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Improved darunavir genotypic mutation score predicting treatment response for patients infected with HIV-1 subtype B and non-subtype B receiving a salvage regimen

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Objectives: The objective of this study was to improve the prediction of the impact of HIV-1 protease mutations in different viral subtypes on virological response to darunavir.

Methods: Darunavir-containing treatment change episodes (TCEs) in patients previously failing PIs were selected from large European databases. HIV-1 subtype B-infected patients were used as the derivation dataset and HIV-1 non-B-infected patients were used as the validation dataset. The adjusted association of each mutation with week 8 HIV RNA change from baseline was analysed by linear regression. A prediction model was derived based on best subset least squares estimation with mutational weights corresponding to regression coefficients. Virological outcome prediction accuracy was compared with that from existing genotypic resistance interpretation systems (GISs) (ANRS 2013, Rega 9.1.0 and HIVdb 7.0).

Results: TCEs were selected from 681 subtype B-infected and 199 non-B-infected adults. Accompanying drugs were NRTIs in 87%, NNRTIs in 27% and raltegravir or maraviroc or enfuvirtide in 53%. The prediction model included weighted protease mutations, HIV RNA, CD4 and activity of accompanying drugs. The model's association with week 8 HIV RNA change in the subtype B (derivation) set was $R^2 = 0.47$ [average squared error (ASE) = 0.67, $P < 10^{-6}$]; in the non-B (validation) set, ASE was 0.91. Accuracy investigated by means of area under the receiver operating characteristic curves with a binary response (above the threshold value of HIV RNA reduction) showed that our final model outperformed models with existing interpretation systems in both training and validation sets.

Conclusions: A model with a new darunavir-weighted mutation score outperformed existing GISs in both B and non-B subtypes in predicting virological response to darunavir.

Introduction

HIV-1 drug resistance is a major limitation to the efficacy of combination ART (cART).[1,2] With several available drug options, the aim of cART is to achieve full virological suppression even in treatment-experienced patients with multi-class drug resistance.[3–5] Darunavir is a PI requiring pharmacokinetic boosting with ritonavir, with preserved antiviral activity against several PI-resistant HIV-1 isolates,[6–9] and has a documented activity against non-B HIV-1 subtypes.[10] The tight binding between darunavir and the active viral protease site and its ability to inhibit protease dimerization form the basis for this preserved activity and high genetic barrier to resistance.[6–15]

The higher efficacy of darunavir/ritonavir as compared with other PIs has been demonstrated in highly treatment-experienced patients with extensive drug resistance.[12] Nonetheless, in the presence of a high number of resistance mutations in the viral protease, combination regimens with darunavir/ritonavir will ultimately fail. It is therefore important to identify the combinations of protease substitutions that result in a loss of darunavir activity. Several scores and algorithms for the interpretation of darunavir genotypic resistance [genotypic resistance interpretation systems (GISs)] have been constructed.[16–25] However, different GISs vary with respect to the mutations that are taken into account and the weights assigned to the individual mutations. This variation may be explained by the inclusion of different source studies and the use of different analytical approaches. Moreover, although most knowledge on darunavir resistance is based on subtype B viruses, other subtypes are becoming increasingly relevant due to the extension of cART coverage in African and Asian countries where non-B subtypes prevail, and to the spread of non-B viruses in Europe and North America.[26–28] Therefore, the precise impact of HIV-1 protease mutations and their combinations on virological response to darunavir remains to be fully elucidated, particularly with different subtypes.

We aimed to derive a refined genotypic interpretation score for darunavir based on virological response in patients harbouring subtype B HIV-1, and to validate its performance for non-B HIV-1 subtypes.

Methods

Case selection and definition of treatment change episodes (TCEs)

We selected darunavir-containing TCEs from European observational cohort studies sharing their data through the CHAIN project,[29] namely EuResist (gathering data from Italy, Germany, Sweden, Belgium and Portugal),[30] the Swiss HIV cohort study,[31] the Aquitaine cohort[32] and cohorts from Paris and Rome, and through the Treatment Change Episodes Database of COHERE in EuroCoord, a collaboration of European cohorts (www.cohere.org). COHERE's Treatment Change Episodes Database was pooled within the EuroCoord network in 2012 (www.EuroCoord.net).

Cases were required to be HIV-infected adults (≥18 years old). A TCE was defined as the start of a darunavir-containing regimen including at least two additional antiretroviral agents in patients with a previous history of virological failure to at least one PI (HIV RNA >1000 copies/mL after at least 3 months of treatment). Not all participating cohorts were able to provide information on darunavir dose; for those that did, only cases receiving 600 mg twice daily were included. Cases were required to have an available drug resistance genotype (protease and reverse transcriptase sequences obtained through standard population sequencing) and an HIV-1 RNA quantification and CD4 count performed within the period from 6 months before to 1 week after baseline (the start date of the darunavir regimen). Additional variables necessary for inclusion were prior treatment history and drugs accompanying darunavir and HIV-1 RNA at week 8 (window ±4 weeks) after baseline; gender and age were not required for inclusion but were collected. No regimen change was allowed between the date of sample collection for pre-baseline viral load and resistance genotyping and the baseline, and the darunavir-containing regimen had to be prescribed for at least 12 weeks without changes or interruptions. All cases gave written informed consent within the respective cohorts. Viral subtype was determined using the Rega 2.0 subtyping system. Unassigned subtypes were classified as non-B.

Statistical analysis

Definition of main outcome variable

The main outcome was change in $\log_{10}$ HIV RNA from baseline to week 8. An undetectable viral load at week 8 was imputed with a value of 50 copies/mL (main analysis) or 25 copies/mL (sensitivity analysis).

Definition of PI mutations

Any protease substitution at any codon (compared with consensus B reference sequence) with a frequency >2% was considered as a candidate PI mutation.

Prediction model derivation strategy

We used a standard linear regression model applied to the derivation dataset to identify predictive mutations from the candidate protease mutations. Regression models included candidate protease mutations, adjusting for the following variables: baseline $\log_{10}$ viral load; baseline CD4 cell count; and genotypic susceptibility score (GSS) of drugs prescribed with darunavir. The GSS of the drugs accompanying darunavir was calculated by summing the number of active drugs using the interpretation of the ANRS 2013 algorithm (HIVdb 7.0 with three resistance categories[21] was used in sensitivity analyses); individual drug activity was scored as 1 if there was no evidence of resistance, 0.5 if there was intermediate resistance and 0 if there was resistance. New and recycled use of raltegravir, enfuvirtide and maraviroc was scored 1 and 0, respectively.

The derivation set comprised all TCE cases with subtype B virus and the validation dataset comprised all non-B subtype cases.

Least squares estimation (LSE) was used to select the 'best' subset of mutations. The LSE chosen to minimize the average squared error (ASE) was based on a 5-fold cross-validation (CV). Briefly, 5-fold CV works by dividing the derivation dataset randomly into five equal parts. The method fits the model to four-fifths of the data and then computes the prediction error on the remaining one-fifth. This procedure was applied to each fifth of the dataset and the five prediction error estimates were averaged. From this procedure we obtained an estimated prediction of the 5-fold CV error (CV PRESS) curve, which was used to establish a threshold for stopping the inclusion of the covariates. In sensitivity analyses, we also investigated the effect of allowing for 2-way interactions between mutation terms and the use of 10-fold CV. CV was applied to the derivation dataset to select the best model providing the final set of mutations defining the score. The final prediction model included the individual selected mutations, along with baseline CD4 and HIV RNA and GSS of the accompanying drugs, which were forced into the model.

Validation of the score model

The performance of the final model was evaluated in different ways. First, the ASEs on both derivation and validation sets were compared. For

completeness the R^2 on the derivation set was also shown. Second, we computed prediction accuracy of the final model and compared it with a model including only the adjustment variables (the 'base model' with baseline $\log_{10}$ viral load, baseline CD4 cell count and GSS of the accompanying drugs) and with models including adjustment variables and the darunavir level of resistance from three main existing GISs (ANRS 2013, Rega 9.1.0 and HIVdb 7.0) using area under the receiver operating characteristic (AUROC) curves. This was applied to both the derivation and the validation set. For each existing GIS a dummy variable indicating resistance was created: for ANRS and Rega 'no resistance' and 'intermediate resistance' were considered as 'no resistance'; for HIVdb, 'susceptible', 'potential low-level resistance' and 'low-level resistance' were considered as 'no resistance'. This required also the transformation of the viral load reduction at week 8 into a binary outcome (response) using three levels of HIV-1 RNA reduction from baseline (>1, >1.5 and >2 $\log_{10}$ copies/mL). Statistical analyses were performed using procedures GLMSELECT and LOGISTIC in SAS 9.3 (SAS Institute, Cary, NC, USA, 2013).

Results

Baseline patient characteristics

Eight hundred and eighty cases fulfilled the selection criteria: 681 (77.4%) were infected with a subtype B virus (derivation dataset) and 199 (22.6%) were infected with a non-B virus (validation set). Non-B subtypes consisted mainly of subtype A (19.6%), CRF02_AG (18.6%), C (14.1%), D (7%), F (6.5%), unique recombinant forms (5.5%), G (4.5%), BF recombinants (2.5%), others (12.5%) and unassigned (9%). The main baseline characteristics of study patients overall and in the derivation and validation datasets are summarized in Table 1. The numbers contributed by each cohort are illustrated in Table S1 (available as Supplementary data at *JAC* Online). The patients were heavily pretreated, with previous experience of a median of five different NRTIs and three different PIs, including tipranavir in one-quarter. The majority had also experienced NNRTIs and almost one-half other drug classes. Patients infected with non-B viruses differed from those infected with subtype B viruses, with a higher proportion of females, a lower median HIV-1 RNA, slightly lower CD4 counts and a smaller number of previously experienced antiretroviral drugs.

The distribution of protease substitutions from consensus B virus by viral subtype category is illustrated in Figure 1. Among the mutations included in the IAS-USA list of major and minor darunavir resistance mutations[16] (Figure 1a), V32I, L33F, I47V and I84V were more frequent in the subtype B subset as compared with the non-B, while L89V was more frequent in non-B subtypes. For the other mutations (Figure 1b and c) there were differences in frequency at several polymorphic positions, while among substitutions associated with resistance to PIs there was a higher frequency for L10I, M46I/L, A71V, G73S, V77I, V82A and L90M in subtype B, whereas K20I, M36I, K70R, T74S, L89I/M prevailed among the non-B subtypes. Figure 1(d) illustrates the proportion of cases with full resistance to at least one drug in each class and the proportion with resistance to at least one drug to all three historical classes, in subtype B and non-B subtype TCEs, according to HIVdb interpretation. Subtype B cases had a higher proportion with resistance to NRTIs and PIs and a higher proportion of three-class resistance compared with non-B subtypes.

Antiretroviral drugs accompanying darunavir

The antiretrovirals used in combination with darunavir/ritonavir and their GSS are summarized in Table 2. About one in four patients used NNRTIs and around one-half were prescribed an integrase inhibitor or entry inhibitor. The single drugs more frequently used in combination with darunavir/ritonavir were tenofovir, raltegravir, enfuvirtide and etravirine, most of these reflecting the treatments employed in heavily experienced patients with multi-class resistant viruses. Individuals with non-B viruses were treated less frequently with raltegravir, enfuvirtide and etravirine, while showing a slightly higher GSS for the accompanying drugs.

Association of protease mutations with 8 week viral load changes in the derivation set (subtype B) and model derivation

In the derivation dataset, median week 8 HIV-1 RNA change from baseline was −2.01 (IQR −2.68, −1.03) $\log_{10}$ copies/mL, with 41.4% achieving <50 copies/mL at this timepoint. In the

Table 1. Patient characteristics at baseline

	All (n=880)	Subtype B (n=681)	Non-B subtypes (n=199)
Age (years), median (IQR)	42.7 (41.6–44.6)	42.6 (41.5–44.6)	43.4 (42.1–44.7)
Male, *n* (%)	688 (78.2)	583 (85.6)	105 (52.8)
HIV RNA ($\log_{10}$ copies/mL), median (IQR)	4.3 (3.3–4.9)	4.4 (3.5–4.9)	3.9 (3.1–4.8)
CD4 count (cells/mm^3), median (IQR)	215 (96–363)	220 (98–370)	197 (90–330)
Number of previously used antiretroviral drugs, median (IQR)	9 (4–13)	10 (4–13)	7 (3–11)
Number of previously used NRTIs, median (IQR)	5 (2–6)	5 (3–6)	4 (2–6)
Number of previously used NNRTIs, median (IQR)	1 (0–2)	1 (0–2)	1 (0–1)
Number of previously used PIs, median (IQR)	3 (1–5)	3 (1–5)	2 (1–4)
Previous lopinavir use, %	70.1	70.2	69.8
Previous tipranavir use, %	25.3	26.7	20.6
Previous etravirine use, %	8.8	9.1	7.5
Previous enfuvirtide use, %	27.3	29.4	20.1
Previous raltegravir use, %	10.0	10.0	10.1
Previous maraviroc use, %	8.1	7.9	8.5

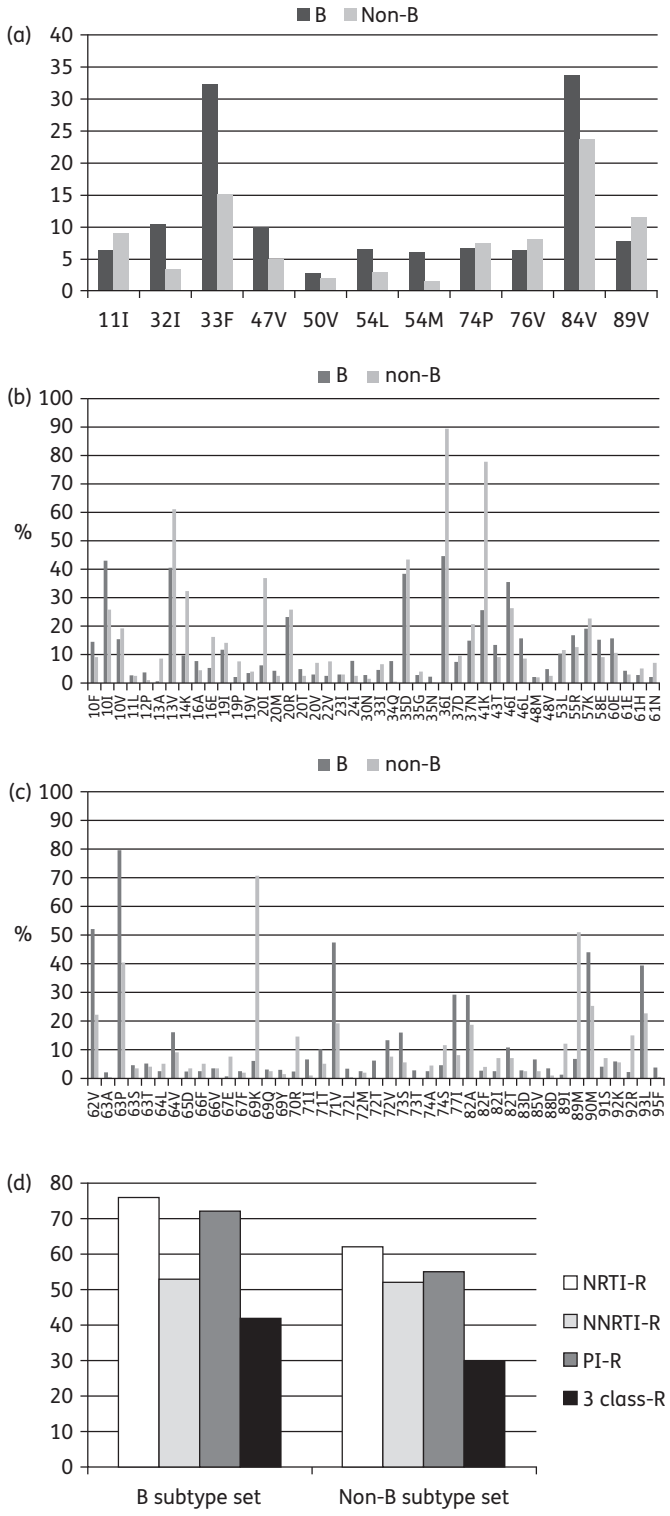

Figure 1. Resistance mutations and interpretations in baseline genotypes of TCEs with subtype B virus (derivation set) and non-B subtypes (validation set): proportion (%) of genotypes with IAS-USA 2014 darunavir mutations (a), proportions with any protease mutation from consensus B with a frequency of >2% (b and c) and proportion with full resistance (R) to at least one drug in each class and to the three historical classes, based on HIVdb 9.0 interpretation (d).

Table 2. Antiretrovirals accompanying darunavir/ritonavir

	All (n=880)	Subtype B (n=681)	Non-B subtypes (n=199)
Any NRTI (%)	87.2	86.9	87.9
Any NNRTI (%)	27.5	28.3	24.6
Any raltegravir, enfuvirtide or maraviroc (%)	52.8	54.6	46.7
Tenofovir (%)	62.5	62.3	63.3
Nevirapine (%)	1.8	1.5	3.0
Efavirenz (%)	3.0	3.2	2.0
Etravirine (%)	23.1	24.1	19.6
Enfuvirtide (%)	24.2	25.6	19.6
Enfuvirtide first-time use (%)	13.4	13.7	12.6
Raltegravir (%)	28.8	29.2	27.1
Raltegravir first-time use (%)	24.2	24.8	23.1
Maraviroc (%)	5.9	5.9	6.0
Maraviroc first-time use (%)	4.5	5.0	3.0
GSS of the accompanying drugs			
0–1	41.3	43.4	34.2
>1–2	41.3	39.4	48.2
>2	17.3	17.3	17.6

validation set the week 8 HIV-1 RNA change was −1.57 (IQR −2.43, −0.52) $\log_{10}$ copies/mL, with 41.1% achieving <50 copies/mL. Five mutations (L10F, V11L, I54M, T74P and V82I) were associated with reduced response while six substitutions (K20T, E34D, I64L, V82A, I85V and I93L) correlated with improved response (Table 3). The final model included the selected protease mutations, baseline CD4, HIV RNA and GSS for the accompanying drugs. The parameters of the model are reported, with the regression coefficient of each individual component indicating its predicted contribution to the 8 week change from baseline HIV RNA (in $\log_{10}$ copies/mL): intercept +1.499; baseline HIV RNA (per 1 log higher) −0.728; baseline CD4 count (per 100 cells/mm^3 higher) −0.048; GSS (by ANRS 2013, per 1 point higher) −0.083; L10F +0.319; V11L +0.405; I54M +0.747; T74P +0.401; V82I +0.537; K20T −0.335; E34D −0.752; I64L −0.471; V82A −0.194; I85V −0.276; I93L −0.161. For each individual case, the predicted 8 week HIV RNA change (in $\log_{10}$ copies/mL) is the result of the algebraic sum of the products of the individual components value times the respective regression coefficient.

Derived model performance and comparison with available GISs

The correlation between the 8 week viral load changes predicted by the derived score and those observed in the derivation set is illustrated in Figure 2. Performance of the final regression model and the different models from sensitivity analyses is summarized in Table 4. As expected, the derived score performed better in training than in validation sets. In the training set, our final model performed much better than the model without any resistance variables (base model) and better than models with existing GISs. There was no such difference in the validation set. In

Table 3. Linear regression model of the association of protease mutations with 8 week viral load changes; subtype B is the derivation set ($n=681$)

Protease substitution from consensus B	Frequency (%)	Adjusted mean difference in viral load change at week 8 ($\log_{10}$ copies/mL)[a]	SEM[a]	*P*
L10F	14.5	+0.32	0.09	0.0006
V11L	2.6	+0.40	0.20	0.045
I54M	6.1	+0.75	0.14	<0.0001
T74P	6.9	+0.40	0.13	0.0022
V82I	2.5	+0.54	0.21	0.011
K20T	4.9	−0.34	0.15	0.026
E34D	2.1	−0.75	0.23	0.001
I64L	2.5	−0.47	0.21	0.029
V82A	29.2	−0.19	0.07	0.007
I85V	6.6	−0.28	0.13	0.036
I93L	39.5	−0.16	0.07	0.017

[a]Results represent mean ± SEM of 5-fold CV. Positive values indicate less virological response (resistance) and negative values indicate better virological response (hypersusceptibility). Associations are adjusted for baseline HIV RNA and CD4 and GSS of the companion drugs.

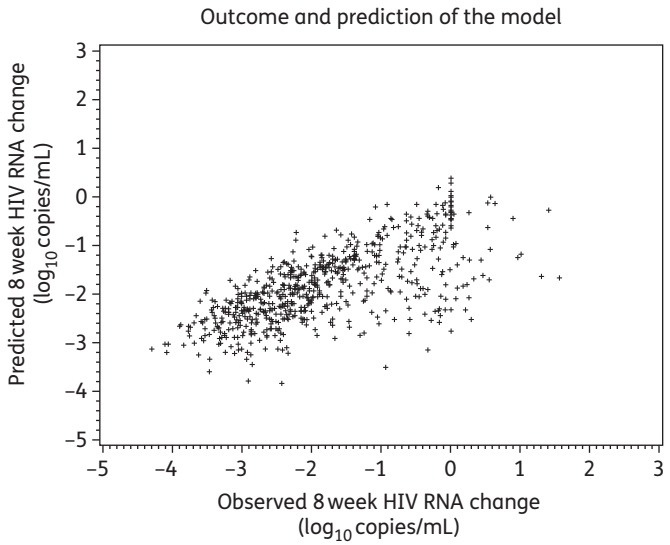

Figure 2. Eight-week HIV RNA changes from baseline: predicted versus observed outcomes in the derivation set (subtype B TCEs). $R^2=0.47$.

sensitivity analyses, the performance of these different models was broadly similar, except when undetectable viral load was fixed at 25 copies/mL, and most of the mutations kept in the final set were similar.

Table 5 summarizes the accuracy of the prediction of viral load 'response' at 8 weeks (using a threshold of 1.5 $\log_{10}$ copies/mL HIV-1 RNA reduction) for the model with the newly derived darunavir score and three existing GISs (see Table S2 for results with threshold values of 1 and 2 $\log_{10}$ copies/mL) using AUROC curves. Among the 681 patients infected with a subtype B virus,

Table 4. ASE and R^2 for the final model and models investigated in sensitivity analyses on the derivation and validation sets

	Derivation set		
Model	ASE	R^2	Validation set ASE
Final model[a]	0.67	0.47	0.91
Base model[b]	0.76	0.40	0.92
ANRS 2013[c]	0.72	0.44	0.94
HIVdb 7.0[c]	0.73	0.43	0.95
Rega 9.1.0[c]	0.74	0.42	0.91
Sensitivity analyses on the derived model			
10-fold[d]	0.67	0.47	0.91
25 copies/mL	0.77	0.41	1.04
2-way interactions[e]	0.68	0.48	0.91
GSS HIVdb[f]	0.68	0.46	0.93

[a]LSE using the model including the derived weighted darunavir mutations, baseline CD4 and HIV-1 RNA and GSS of the accompanying drugs (according to ANRS 2013 interpretation).
[b]LSE using the final model without the derived weighted darunavir mutations (i.e. using only baseline CD4 and HIV-1 RNA and GSS of the accompanying drugs).
[c]LSE models including the available darunavir genotypic interpretations, baseline CD4, HIV-1 RNA and GSS of the accompanying drugs.
[d]10-fold CV was used instead of 5-fold; 25 copies/mL, undetectable value replaced with 25 copies/mL.
[e]Significant 2-way interactions between mutations terms retained in the final model.
[f]The final model is adjusted for the GSS of the drugs accompanying darunavir computed according to HIVdb 7.0 instead of ANRS 2013.

444 (65%) had a >1.5 $\log_{10}$ copies/mL reduction (response) at 8 weeks and 101 (51%) among the 199 patients infected with a non-B virus. Two sets of comparisons were performed. First, we compared the accuracy of the response prediction by the different models including darunavir genotypic resistance interpretation ('final model' in Tables 4, 5 and S2 and three available GIS models) with the base model, which incorporated only CD4, HIV-1 RNA and GSS of the accompanying drugs. Of note, all darunavir resistance interpretation systems showed a better prediction than the base model, demonstrating the added value of the different darunavir resistance interpretations. Second, we compared the existing darunavir GISs with the final model. In the derivation set, the new score of the final model consistently out-performed the existing GISs in terms of accuracy, regardless of the definition of viral load response.

In the validation set, consisting of non-B subtypes, the greater accuracy of the new score was evident in several analyses. Indeed, the final model was the only model showing consistently higher AUROC values as compared with the base model in predicting the 8 week virological response, using all viral load reduction thresholds (Tables 5 and S2). Moreover, the final model showed higher AUROC values as compared with the models of the existing darunavir GIS, with significant differences over ANRS and HIVdb at the 1.5 log copies/mL threshold (Table 5), over ANRS at the 1.0 log copies/mL and over all three GISs at the 2.0 log copies/mL threshold (Table S2).

Table 5. Comparative AUROC curves of different models using a binary outcome at week 8 (HIV-1 RNA reduction < or ≥1.5 $\log_{10}$ copies/mL)

Model	AUROC	Difference in AUROC	P^a	Difference in AUROC	P^b
Subtype B (derivation set)					
base model	0.804	reference			
final model	0.844	0.040	<0.001	reference	
ANRS 2013	0.830	0.026	0.003	−0.014	0.108
HIVdb 7.0	0.824	0.020	0.002	−0.020	0.025
Rega 9.1.0	0.817	0.013	0.009	−0.027	0.002
Subtype non-B (validation set)					
base model	0.838	reference			
final model	0.872	0.034	0.015	reference	
ANRS 2013	0.840	0.002	0.390	−0.032	0.016
HIVdb 7.0	0.840	0.001	0.690	−0.032	0.015
Rega 9.1.0	0.853	0.015	0.140	−0.019	0.190

All models include baseline CD4 and HIV-1 RNA and GSS of the accompanying drugs. The base model includes only baseline CD4 and HIV-1 RNA and GSS of the accompanying drugs. The final model includes the weighted mutations as listed in Table 3 and the other models include a dummy variable indicating susceptibility or resistance according to the three existing interpretation systems (see the Methods section).
[a]Comparison with the base model.
[b]Comparison with the final model.

Discussion

Darunavir with ritonavir is one of the most widely used drugs in patients with virological failure, and is an essential component of salvage therapy for those who have previously failed other PI therapies.[33] Accurate information on the predicted activity of darunavir based on the results of resistance genotyping enables decisions on whether it should be prescribed in the new regimen, as well as the requirement for active companion drugs. Several previous studies have focused on the interpretation of darunavir resistance.[17–20,24,34,35] However, analyses were either based on genotypic analysis of clinical trials, which used specific selection criteria, or were performed using observational data with a limited size[24] or without external validation. Moreover, the performance of predictions was mostly based on sets of cases infected with subtype B virus, yet non-B variants predominate in resource-limited settings and are spreading in Europe. Given the polymorphic variability in the viral protease across different subtypes, it is essential to test how darunavir resistance interpretation performs in non-B subtypes.

In this study, we used the largest dataset of darunavir-based TCEs from PI-experienced HIV-1-infected patients collected to date. This allowed us to use two independent sets of data: one consisted of subtype B TCEs, to derive a new weighted score, and a separate second set consisted of non-B subtype TCEs, for validation.

The main finding of this study was that a model including the new weighted score was more accurate in predicting the virological response at 8 weeks compared with three popular existing GISs for darunavir. While this was expected for B subtypes, due to training on the same dataset, the model including the new darunavir score also outperformed GIS prediction of virological outcome in an independent validation set of non-B subtypes.

Previously existing GISs were consistently less accurate in predicting virological response in non-B subtypes. In particular, none of the existing GISs significantly improved prediction of response to darunavir over a prediction made on the basis of CD4, viral load and GSS of the accompanying drugs (the base model). This indicates that renewed efforts are necessary to improve the understanding of protease resistance interpretation in non-B subtypes. Notably, the weighted darunavir resistance score developed in the present study and included in the final prediction model resulted in an improved prediction of 8 week viral load outcome in non-B subtypes compared with both the base model and other existing GISs, using different virological response thresholds, indicating that the weighted mutation score included in the models is of relevance in this context.

The derived score includes five protease mutations that were associated, with different weights, with reduced response to the darunavir regimens. Two, I54M and T74P, are included in the IAS-USA 2014 mutation list as a major and a minor darunavir resistance mutation, respectively.[16] Consistent with this, our score gives the highest resistance weight to I54M and a somewhat lower weight to T74P. On the other hand, V11I but not V11L, which we included in our score, is considered a minor resistance mutation in that list. However, V11L and L10F are considered accessory PI-resistance mutations that are associated with minimal reductions in darunavir susceptibility at the same level as V11I.[21] Finally, V82I is a highly polymorphic mutation that is not selected by PIs and is the consensus amino acid in subtype G viruses.[21] We hypothesize that it may represent a marker or be in linkage disequilibrium with other mutational haplotypes, either within or outside the viral protease, translating into reduced *in vivo* susceptibility to darunavir. Six additional protease mutations correlated with improved response to darunavir and received negative weights in the derived score. With the exception of V82A, which has already been associated with improved response to darunavir,[19,36] no other of these mutations has been associated with decreased or increased susceptibility to darunavir. Three, E34D, I64L and I93L, are considered natural polymorphisms, K20T is associated with variable resistance to other PIs, and I85V is selected by PIs but has no impact on susceptibility to

any of them.[21] It should be underscored that the developed prediction model includes baseline viral load, CD4 counts and GSS of the antiretroviral drugs co-prescribed with darunavir and that each contributes to improvement of prediction, as previously shown by others,[36] but it is noteworthy that inclusion of the darunavir mutational score improved the prediction over the simple use of these background features.

The findings of this paper are subject to several limitations. First, we were not able to develop a subtype-specific tool predicting virological response to darunavir. Indeed, although this study included the largest standardized darunavir TCE set ever analysed, the number of cases with individual non-B subtypes was limited and did not allow specific predictions. In order to overcome this limitation, future studies will need to address the role of specific protease mutations in the context of the most common non-B subtypes, such as C, A and D, and recombinants AE and AG, either *in vitro* using site-directed mutagenesis experiments or *in vivo* using well-defined TCEs from large cohorts in specific African or Asian settings, as darunavir becomes more widely employed as rescue therapy in these countries. Second, some of the established darunavir resistance mutations did not appear in the derived score. As a possible explanation, we hypothesize that since patients from this study were selected from clinical practice, most were prescribed darunavir based on results of a genotypic resistance test, as indicated by the manufacturer, and thus darunavir was included in the salvage treatment mostly in the absence of known darunavir resistance mutations. Since a selection bias against these mutations has been applied in this dataset, their absence in this newly derived score should be interpreted with caution. Indeed, a prudent approach would be not to ignore mutations that were shown to be predictive of reduced response based on previous clinical trial analyses[16,17,19,35] and to use the newly derived score as an additional, refined darunavir genotypic resistance interpretation tool. Moreover, rare mutations, which may play a relevant role in predicting the virological outcome, may have been missed due to their low prevalence in this study dataset. Finally, this study allowed us to only predict the short-term virological outcome, which may differ from longer-term outcomes. However, short-term resistance genotype-guided virological responses are significantly associated with long-term virological outcomes.[37] Moreover, short-term responses are more strongly influenced by the antiviral activity of the regimen and by its relationship with baseline resistance mutations, and less by other confounders such as medication adherence and toxicity, which are more influential on the longer-term.

The strong points of this study are the large number of darunavir TCEs utilized, the uniform standardization of the data used to define eligible TCEs and the significant proportion of non-B subtypes analysed.

In conclusion, using a large, standardized dataset of genotype–response cases including darunavir-based regimens, we derived and validated a new weighted score that outperformed state-of-the-art darunavir GIS in predicting virological response both in B and non-B subtypes. This score may be used for predicting response to darunavir in individuals failing previous PIs with different subtypes and thus be of help in designing the most appropriate salvage regimens. However, mutations found in previous large clinical trials to be predictive of reduced virological response and not included in this score should also be considered in treatment decisions. After predicting treatment outcome of a darunavir-based regimen using the new score, a prudent solution for a user could be to check for the presence of additional major darunavir resistance mutations on the IAS-USA list.[16]

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Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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