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Genotypic Susceptibility Scores and HIV Type 1 RNA Responses in Treatment-Experienced Subjects with HIV Type 1 Infection

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Abstract

This study compared the role of genotypic susceptibility scores (GSS) as a predictor of virologic response in a group (n = 234) of HIV-infected, protease inhibitor (PI)-experienced subjects. Two scoring methods [discrete genotypic susceptibility score (dGSS) and continuous genotypic susceptibility score (cGSS)] were developed. Each drug in the subject's regimen was given a binary susceptibility score using Stanford inferred drug resistance scores to calculate the dGSS. In contrast to the dGSS, the cGSS model was designed to reflect partial susceptibility to a drug. Both GSS were independent predictors of week 16 virologic response. We also compared the GSS to a phenotypic susceptibility score (PSS) model on a subset of subjects that had both GSS and PSS performed, and found that both models were predictive of virologic response. Genotypic analyses at enrollment showed that subjects who were virologic nonresponders at week 16 revealed enrichment of several mutated codons associated with nucleoside reverse transcriptase inhibitors (NRTI) (codons 67, 69, 70, 118, 215, and 219) or PI resistance (codons 10, 24, 71, 73, and 88) compared to subjects who were virologic responders. Regression analyses revealed that protease mutations at codons 24 and 90 were most predictive of poor virologic response, whereas mutations at 82 were associated with enhanced virologic response. Certain NNRTI-associated mutations, such as K103N, were rapidly selected in the absence of NRTIs. These data indicate that GSS may be a useful tool in selecting drug regimens in HIV-1-infected subjects to maximize virologic response and improve treatment outcomes.

Introduction

Antiretroviral therapy (ART) has been well documented to decrease HIV-1 RNA viral load as well as HIV-1-associated morbidity and mortality.[1-5] Unfortunately, virologic rebound commonly occurs in treatmentnaive and treatment-experienced patients.[6-8] Persistent viral replication in the setting of drug selection can lead to the appearance of amino acid substitutions that confer resistance to the current regimen. Thus, identification of patients with acquired drug resistance mutations is critical to achieve virologic suppression and

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improve patient outcome. To this end, testing for resistance to antiretrovirals is standard of care, and current guidelines recommend resistance testing for treatment failure during chronic infection.[9,10]

Resistance testing is important in guiding the medical management of HIV-1-infected individuals and has been shown to improve virologic response,[11-15] but it remains unclear which method of resistance testing is most useful. Currently, there are three methods to evaluate HIV-1 resistance: genotype, phenotype, and virtual phenotype. Determining which interpretation model is most sensitive and valid is a subject of ongoing intense investigation as there are advantages and disadvantages to each. Improved strategies to interpret viral resistance are necessary to predict the complex relationship between drug effects and virologic and immunologic outcomes.

In the current study, we extended our analyses of the AIDS Clinical Trials Group (ACTG) Study 359, a study of treatment-experienced patients who underwent drug resistance testing prior to receiving a regimen based on a combination of two new protease inhibitors (PIs) (a detailed summary is given in Materials and Methods). In doing so, we have tested two scoring methods [discrete genotypic susceptibility score (dGSS) and continuous genotypic susceptibility score (cGSS)] to explore the relationship between the genotypic resistance pattern at the time of study entry and virologic response through week 16.

Materials and Methods

ACTG 359 study subjects

The results of the original clinical study have been published previously.[16] Written informed consent was obtained from all patients or their guardians, and the human experimentation guidelines of the U.S. Department of Health and Human Services and the individual institutions were followed in conducting this research. Briefly, ACTG 359 was a randomized, partially-blinded, multicenter 2 × 3 factorial trial that was designed to test the efficacy of two new PIs, ritonavir (RTV) and saquinavir (SQV) or nelfinavir (NFV) and SQV, in conjunction with a new nucleoside reverse transcriptase inhibitor (NRTI) [adefovir dipivoxil (ADV)] or NNRTI [delavirdine (DLV)], or both ADV and DLV in treatment-experienced subjects who had experienced virologic failure while taking indinavir (IDV). Eligible subjects had an HIV-1 RNA viral load of 2000–200,000 copies/ml (Amplicor HIV-1 Monitor test version 1.0; Roche Diagnostic Systems). Subjects were assigned to study therapy for a minimum of 24 weeks. Clinical assessments and routine laboratory tests were performed at baseline and every 4 weeks through week 16, and at 8 week intervals thereafter.

Genotypic analyses

Plasma was obtained at each visit and assayed in real time for HIV-1 RNA (Roche Amplicor HIV-1 Monitor test) at a central laboratory at University of North Carolina or University of Washington. HIV-1 viral RNA was extracted from EDTA-treated plasma using the QIAamp viral RNA isolation kit (Qiagen). RT-PCR and sequencing were performed using the TRUGENE™ HIV-1 Genotyping kit from Bayer Diagnostics, according to the manufacturer's instructions, to identify the presence of resistance-associated mutations in the *pro* gene (encoding the viral protease) and over the first 240 codons of the *pol* gene (encoding the viral DNA polymerase reverse transcriptase). Sequences were analyzed using the Bayer Diagnostics OpenGene system, version 3.1.5 software. Amino acid sequences were deduced and mutations associated with NRTI, NNRTI, or PI resistance were reported with respect to wild-type virus HXB2.

Statistical analyses

We explored two scoring methods to describe the relationship between antiretroviral resistance and therapy outcome. In each method, a resistance score was calculated using the Stanford Inferred Drug Resistance Scores,[17] version 4.2.1, to assess the impact of resistance mutations on predicted drug susceptibility; GSS to RTV was based on version 4.2.0, the last version that still included RTV as a PI drug. Susceptibility to each drug in the study regimen was considered. In the first method, a discrete value of 1 or 0 was given if the Stanford score was below 30 (susceptible) versus 30 or above (resistant). The values (0 or 1) for each of the drugs in the subject's regimen were summed to create dGSS. Since resistance scores to ADV are unavailable in Stanford rules and previous phenotypic analyses have shown ADV to be a less potent drug, a resistance score for ADV was set to 0 in the dGSS calculation; RTV was assigned a weight of 0.5 for being used as a PI booster.[18]

In the second method, the cGSS was generated to reflect partial susceptibility to a drug. Intermediate susceptibility scores between 1 and 0 were calculated if the Stanford resistance score ranged from 10 to 60 [(60 – Stanford resistance score) ÷ (60–10)]. A score of 1 was assigned to a sequence if the Stanford resistance score was less than 10, and 0 if 60 or greater. Similar to the dGSS scoring method, a weighted sum of the individual values for each drug was generated for the cGSS.

Univariate and multivariate logistic-regression models were used to examine the association between the GSS and the primary study end point (HIV-1 RNA ≤500 copies/ml at week 16). The predictive value of the GSS was evaluated by use of univariate and multiple censored regression models under the assumption of normal error distribution.[19] Descriptive analyses of mutation patterns were performed at baseline and in the subjects who were virologic nonresponders at week 16 (HIV-1 RNA >500 copies/ml at week 16). Exact Chi-square test or Fisher's exact test was used for comparison of proportions across groups and the Kruskal–Wallis test for comparison of continuous variables across groups. Significance levels were set at $p = 0.05$. To relate baseline mutational patterns to week 16 virologic response, two sets of analyses were performed by either excluding subjects whose week 16 virologic responses were missing, or treating missing week 16 virologic responses as nonresponders.

To dissect further the relationship between GSS, magnitude of entry HIV-1 RNA, and virologic response, additional analyses were performed on subjects who were identified as compliant failures (subjects with a transient decrease in viral RNA load and the maintenance of baseline resistance mutations and/or the appearance of new resistance mutations) or virologic responders; compliant failures had either new mutations at week 16 compared to baseline or retained baseline PI mutations at week 16. Subjects with low baseline viral loads (<10,000 copies/ml) were excluded. The remaining subjects were categorized into two groups according to baseline HIV-1 viral RNA (10,000 to 50,000 copies/ml or >50,000 copies/ml). Since the nadir HIV-1 viral RNA during the first 16 weeks for many subjects was below 500 copies/ml, the maximum viral load reduction was left censored. Censored regression models were used by assuming a normal error distribution. No *p*-value was adjusted for multiple testing.

Results

Baseline genotypic resistance analysis

Baseline sequence data were obtained from 234 of the 277 subjects enrolled in the trial (84%), with the sequences well distributed between the arms [$N = 34$ to 42 among the different arms with 34 in Arm A (SQV + RTV + DLV), 42 in Arm B (SQV + RTV + ADV), 37 in Arm C (SQV + RTV + DLV + ADV), 41 in Arm D (SQV + NFV + DLV), 41 in Arm E (SQV + NFV + ADV), and 39 in Arm F (SQV + NFV + DLV + ADV)]. Of note, the

treatment-experienced ACTG 359 study subjects had a median prior IDV use of 13.8 months, median CD4 cell count at entry of 228 cells/mm^3, and mean HIV-1 viral RNA load at entry of 32,297 copies/ml (4.51 $\log_{10}$ copies/ml). Overall, 30% (77 of 254) of subjects had HIV-1 RNA levels of ≤500 copies/ml at week 16 (virologic responders). In addition, sequence data were obtained for 141 of the 177 subjects (80%) who had experienced virologic failure with plasma viral RNA levels greater than 500 copies/ml at week 16 (virologic nonresponders). The drug regimens in this trial had the potential for strong selective pressure from the PIs and from a potent NNRTI. Thus the analysis of the impact and evolution of resistance using this trial represents an opportunity to examine relevant descriptive and statistical approaches that are generally applicable.

The NRTI resistance mutations and PI resistance mutations present at baseline are summarized in Tables 1 and 2. Resistance mutations in RT to NRTIs were largely to lamivudine (M184V) and zidovudine (M41L, K70R, L210W, T215F/Y, and K219Q).[20] The M184V mutation was present in 85% of subjects and a mutation at position 215 was present in 59% of subjects. These mutation patterns are consistent with high levels of resistance to lamivudine and a wide range of resistance to zidovudine seen in the phenotypic resistance analysis of a subset of these same subjects.18 The D67N mutation occurred in 40% of the subjects. In contrast, the 69 insertion complex, associated with multi-NRTI resistance,21 was not detected in any of the baseline sequences. Resistance mutations to NNRTIs were absent as would be expected given the NNRTI-naive status for trial entry.

The baseline resistance mutations in protease were dominated by primary resistance mutations M46I/L, I54V, V82A/F/T, and L90M (Table 2). Secondary or compensatory mutations that were most notable were L10I, L24I, M36I, L63P, and A71V/T. Positions 46 and 82 both had approximately 50% mutation prevalence in baseline samples. These observations are consistent with phenotypic studies, which reveal that a substitution at residue 82 is most frequently associated with IDV resistance,[22] and that a sensitive predictor of IDV resistance can be obtained if substitutions at either residue 46 or 82 are considered. Havlir *et al.* have previously shown that virologic failure to an IDV-containing regimen can occur in the absence of resistance mutations, again consistent with only 50% of subjects in this cohort having resistance mutations in the protease coding domain at entry.[23]

Correlation between GSS and therapeutic response

A series of logistic regression models were used to determine the relationship between baseline GSS and week 16 virologic response. With dGSS or cGSS as the only variable for week 16 outcome, we found that both scoring schemes strongly predicted virologic response at week 16 ($p = 0.0022$ or 0.0015, respectively). Each additional unit increase in the dGSS increased the odds of virologic response by about 2-fold, and 2.5-fold with the cGSS. However, entry viral RNA load was a strong determinant of virologic response and an effect modifier of drug resistance markers. Thus, when the dGSS and the baseline RNA were assessed as independent variables, both were found to be independent predictors of virologic response ($p = 0.002$ and 0.0007, respectively). Like-wise, a similar result was obtained when the cGSS and baseline viral RNA load were assessed as independent variables, with even smaller p values ($p = 0.009$ and 0.005). Therefore, both the dGSS and cGSS were significant predictors of virologic outcome at week 16, independent of entry viral RNA loads. The logistic regression models excluded the 18 subjects with missing week 16 HIV RNA outcomes (missing excluded). When identical models were generated treating the missing 18 subjects' week 16 outcomes as nonresponders, similar results were obtained, although the p values for the GSS increased 3- to 4-fold (data not shown).

There are a variety of clinically available genotypic drug-resistance interpretation systems, and the most commonly used are the Stanford HIV RT and Protease sequence data-base,

Geno2pheno, Rega, ANRS, and virtual phenotype.[24-26] These are based on genotype–phenotype correlations, known clinical outcomes, rule-based algorithms, and/or expert opinion. In the current study, we found that two different GSS based on the Stanford HIV RT and Protease sequence database, the dGSS and cGSS, were independent predictors of virologic response. However, there are some limitations in using the GSS to predict viral outcome. First, scores are scaled from 0 to 1 for all drugs, and do not adjust for individual or combined drug potency. Second, since resistance scores to ADV are unavailable in Stanford rules and ADV has been shown to be a much less potent drug *in vivo*, a score for ADV susceptibility was set to zero. However, any ADV effect was likely small, as our data did not reveal any significant difference in the appearance or disappearance of NRTI mutations in the groups treated with or without ADV (see below). Third, since RTV was used as a PI booster, it was assigned an intermediate weight of 0.5 for both GSS models. Fourth, neither model can account for complex virus-host interactions or host differences in pharmacokinetics. Fifth, minor variants, i.e., those comprising less than 30% of the viral population, were not accounted for in the genotypic test used, and this is especially relevant as 50% of subjects taking IDV at entry showed no resistance mutations in the protease gene. Even with these limitations the GSS models were significantly correlated with outcome, confirming previous studies of the utility of genotypic information in assessing the likelihood of therapeutic response.[11,12,27-29]

Comparison of the predictive value of GSS versus PSS

Previously, we described a retrospective analysis of the utility of phenotypic characterization of drug resistance at entry as a predictor of therapy outcome;[18] a phenotypic susceptibility score (PSS) was determined on a total of 142 subjects within the ACTG 359. The PSS was found to be a significant predictor of virologic response at week 16. There were 125 subjects with both baseline genotypic and phenotypic analyses, and week 16 HIV-1 RNA results available. The Spearman correlation between the value of the cPSS and the value of the cGSS was 0.79 ($p = 0.0001$). Using logistic regression analysis and correcting for baseline viral RNA load, we found that both the cPSS and cGSS were significant predictors of virologic response in this subset of subjects, with the cGSS having a moderately smaller *p*-value in this analysis (0.009 vs. 0.025) (Table 3).

The methodology of genotypic or phenotypic resistance testing has been a subject of previous study.[18,25,30-38] Our analysis adds to several trials that have attempted to compare directly these two different methods. For the majority of these studies, the clinical outcomes have been similar regardless of the methodology: phenotype vs. virtual phenotype[39,40] and genotype vs. virtual phenotype;[33,41] however, in a subset of HIV-1-infected subjects with PI experience, a greater proportion of subjects achieved virologic suppression using genotypic testing vs. phenotypic testing[42] to guide antiviral selection. Although the optimum interpretation system has yet to be determined, our data suggest that the genotypic susceptibility scores perform similarly to the phenotypic susceptibility score in being correlated with virologic outcome.

Resistance mutations present at entry that are overrepresented, lost, or newly evolved in the subjects who experienced virologic failure at week 16

Among the 216 subjects with available baseline genotypic data and week 16 HIV-1 RNA data, 67 of 216 (31%) had a favorable virologic response (week 16 HIV RNA ≤500 copies/ml). To determine the relationship between resistance mutations present at entry and virologic response, we compared the frequencies of wild-type or mutant codons at week 16 for those subjects with and without a virologic response (Tables 1 and 2). For all comparisons, a two-sided Fisher's exact test was used, and missing data were excluded. Consistent with the overall week 16 response rate of 30% in the trial, the baseline wild-type

sequences partitioned at a 2:1 ratio between the groups that experienced virologic failure vs. response (except as outlined below). Of note, there were no NNRTI resistance mutations present at entry to include in the analysis, and all subjects discontinued NRTI therapy with the exception of subjects who received the minimally potent ADV.

NRTI resistance mutations

At baseline, there were several positions in the RT coding domain that were overrepresented in the subjects who were virologic nonresponders at week 16 (Tables 1 and 4). Subjects with mutations at position 67 (D to E/G/N), 69 (T to A/D/N/S), 70 (K to R/G/E), and 118 (V to I) were significantly enriched in those who were virologic nonresponders ($p = 0.005$, 0.005, 0.02, and 0.013, respectively). In addition, although at positions 215 and 219, there were similar rates of wild-type vs. mutant codons present in the virologic responders and nonresponders, there was a statistically significant overrepresentation of the thymidine analog resistance mutations T215F (16% vs. 1.5%) and K219Q (17.5% vs. 4.5%) in the virologic nonresponders vs. the virologic responders ($p = 0.002$ and 0.015, respectively). However, the more common T215Y mutation partitioned equally among the responders and nonresponders. The enrichment of T215F and K219Q in the subjects who were virologic nonresponders correlates with the differential evolution of the TAM-1 cluster (M41L, L210W, and T215Y) vs. TAM-2 cluster (K70R, T215F, and K219Q),[43-45] and is possibly due to steric constraints on the ATP site as assessed by molecular modeling approaches and known ATP-protein interactions or fitness differences (see below).[46]

In comparing the week 16 sequences of the virologic non-responders to their entry sequences there were three codons associated with NRTI resistance that showed changes not involving a reversion to wild type. The most frequent position with new mutations was at position 215, where changes were detected in 18 sequences (13% of subjects). However, the substitutions were not the common TAMs (215 T to F or Y). Instead, the 215 position was enriched for the phenotypic reversion substitutions S/Z/I/D/C/V. In addition, two new polymorphisms were detected in the TAM position 219. At position 184, there were two subjects who gained the M184I substitution. Like M184V, this substitution is associated with high level resistance to 3TC and FTC. The only other new NRTI resistance mutation was a L74V substitution (associated with resistance to DDI and ABC) in one subject in the DLV arm. The appearance of these latter substitutions (M184I and L74V) likely represents the fortuitous outgrowth of viral subpopulations that were initially present at baseline and evolved linked resistance mutations for which there was selective pressure. Although ADV is known to have minimal NRTI activity, as expected we did not detect any significant difference in the gain or loss of any TAM at week 16 when comparing groups with or without ADV; *p*-values at baseline and 16 weeks for these NRTI resistance mutations in the DLV or ADV arms are all >0.16.

NNRTI resistance mutations

Multiple NNRTI mutations have been associated with DLV resistance: K103N, V106M, Y181C, Y188L, and P236L.[20] Although none of these was present at entry, a total of 53 NNRTI resistance mutations were detected in the 141 virologic nonresponders in the DLV-containing arms (Tables 1 and 4). As expected, none of the subjects treated with ADV, but not DLV, developed any NNRTI-associated mutations. The most frequently observed mutation was K103N, which was detected in 34 subjects. Y181C arose in 17 subjects, and P236L was seen in two subjects.

We also wanted to determine whether potent NNRTI drug selection had an effect on reversion of TAMs or loss of M184V in the absence of ongoing NRTI drug selection. To address this question, we analyzed resistance mutations at baseline and week 16 in the

subgroup of patients that received only DLV, but not ADV (in addition to the PIs) and fixed an NNRTI-resistance mutation (19 subjects). In the subjects that developed either K103N or Y181C in the DLV arm, 12 of 17 subjects (71%) maintained all of their baseline TAM mutations. In addition, the rate of M184V retention in the DLV arm was relatively similar (65% retained M184V). These data indicate that in the absence of potent NRTI drug selection, there is no strong tendency for unselected NRTI resistance mutations to revert back to or be replaced by the wild-type *pol* sequence while fixing a strongly selected NNRTI mutation. However, when NRTI resistance mutations were lost, this was more likely to occur in arms that contained DLV compared to the arms without DLV; 24% of the week 16 sequences in the DLV-containing arms lost one or more TAMs compared to only 8% in the DLV-negative arms ($p = 0.01$). M41L was the most common TAM that was lost (either alone or in combination, at a frequency of 20%). The persistence of NNRTI mutations has been well documented despite the absence of drug selection for up to 12 months, indicating minimal effect of NNRTI mutations on viral fitness.47 Thus, while NNRTI resistance mutations were easily added to the background NRTI mutations in many cases, a presumed cumulative effect of fitness loss was also suggested with the more pronounced loss of NRTI mutations in the DLV arms. A potential factor leading to the relative enrichment of various NRTI mutations in the subjects who were virologic nonresponders is NNRTI hypersusceptibility.[48,49] The presence of mutations at three RT codons (215, 208, and 118) is independently associated with hypersusceptibility to NNRTIs.[50,51] Of note, T215Y was highly predictive of NNRTI hypersusceptibility, whereas T215F was not predictive. Our observation of the overrepresentation of T215F but not T215Y in the subjects who were virologic nonresponders is consistent with the T215Y mutation conferring NNRTI hypersusceptibility.

PI resistance mutations

At baseline, there were several mutations within *pro* that were overrepresented in the virologic nonresponders (Tables 2 and 4). Mutations at position 10 (L to F/I/R/V), 24 (L to I), 71 (A to T/V/I), 73 (G to C/S/T), and 88 (N to D/S/T) were enriched in the nonresponder samples relative to the wild type (all p values < 0.05). There were relatively few examples of I47V, but these were overrepresented in the virologic responders ($p = 0.038$) indicating a protective effect. The M46I mutation approached statistical significance ($p = 0.057$) for being overrepresented in the nonresponders, while the M46L mutation had the same partitioning as the wild type. As expected, the position most frequently substituted among this group of IDV-experienced subjects was position 82 (present in approximately 50% of subjects); however, mutations at this position were partitioned equally among the virologic responders and nonresponders. The I84V and L90M mutations would be expected to contribute to resistance to regimens containing SQV and NFV, and both of these primary resistance mutations showed a trend in the nonresponders.

The differential partitioning of baseline PI resistance-associated mutations in the viral strains of nonresponders largely paralleled increases seen in the frequency of protease mutations in the week 16 sequence of these subjects, especially for primary resistance mutations (Table 2). The following mutations were significantly increased at week 16 compared to their paired entry sample: L10I, M36I, F53L, I54V, A71V, G73S, I84V, and L90M (all p values <0.02). In general, there was a trend for higher incidence of PI resistance mutations at week 16, with two exceptions. There was a significant decrease in I93L from 52% to 43% ($p < 0.004$) from 73 of 141 subjects at baseline to 61 of 141 subjects at week 16. In addition, there was a trend for the loss of V82A ($p = 0.08$) even though I54V, which is frequently seen as a compensatory mutation to V82A,[52,53] increased. There was no statistically significant increase in L24I, M46I, L63P, or N88S, suggesting that although they could contribute to resistance as evidenced by their enrichment in the nonresponder subset, they

were not selected for resistance pathways when more mutations appeared. These data suggest that the V82A pathway commonly seen with IDV (and RTV) did not participate in the evolution of resistance to the SQV and NFV-based PIs. Consistent with these data, mutations at position 82 were associated with greater reductions in viral RNA load, while mutations at positions 24 and 90 were associated with smaller reductions in viral RNA load (see below).

Discussion

Both GSS and viral RNA load predict the magnitude of the viral RNA load decrease

In the previous models, we used the primary endpoint of the ACTG 359 study (viral load ≤500 copies/ml at week 16) to define virologic response. We also generated a series of logistic regression analyses using as the endpoint a 1.5 $\log_{10}$ decrease in viral RNA load or viral load ≤500 copies/ml at any point during the first 16 weeks since the viral load nadir could occur prior to week 16 and then rebound. In these analyses, both the dGSS and the cGSS continued to be predictors of virologic outcome (data not shown). However, the baseline viral load remained a significant predictor of the magnitude of the viral RNA decrease. This observation suggests that the magnitude of the drop in viral RNA load is in part dependent on the baseline level of viral RNA, with the drop being proportionately smaller with higher viral RNA loads. To dissect the relationship between GSS, entry viral load, and virologic outcome, we used censored regression models of subjects with entry HIV-1 RNA between 10,000 and 50,000 copies/ml, and those with HIV-1 RNA >50,000 copies/ml. As expected, the cGSS was a strong predictor of the magnitude of the viral RNA drop. However, the magnitude of the drop as predicted by the cGSS was reduced by 0.57 $\log_{10}$ among the subjects with higher entry viral RNA levels, suggesting less drug efficacy per unit cGSS among subjects with high viral RNA loads. One limitation in this analysis is that subjects with baseline viral loads greater than the median had dGSS and cGSS significantly lower than those subjects with viral loads less than the median ($p = 0.004$ and 0.002, respectively); thus inaccuracies in assigning the GSS values would be biased between the two groups.

Scaling the viral RNA load decrease to the GSS does not predict the virologic response

We created a dataset with a subset of the virologic nonresponders at week 16 and for whom there was clear evidence of new drug exposure and some measure of treatment compliance (a transient decrease in viral RNA load and the maintenance of baseline resistance mutations and/or the appearance of new resistance mutations). The goal in making this data set was to be able to evaluate the magnitude of change in viral RNA load in subjects who were largely adherent. In comparing the arms that either did or did not include DLV there was a 0.4 log difference in the extent of viral RNA load drop (1.08 $\log_{10}$ with DLV vs. 0.68 $\log_{10}$ without DLV). We next used regression analysis after adjusting for the effect of DLV to determine which PI resistance mutations most impacted virologic response as measured by maximum decline in HIV-1 RNA. Three positions were found that impacted outcome using a backward or forward selection model. Position 82 was associated with enhanced virologic response by 0.47 $\log_{10}$ ($p = 0.03$). In contrast, mutations at positions 24 and 90 were associated with an increase in HIV-1 RNA by 0.71 and 0.75 $\log_{10}$ ($p = 0.005$ and 0.0004, respectively). Finally, we used the model developed based on data from the virologic nonresponders to examine the magnitude of the viral RNA drop in the virologic responders. However, the parameters derived from the subjects who were nonresponders did not predict the magnitude of the drop in the virologic response (data not shown). It is not clear why we were unable to link the empirically derived data for drug potency, as defined by viral RNA load drop in the compliant nonresponders, to model viral RNA load drop in the virologic response. The strong linkage between entry viral load and therapeutic response suggests this

relationship should exist. The scaling of the resistance mutations to account for differences in the level of viral RNA drop may be too inaccurate to have predictive power in the absence of resistance mutations. Also, we did not measure the additional host cellular, humoral, or innate immunologic responses, variation in pharmacogenomics and drug metabolism, or genetic regulation of viral replication among individuals, each of which may contribute to the effectiveness of antiretroviral therapy and viral suppression.

In summary, we have shown the utility of a GSS as a significant predictor of therapy outcome and compared this approach to a PSS. We were able to examine the role of resistance mutations in the context of mutations at entry and their over- or underrepresentation in subjects who were virologic nonresponders, to compare these patterns to the appearance of new mutations, to define mutations that enhanced or reduced the virologic response, and to show that potently selected new mutations can be added onto the backbone of previous mutations even in the absence of the previous selective drug pressure (e.g., subjects that developed K103N or Y181C in the DLV arm who maintained baseline TAMs, $n = 12$ of 17 subjects). Finally, we explored the relationship between the GSS and the magnitude of viral RNA drop in subjects with a successful virologic response or lack thereof, and found discrepancies in comparing these two groups. Collectively, these studies represent a comprehensive exploration of the role of baseline resistance mutations in this cohort during salvage therapy.

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Table 1

Frequency of Wild-Type (wt) and RTI Resistance Mutations

Position[a,b]	Baseline count (n = 234)	Baseline RTI mutations in virologic nonresponders at week 16 (n = 149)[c,d]	Baseline RTI mutations in virologic responders at week 16 (n = 67)[c,e]	Week 16 RTI resistance mutations in virologic nonresponders (n = 141)[c,d]
41M (wt)	128	79	36	81
41L	106	70	31	60
67D (wt)	132	73	47	76
67E/G/N	2, 6, 94	**1, 5, 70**	1, 1, 18	1, 2, 62
69T (wt)	195	118	63	112
69A/D/N/S/Z	2, 20, 17, 1, 2	**2, 16, 13, 1, 1**	0, 2, 2, 0, 0	1, 15, 12, 2, 1
70K (wt)	171	101	56	99
70R/G/E	62, 2, 1	**47, 2, 1**	11, 0, 0	41, 2, 1
103K (wt)	234	149	67	107
103N	0	0	0	**34**
118V (wt)	199	120	63	113
118I	35	**29**	4	28
181Y (wt)	234	149	67	124
181C	0	0	0	**17**
184M (wt)	35	21	8	68
184I/V	0, 199	0, 128	0, 59	2, **73**
210L (wt)	162	107	47	103
210W/Z	63, 2	41, 1	19, 1	37, 1
215T (wt)	96	58	29	62
215C/D/F/I/V/S/Y/Z	0, 0, 26, 2, 1, 5, 111, 4	0, 0, **24,** 1, 1, 3, 68, 2	0, 1, 1, 0, 2, 35, 1	1, 1, 21, 5, 2, 14, 60, 7
219K (wt)	185	112	57	103
219E/N/Q/R/Z	12, 4, 30, 2, 2	6, 3, **26,** 1, 2	6, 1, 3, 0, 0	6, 3, 25, 1, 4
236P (wt)	234	149	67	139
236L	0	0	0	2

[a] Z represents any other polymorphism different from wild-type.

[b] Positions with low frequencies of substitutions that were not significantly different or associated with a virologic response include codons 44, 62, 65, 74, 75, 77, 100, 115, 116, and 151.

[c] Numbers in bold font (p value <0.05).

[d] Virologic nonresponders = subjects with HIV-1 viral RNA load >500 copies/ml.

[e] Virologic responders = subjects with HIV-1 viral RNA load ≤500 copies/ml.

Table 2

Wild-Type (wt) and PI Resistance Mutations

Position[a,b]	Baseline count (n = 234)	Baseline PI resistance mutations in virologic nonresponders at week 16 (n = 149)[c,d]	Baseline PI resistance mutations in virologic responders at week 16 (n = 67)[c,e]	Week 16 PI resistance mutations in virologic nonresponders (n = 141)[c,d]
10L (wt)	127	71	46	60
10F/I/R/V/Z	14, 87, 6, 9, 2	**10, 62, 4, 8, 1**	3, 18, 1, 0, 0	10, **72,** 1, 6, 0
24L (wt)	210	130	65	118
24I/F/Z	24, 0, 0	**19,** 0, 0	2, 0, 0	22, 1, 1
36M (wt)	194	122	56	105
36I/L	38, 3	25, 2	10, 1	**34,** 2
46M (wt)	117	67	39	74
46I/L/V	84, 49, 0	62, 28, 0	19, 15, 0	49, 25, 1
47I (wt)	221	145	60	140
47V	13	4	7	1
48G (wt)	234	149	67	128
48V/Z	0, 0	0, 0	0, 0	**11, 2**
53F (wt)	229	146	67	118
53L/Y/Z	4, 0, 1	2, 0, 1	2, 0, 0	**21,** 1, 1
54I (wt)	190	118	57	92
54T/V/A/L	1, 43, 2, 0	0, 30, 1, 0	1, 10, 1, 0	0, **47,** 0, 2
63L (wt)	28	15	11	13
63P/Z	181, 33	122, 16	46, 12	117, 16
71A (wt)	139	83	47	66
71T/V/I	32, 64, 5	**21, 46, 4**	8, 12, 1	16, **59,** 6
73G (wt)	202	122	65	103
73C/S/T/Z	4, 23, 5, 1	**4, 19, 4, 1**	0, 1, 1, 0	5, **30,** 9, 4
82V (wt)	117	77	33	82
82A/F/I/S/T	96, 11, 3, 2, 17	61, 4, 1, 2, 11	26, 6, 0, 0, 5	49, 2, 2, 1, 7
84I (wt)	222	139	65	101
84V/A/Z	12, 0, 0	10, 0, 0	2, 0, 0	**40,** 3, 2
88N (wt)	219	135	66	128
88D/S/T/Z	9, 5, 1, 1	**8, 5, 1, 1**	1, 0, 0, 0	7, 5, 1
90L (wt)	191	115	59	77
90M	43	34	8	**65**
93I (wt)	114	72	33	80
93L/Z	120, 1	77, 0	34, 1	**61,** 0

[a] Z represents any other polymorphism different from wild-type.

[b] Positions with low frequencies of substitutions that were not significantly different or associated with a virologic response include codons 20, 30, 32, 33, and 50.

[c] Numbers in bold font (p value <0.05).

[d] Virologic nonresponders = subjects with HIV-1 viral RNA load >500 copies/ml.

[e] Virologic responders = subjects with HIV-1 viral RNA load ≥500 copies/ml.

Table 3

Logistic Regression Relating Baseline GSS or PSS to Week 16 RNA Responses

Model	Variables in model	OR (95% CI)	*P*-values
1	cPSS	2.34 (1.27, 4.30)	0.006
2	cGSS	2.50 (1.42, 4.39)	0.0015
3	cPSS	2.04 (1.10, 3.81)	0.025
	$\log_{10}$ baseline RNA	0.33 (0.19, 0.69)	0.003
4	cGSS	2.22 (1.22, 4.03)	0.009
	$\log_{10}$ baseline RNA	0.36 (0.17, 0.73)	0.005

Table 4

Partitioning of Mutant and Wild-Type Positions

Baseline mutations enriched at week 16 in virologic nonresponders	**Mutation evolution at week 16 in virologic nonresponders**	
NRTI	NRTI gain	NRTI loss
67, 69, 70, 118,	T215S/Z/I/D/C/V	M41L
T215F	K219Z	M184V
K219Q	M184I	
	L74V	
	NNRTI gain	
	K103N	
	Y181C	
	P236L	
PI	PI gain	PI loss
10, 24, 71, 73, 88	Secondary = L10I, M36I, F53L, A71V	I93L, V82A
M46I, I84V, L90M	Primary = G48V, I54V, G73S, I84V, L90M	