assumptions, and therefore a statistician is needed.

Time-independent virologic response

This virologic response is defined as HIV-RNA below a certain threshold value, or having a drop ≥ 1 $\log_{10}$ copies/ml. In this situation, the Fisher's exact test is often used to compare the proportion of virologic response according to some categorical variables. The Wilcoxon test is used to investigate whether a continuous variable (e.g. the baseline HIV-RNA) is associated to the virologic response. As in the above situation, nonparametric tests are limited to univariate analysis, although when the sample size is large, stratified tests can be used to investigate the interaction between two or more variables. When multivariate analysis is needed, logistic regression is frequently used. Note that logistic regression can be used when the response variable is binary (virologic or non-virologic response), but also when the response is defined with more than two categories, e.g. complete response, partial response, and no response. When a final multivariate model is needed, the first step is to select in a series of univariate models the variables that are predicted to the virologic response ($p < 0.20$ or 0.10) and then to use a stepwise or backward selection to retain the final multivariate model.

New methods adding pharmacologic information to enhance the prediction of virologic response: the use of inhibitory quotients (IQ) of PIs

Combining the information of therapeutic drug monitoring (TDM) and resistance tests is likely to be of greatest clinical utility in ARV-experienced patients harboring HIV strains with reduced susceptibility. The combination of pharmacologic and virologic parameters as a predictor of virologic response has been combined into the parameter defined IQ[37]. The use of this PK/PD parameter may overcome one of the reasons for not performing TDM in experienced patients, which consists of the fact that determination of optimal drug concentrations in salvage therapy settings remains complex, with experts not agreeing on optimal target values. While the numerator of the formula to calculate the IQ has most frequently been the C_{trough}, taken as the concentration measured at the end of the dosing interval, debate is still ongoing on the denominator. Different IQ have been measured in different studies, showing that these are related to ARV effect and able to predict virologic responses[8,11,13,37,38].Originally, the IQ was considered as the ratio between the C_{trough} and the IC_{50} of the virus isolated from the patient. However, the cost and the lack of a standardized method to perform phenotype tests are major drawbacks for the use of the IC_{50} in routine clinical practice. Despite this, a recent study performed in multiple NNRTI- and PI-experienced patients on high doses of lopinavir/ritonavir aimed at investigating the predictors of response at week 48, has shown that in such a population, significant independent predictors of response were the number of NRTI-related mutations and the IQ value[38].

A different approach may be based on the virtual IQ (vIQ), calculated by dividing the C_{trough} value by the virtual phenotype (as the expected fold change in viral susceptibility associated with a particular genotypic mutation pattern) multiplied by the serum-adjusted wild-type HIV-IC_{50} value[37]. A further way to calculate the IQ, which involves results from phenotypic assays as a denominator, but does not require protein binding corrections, is the normalized IQ (nIQ). This concept reflects the ratio of measured drug concentration to drug susceptibility divided by the ratio of reference drug concentrations to drug susceptibility, where drug concentrations are obtained from reference studies or from population-based reference measures, and reference drug susceptibility is the resistance cutoff given by the phenotypic assay[39,40]. The nIQ of amprenavir has been shown to predict virologic outcome in experienced patients on amprenavir plus lopinavir/ritonavir and an NRTI backbone, previously failing three drug classes[40]. The importance of the genotypic IQ (GIQ), (the ratio between drug C_{trough} and the number of mutations in the protease gene) has also emerged in different studies[13-15]. The IAS guidelines for drug-resistance testing[24] report that prospective randomized trial data indicate short-term virologic benefits for both genotypic and phenotypic drug-resistance testing[6,41-47], although evidence is stronger for genotypic testing. There is, therefore, a trend to think that genotype testing provides better results and this would support the use of GIQ, which is less costly. Currently used GIQ take into consideration all protease mutations known to be associated with resistance. Despite the potential limitation that some mutations impact more than others on the virologic response, the usefulness of the GIQ as the predictor of response has been already shown by different studies and for different drugs. For amprenavir, the use of GIQ in experienced patients is better at predicting virologic response at week 12 than the use of genotype or drug concentrations independently[15]. In the CONTEXT study, a GIQ12 > 250 at week 12 was independently associated with virologic response to fosamprenavir/ritonavir[48]. For lopinavir in highly experienced patients, a positive correlation was found between GIQ and the drop in viral load at 12 months[13]. This was confirmed by a study in 74 patients where a higher GIQ was associated with a higher probability of achieving an undetectable viral load after six months of treatment[49]. For atazanavir (unboosted), a higher HIV-RNA reduction was associated with a higher GIQ[11]. Finally, for lopinavir/ritonavir/saquinavir, plasma concentrations impacted on virologic response consistently, with nonresponders having significantly lower saquinavir AUC, C_{min} and C_{max}, lopinavir C_{min} and ritonavir C_{min}, confirming that when higher concentrations constitute the numerator of the IQ, therapeutic response is more likely to be achieved[50]. Reduced drug susceptibility may be overcome by achieving sufficient drug potency and exposure. Therefore, a logical and simple approach to manage resistance would be to increase drug exposure to a level sufficient to inhibit any HIV-resistant strain. This should be possible, since resistance to the majority of existing PIs has been shown *in vitro* to be a continuum rather than a discrete phenomenon[51]. However, despite drug exposure being the primary determinant of virologic response to treatment, the development of drug-related toxicity must be taken into consideration. As discussed, different ARV have shown a clear concentration-related toxicity[52,53]. For these agents, doses must not be increased above a certain limit. Whether higher doses of drugs are associated with a more durable HIV suppression and confer a lower probability of the emergence of resistance is still unclear. As such, when C_{trough} are achieved and maintained over time, these may not ensure an optimal long-term treatment response when just over the effective minimum concentration (C_{min}), compared with those drugs which achieve concentrations much higher than the minimum needed to inhibit viral replication. In this scenario, it is worth noting that drugs such as boosted PIs are characterized by a high genetic barrier. Moreover, it has been shown that when treatment-naive patients fail lopinavir/ritonavir twice daily, fosamprenavir/ritonavir once daily and atazanavir/ritonavir, this happens without any evidence of genotypic or phenotypic resistance[54-56]. It has been speculated that the reason relates to the high genetic barrier associated with drugs characterized by short half-lives (such as PIs/ritonavir), and which persist in plasma for short periods of time after interruption. This confirms the importance of achieving high IQ values.

In the context of giga-HAART therapy, using several boosted PIs, it has been shown that the GIQ of each PI used in the regimen was not associated with virologic success. However, the sum of PI GIQs was predictive of a virologic response, suggesting that pharmacologic enhancement might overcome viral resistance and that there is some benefit in adding the activity of several boosted PIs to improve the response to a salvage regimen[57].

Conclusion

There is increasing evidence that virologic parameters measuring resistance to ARV and PK parameters are involved as determinants of virologic response to boosted PI-containing regimens. This has to be taken into account in analyses performed to determine new algorithms for the interpretation of genotypic resistance tests and to analyze the determinants of virologic response in clinical trials.

There still are gaps and challenges for the use of TDM in clinical practice. This includes the knowledge of therapeutic ranges for ARV. These ranges of concentration need to be established for wild-type HIV and for viruses with reduced susceptibility. Thus, this approach could be used in therapeutic drug monitoring to define the plasma concentration needed to control replication of viruses at different stages of PI resistance. The GIQ approach, using results of assays widely available (C_{min} measurements and genotypic resistance testing), has shown its usefulness in retrospective studies. Randomized prospective studies are now warranted to confirm the IQ role in preventing virologic failure.

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