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Original article

Interpretation of genotypic HIV-1 resistance to darunavir and virological response: validation of available systems and of a new score

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Background: There is not yet consensus on interpretation of genotypic HIV-1 resistance to darunavir (DRV). We validated existing rules and a newly derived score.

Methods: Protease inhibitor (PI)-failing patients starting a DRV/ritonavir-based regimen, with available baseline resistance genotypes, were extracted from three Italian databases. Virological response (VR) was analysed between 4 and 32 follow-up weeks, defined as a drop from baseline HIV RNA of ≥2 $\log_{10}$ or a value <50 copies/ml if the last measurement had been obtained at ≤12 weeks and as HIV RNA<50 copies/ml if it had been obtained at >12 weeks of follow-up. DRV/ritonavir resistance was interpreted by seven algorithms. A new weighted score (DRV-2009) was derived and validated, analysing associations of protease mutations with VR.

Results: A total of 217 patients were analysed, with a mean (±sd) follow-up time of 17 (±9) weeks. At baseline, median HIV RNA was 4.26 $\log_{10}$ copies/ml (IQR 3.11–5.03); VR was achieved in 135/217 (62%) patients. Adjusting for use of a new drug class, number of previous PIs experienced, CD4+ T-cell count and HIV RNA, only the Rega DRV/ritonavir interpretation was significantly associated with VR (per increase in susceptibility category, OR 1.94, 95% CI 1.32–2.86; $P<0.001$). The DRV-2009 score V11I+L33F+R41K+I47V+2*I-50V+2*I54M+K55R+D60E+L74P+L76V+N88D+2*L89V–L10I/V–I13V–G16E–G48V–F53I/L–I62V–I66F–V77I (<0 indicating susceptibility, 0–1 intermediate resistance and ≥2 resistance) correlated with VR in the derivation set ($n=132$, $R=0.395$; $P<0.001$). In the validation set ($n=85$), after adjusting for mutual interpretation and new use of enfuvirtide, DRV-2009 ($P=0.017$) and Rega ($P=0.013$) were both independently associated with VR.

Conclusions: In contrast to the other algorithms, both the DRV-2009 score and Rega interpretation showed a robust predictive capacity of VR to DRV/ritonavir-containing regimens.

Introduction

Virological failure due to the development and subsequent accumulation of HIV-1 drug resistance is a major limitation to the efficacy of combination antiretroviral therapy. The accumulation of drug resistance mutations with extensive cross-resistance within drug classes has a negative effect on disease prognosis [1,2] and limits further treatment options [3].

The availability of new classes of antiretroviral agents with novel mechanisms of action as well as newer generations of drugs for the historical classes of antiretrovirals

changed this scenario, allowing one to aim at full virological suppression even in treatment-experienced patients with multi-class drug resistance [4–7]. Darunavir (DRV) is a third-generation protease inhibitor (PI) requiring pharmacokinetic boosting with ritonavir (RTV), which appears effective against several HIV-1 isolates with broad cross-resistance to the previously licensed PI [8,9] and has a documented activity also against non-B HIV-1 subtypes and HIV-2 [10]. The favourable characteristics of DRV are mostly due to the high number of contacts between the molecule and the active site of viral protease that produce a very tight bond between the compound and the enzyme [8,10,11]. In addition, DRV also inhibits dimerization of the protease, a post-translational step required for enzyme function [12].

The efficacy of DRV/RTV in combination with an optimized background therapy has been demonstrated in highly treatment-experienced patients with extensive drug resistance and very limited treatment options [13] and proved to be superior to lopinavir/RTV both in patients with less extensive treatment experience [14] and in treatment-naive patients [15,16]. Nonetheless, treatment failure of DRV/RTV-containing combination antiretroviral therapy can occur in the presence of a high number of resistance mutations. The IAS–USA HIV-1 drug resistance panel describes six major (I47V, I50V, I54M/L, L76V and I84V) and five minor (V11I, V32I, L33F, T74P and L89V) protease mutations associated with DRV resistance, whereby the presence of three or more of these substitutions is expected to cause reduced susceptibility [17]. A different score for the interpretation of resistance mutations to DRV/RTV was derived from the pooled analysis of the Power 1, 2 and 3 studies, performed in patients with extensive treatment experience and PI resistance [18]. Other predictive algorithms were subsequently derived from observational data by other experts and major research groups [19–23] and are still awaiting clinical validation with independent datasets. Differences in the mutations included in the algorithms have been explained by their developers with differences in the size and frequency of mutations observed in the derivation datasets as well as in the different approaches used for the construction of the interpretation rules [19,24]. Therefore, full consensus on genotypic DRV resistance interpretation is presently lacking. The aim of our study was to evaluate existing rules, derive a new DRV genotypic score and validate it by head-to-head comparison with the best performing scores in an independent validation set.

Methods

Study patients

HIV-1-infected patients with previous failure of combination antiretroviral therapy (defined as the concomitant use of three or more antiretrovirals, excluding RTV at subtherapeutic doses) undergoing DRV/RTV-containing salvage regimens were extracted from those enrolled in three Italian cohorts: the clinical cohort of the Department of Infectious Diseases of the Catholic University in Rome, Italy (UCSC); the ARCA cohort, a multicentre database of HIV-infected individuals undergoing drug resistance testing; and the Italian DRV/RTV Early Access Protocol (study TMC114-C226). Patients were evaluable for the analysis if they never underwent a mono- or dual nucleoside reverse transcriptase inhibitor (NRTI)-based therapy, had an available baseline resistance genotype performed on-therapy from 180 days prior to DRV/RTV initiation (baseline), had a viral load determined ≤8 weeks before baseline and had ≥1 follow-up viral load determined between 4 and 32 weeks after baseline without changes in the regimen. When >1 viral load was available, the latest was chosen. Patients and HIV-related characteristics, baseline viral sequences, HIV-1 RNA values at baseline and follow-up as well as the previous and the new treatment regimens were retrieved from the individual databases and merged centrally. Virological response was analysed on the latest available viral load and defined as a drop from baseline HIV RNA of ≥2 $\log_{10}$ copies/ml or a value <50 copies/ml if the last data available had been obtained at 12 weeks or earlier and as a viral load <50 copies/ml if they had been obtained at >12 weeks of follow-up.

Genotypic resistance testing and interpretation

Genotypic resistance testing was performed by sequencing the protease and part of the reverse transcriptase gene by the local laboratories using different commercially available assays. In the ARCA database, the backup of the data is automatically performed on tape units on a daily basis using the PS3.01 Veritas Server Edition software package (Symantec, Mountain View, CA, USA). In particular, HIV sequences are checked for the inclusion of the minimum required regions, the presence of frameshifts, stop codons and highly (three-base codes B, D, H and V) or completely (N) degenerate base calling, all indicators of poor accuracy.

DRV/RTV resistance interpretation was evaluated on the whole patient dataset using the mutations listed in the IAS–USA HIV drug resistance figures (version December 2008) [17], the DRV/RTV score from Tibotec [18], the rules from the PREDZISTA study [19], the rules from the study by Descamps *et al.* [20] (Agence Nationale de Recherche sur le SIDA et les hépatites virales [ANRS] DRV-ATU), the Stanford HIVdb algorithm version 5.0.1 [22], the Rega version 8.0.1 [23] and the ANRS AC11 algorithm version 17 [25]. For ANRS, HIVdb and Rega, individual

Table 1. Available rules for the interpretation of genotypic resistance to DRV/ritonavir

System and version or rule	DRV score and susceptibility categories	Reference
ANRS AC11 version 17	Susceptible: <3 mutations among V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V and L89V. Possible resistance: 3 mutations among V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V and L89V. Resistance: ≥4 mutations among V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V and L89V	[25]
Stanford HIVdb version 5.0.1	Incorporates, with different weights, the following mutations: L10F, V11I, V32I, L33F, M46I/L/V, I47V/A, I50V, F53L, I54L/M/S/T/V/A, G73A/C/S/T, T74P, L76V, V82A/F/S/T/M/I/L/C, I84A/C/V, L89V and L90M. Favourable mutations (negative scores): I50L and N88S. Algebraic sum: total score <15: susceptible; 15–60: intermediate resistance; >60: resistance.[a]	[22]
Rega version 8.0.1	Add 1.5 to the score for every mutation in the following list: I50V, I54M, L76V and I84A/C/V. Add 1 to the score for every mutation in the following list: V11I, V32I, L33F, R41T, I47A/V, I54L, 70E, T74P and L89V. Add 0.5 to the score for every mutation in the following list: V32L, L33M/V, 34V, E35G, R41I, M46I/L, G48M, I54/S/T/V, G73I/A/C/F/S/T7V, T74E, V82F/L, 85V and L89T. Add 0.25 to the score for every mutation in the following list: L33I, E35N and L89I. Subtract 0.25 from the score for every mutation in the following list: I50L and N88S. Algebraic sum: total score <2: susceptible; total score ≥2 but <3.5: intermediate resistant; total score ≥3.5: resistant.	[23]
IAS–USA table	The following mutations (continuous score): V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V and L89V.	[17]
Tibotec score	The following mutations (continuous score): V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, T74P, L76V, I84V and L89V.	[18]
PREDZISTA score	The following mutations (continuous score): I13V, V32I, L33F/I/V, E35D, M36I/L/V, I47V, F53L and I62V	[19]
ANRS DRV-ATU score	The following mutations (continuous, algebraic score): K14R+K20I+E34Q+I47V+I54M+K55R+T74P+I84V-E35D-V82A.	[20]

[a]The five original categories were reduced to three (see *Methods*). ANRS, Agence Nationale de Recherche sur le SIDA et les hépatites virales; DRV, darunavir.

drugs were given three levels of activity and scored as 0, 0.5 or 1 according to a three-class categorization of resistant, intermediate resistant or susceptible, respectively. The five levels of activity reported by the HIVdb were translated into the three new levels by classifying the levels 'susceptible' and 'potential low-level resistance' as susceptible (1), the levels 'low-level resistance' and 'intermediate resistance' as intermediate resistant (0.5), and the level 'high-level resistance' as resistant (0). The other interpretation scores were treated as linear variables by analysing the positive unit increments of the scores. The detailed description of the different interpretation rules is reported in Table 1.

The backbone drugs' genotypic susceptibility score (GSS) for drugs belonging to the three historical classes was obtained by the sum of the number of active drugs, as reported previously [26], evaluating different genotypic interpretation systems and choosing that with the highest association under univariable analysis, which was ANRS.

The activity of enfuvirtide, raltegravir and maraviroc were scored as 0 or 1, according to whether the drugs had been previously employed by the patient or not, assuming that maraviroc was newly administered after confirming a pure R5 viral strain by a phenotypic or genotypic tropism prediction.

Derivation and validation of the new DRV score

The whole dataset was randomly split, using an automated procedure included in the two distinct datasets: the first, consisting of 60% of the cases, was used for the derivation of the new rule (hereafter denominated the derivation dataset); and the second, with the remaining 40% of the dataset, was used for the validation (hereafter denominated the validation dataset). A new genotypic DRV/RTV rule was derived, analysing associations of all protease substitutions from consensus B with a frequency >4%. Mutations that were associated with the virological response under univariable logistic analysis with $P<0.30$ were retained. This set of mutations and subsets of this set were considered for a subsequent multivariable logistic analysis, performed with an additional forward-stepwise variable selection. We constructed several linear scores by weighting and combining mutations according to their coefficients obtained from the multivariable analysis. The score with the highest accuracy (for example, percentage of correctly predicted cases) in predicting the virological response was selected. Subsequently, further mutations

known to confer phenotypic resistance to DRV but not included in the score were individually tested and those shown to increase the correlation coefficient with virological response were retained in the final score. The final best-fitting score was then translated into three categorized levels of resistance. The robustness of the correlation between the score and virological response was finally tested on the validation dataset and compared with that of the best performing pre-existing DRV interpretation rule.

Other statistical analyses

HIV-1 RNA values were $\log_{10}$ transformed before calculations. Association between variables, including the different DRV resistance interpretations and categorical outcomes (that is, reaching a virological response), was analysed by univariable and multivariable logistic regression, and additionally by Cox proportional hazard models. The new DRV score, along with the other scores previously presented in the literature, were evaluated with respect to their predictive ability on the success outcome (defined in the *Study patients* section), using receiver operating characteristic analysis and area under the receiver operating characteristic curve (AUROC) comparison. In detail, the new DRV score was tested in the validation test set, along with the other scores, verifying that the obtained AUROC in the test set was statistically higher than the reference value of 0.5 (that is, the value obtained by random guessing). All analyses were performed using SPSS (version 16; SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics, treatment regimens and virological responses of patients

We analysed 217 patients; 132 were included in the derivation set and 85 in the validation set. In total, 35 (16.1%) patients were from UCSC, 97 (44.7%) from the ARCA database and 85 (39.2%) from the Italian DRV/RTV Early Access Protocol. Overall, 76% were male; their risk factor was intravenous drug use in 23.5%, homosexual contacts in 25.8% and heterosexual contacts in 44.2%. At baseline, patients' median age was 44 years (IQR 41–48), median CD4$^+$ T-cell count was 263 cells/μl (IQR 143–442), and median HIV RNA was 4.26 $\log_{10}$ copies/ml (IQR 3.11–5.03); 19.8% of patients had a history of a prior AIDS-defining illness. Prior to baseline, patients had been exposed to a median of five highly active antiretroviral therapy regimens (IQR 3–7). All patients were NRTI- and PI-experienced, with a median time of 7 (IQR 5–9) years of PI exposure. Overall, 87.1% of patients were non-nucleoside reverse transcriptase inhibitor (NNRTI)-experienced, 12.9% of patients experienced two classes and 87.1% three or more classes of antiretroviral drugs. Specifically, 37% had experienced enfuvirtide, 5% raltegravir and 1% etravirine.

The DRV/RTV-containing regimen included NNRTI in 6% of patients (including etravirine in 3.2%), enfuvirtide in 23%, raltegravir in 15% and maraviroc in 5%; 40% used ≥1 new (that is, previously never experienced) drug class (15% an NNRTI, 14% enfuvirtide,15% raltegravir and 5% maraviroc). The median backbone GSS by ANRS algorithm interpretation was 0.5 (IQR 0–1). No significant differences in the median values of the distributions of backbone ANRS GSS by grouping for individual cohorts were detected (P=0.65).

No significant differences in pre-baseline or post-baseline drug use were observed between the derivation and validation sets

The mean (±SD) time of follow-up was 17 weeks (±9). At 8 weeks (range 4–12; n=178 cases), the mean change from baseline HIV-1 RNA level was -1.65 $\log_{10}$ copies/ml (±1.41), with 44.9% of patients achieving a viral load <50 copies/ml. At 24 weeks (range 12–32; n=114 cases), the mean change from baseline HIV-1 RNA level was -1.38 $\log_{10}$ copies/ml (±SD 1.78), with 52.6% of patients achieving a viral load <50 copies/ml. Again, no significant differences between the derivation and validation sets were observed. Overall, the study-defined virological response was achieved in 135 of 217 (62%) patients. In the derivation set, virological response was observed in 81 of 132 (61%) and in the validation set in 54 of 85 (63%) patients. There were significant differences in the percentage of overall virological responses (validation plus derivation sets) among the distinct study cohorts. Specifically, the UCSC cohort had 22/35 (63%), TMC114-C226 had 42/85 (49%) and ARCA 71/97 (73%; P=0.04).

Testing the available DRV interpretation rules and other predictors of virological response in the entire set of patients

The results of the univariable and multivariable analyses associating the different existing interpretation rules and other variables with virological response are summarized in Table 2. At univariable analysis, the higher GSS of the backbone therapy, the use of NNRTI as a new class, the use of new enfuvirtide and the lower number of prior PIs experienced were associated with virological response. DRV susceptibility interpretation using either Stanford HIVdb or Rega rules also predicted a virological response. In multivariable models, variables independently associated with virological response were the backbone therapy GSS and DRV/RTV genotypic resistance interpretation using the Rega rules. No other

Table 2. Predictors of virological response: univariable and multivariable analyses on the entire set of patients

Predictor variable	Univariable analysis[a]		Multivariable analysis[a]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
HIV-1 RNA at GRT, per 1 $\log_{10}$ copies/ml higher	1.11 (0.87–1.42)	0.404	1.16 (0.89–1.52)	0.271
CD4⁺ T-cell count, per 100 cells/mm³ higher	0.89 (0.78–1.01)	0.065	0.96 (0.84–1.10)	0.514
Backbone GSS ANRS, per 1 lower	0.72 (0.53–0.98)	0.037	0.65 (0.46–0.91)	0.013
Backbone GSS Stanford HIVdb, per 1 lower	0.69 (0.48–0.99)	0.046	NC	NC
Backbone GSS Rega, per 1 lower	0.73 (0.52–1.03)	0.071	NC	NC
Number of prior PIs experienced, per 1 additional	0.82 (0.70–0.97)	0.019	0.81 (0.68–0.97)	0.021
Use of new class	1.72 (0.96–3.07)	0.068	0.99 (0.52–1.86)	0.973
Use of NNRTI as a new class	2.10 (0.90–4.91)	0.086	NC	NC
Use of new raltegravir	1.19 (0.54–2.61)	0.670	NC	NC
Use of new enfuvirtide	2.74 (1.07–7.02)	0.036	NC	NC
DRV ANRS score, per higher susceptibility category R, I or S	1.32 (0.97–1.79)	0.070	1.22 (0.88–1.72)	0.241
DRV HIVdb score, per higher susceptibility category R, I or S	1.67 (1.05–2.56)	0.028	1.59 (0.98–2.05)	0.059
DRV Rega score, per higher susceptibility category R, I or S	1.97 (1.37–2.82)	<0.001	1.94 (1.32–2.86)	<0.001
ANRS DRV-ATU score, per one score higher	0.93 (0.73–1.17)	0.518	0.88 (0.69–1.13)	0.330
DRV Tibotec score, per resistance score higher	0.92 (0.77–1.09)	0.329	0.96 (0.80–1.16)	0.662
PREDZISTA score, per resistance score higher	0.53 (0.24–1.15)	0.107	1.14 (0.92–1.42)	0.230
Number of IAS–USA 2008 DRV resistance mutations, per 1 more	0.87 (0.74–1.04)	0.123	0.88 (0.73–1.05)	0.161

Total *n*=217. [a]Other variables tested but found not associated with virological response were age, prior AIDS, prior use of (fos-)amprenavir, duration of prior combination antiretroviral therapy and number of regimens experienced. [b]Each darunavir (DRV) interpretation was individually adjusted for baseline viral load, CD4⁺ T-cell count, prior number of protease inhibitors (PIs), backbone genotypic susceptibility score (GSS; by Agence Nationale de Recherche sur le SIDA et les hépatites virales (ANRS)) and use of a new class; values from the latter variables are those derived from the model including DRV interpretation by Rega. GRT, genotypic resistance test; I, intermediate resistant; NC, not computed in the model; NNRTI, non-nucleoside reverse transcriptase inhibitor; R, resistant; S, susceptible.

interpretation rule for DRV genotypic resistance showed an independent association with the subsequent virological response to a DRV-containing regimen.

Derivation of a new DRV/RTV genotypic resistance interpretation score from virological outcomes

In the randomly selected derivation set of patients (n=132), the following mutations present in >4% of the sample were associated, at the P<0.30 level, with a virological response: L10I, I13V, L33F, I47V, I50V, I54M, I62V, I66F, A71V, L76V and L89V (Table 3). Mutations associated with response were used for a stepwise construction of the new DRV score, as specified in the *Methods* section. Additional protease mutations were individually tested using a set previously selected in a genotypic resistance and DRV response study performed by the authors on a smaller set [27]; finally, mutations known to confer significant phenotypic resistance to the drug were added [18]. The final DRV-2009 score included 22 mutations at 20 codons and showed a strong correlation with virological response (Table 4).

Finally, the derived continuum score was classified into three resistance categories based on visual inspection of the probability of virological response with the individual scores. The classification showing the strongest correlation with the virological response was the one where viruses with scores from -5 to -1 were categorized as being susceptible, those with scores between 0 and 1 were categorized as having intermediate resistance, and those with scores 2 or higher were categorized as being resistant (Table 4). The proportions of virological responders according to the DRV-2009 score in the derivation set are illustrated in Figure 1.

Validation of the DRV/RTV resistance interpretation score and comparison with existing rules

The categorical DRV-2009 score obtained from the derivation dataset was then tested on the independent validation dataset (n=85). The proportions of virological responders according to the three categories of the DRV-2009 score in the validation set are illustrated in Figure 1.

From the receiver operating characteristic analysis on the validation dataset, the DRV-2009 score and the Rega rules showed the highest AUROC (Table 5). All the other scores showed an AUROC significantly (or borderline) higher than the null reference value of 0.5, except for the Tibotec, Predzista and IAS–USA mutation scores, which did not perform better than random guessing. In a multivariable logistic regression model adjusting for mutual interpretation and new use of enfuvirtide in the regimen, the DRV-2009 score (per higher susceptibility category OR for virological response 2.12, 95% CI 1.14–3.94; P=0.017) and the Rega interpretation rule (OR 2.19, 95% CI 1.18–4.05; P=0.013) were both independently predictive of virological response.

Survival analysis

Because the virological response could have been achieved at different time points between the 8th and the 36th week of follow-up, we executed an additional survival analysis accounting for the time needed to reach a virological response, censoring when the virological response was not reached. By performing a multivariable Cox model using the same set of adjusting covariates as for the multivariable logistic regression, we had a confirmation for the Rega score; in addition, the Stanford HIVdb, Tibotec and PREDZISTA scores showed a significant association with the time to achieve a virological response (see Additional file 2).

Discussion

The HIV-1 protease genotypic determinants of DRV/RTV resistance are still incompletely defined. In the present study, we have evaluated all of the different available genotypic resistance interpretation scores in a set of 217 patients from different clinical settings undergoing a DRV/RTV-containing regimen after experiencing HAART failure. We show that the Rega interpretation score is the only one independently predictive of virological response in this set of heavily experienced patients. The external validation of newly derived sets of interpretation rules is a necessary process, particularly for newly developed drugs. This study indicates that the divergence of the existing interpretations for the genotypic resistance to DRV may translate into different capabilities to predict virological response in external patient datasets. In this context, it is reassuring to observe that Rega, one among the most popular HIV drug resistance interpretation systems, confers a robust clinically predictive interpretation of DRV genotypic resistance. The fact that this system employs weighting

Table 3. Association of substitutions in HIV-1 protease from consensus B[a] with virological response at $P<0.30$[b] in the derivation dataset

Mutation	Frequency, %	Beta	Virological response OR (95% CI)	P-value
L10V	9.8	0.81	2.25 (0.59–8.62)	0.235
L10I	35.6	0.45	1.57 (0.74–3.31)	0.240
I13V	19.9	0.59	1.80 (0.69–4.66)	0.229
L33F	45.5	-0.49	0.61 (0.30–1.24)	0.172
I47V	10.6	-0.84	0.43 (0.14–1.32)	0.141
I50V	10.6	-0.69	0.50 (0.16–1.59)	0.242
I54M	6.5	-1.24	0.29 (0.07–1.21)	0.089
I62V	42.6	0.62	1.86 (0.90–3.84)	0.099
I66F	6.8	1.70	5.48 (0.66–45.19)	0.114
L74P	5.5	0.33	1.26 (0.55–3.10)	0.128
L76V	8.3	-0.71	0.49 (0.14–1.71)	0.265
V77I	24.2	0.81	2.26 (0.93–5.53)	0.070
L89V	13.6	-1.07	0.34 (0.12–0.96)	0.041

[a]Frequency >4%. [b]Logistic regression. Total $n=132$.

Table 4. Stepwise construction and performance of the new DRV interpretation score in the derivation dataset

Mutations included in the score	Algebraic expression of mutations	Correlation coefficient with virological response
Mutations associated with virological outcome in the derivation set: L10I/V, I13V, L33F, I47V, I50V, I54M, I62V, I66F, L74P, L76V, V77I and L89V	L33F+I47V+2*I50V+2*I54M+L76V+2*L89V-L10I/V-I13V-I62V-I66F-L74P-V77I	0.359
Additional mutations associated in analysis: V11I, G16E, R41K, G48V, F53I/L, K55R, D60E and N88D [27]	Add to the previous mutations V11I+R41K+K55R+D60E+N88D-G16E-G48V-F53I/L	0.363
L74P [18,21]	Add +L74P and omit -L74V	0.375
Final DRV SCORE 2009: V11I+L33F+R41K+I47V+2*I50V+2*I54M+K55R+D60E+L74P+L76V+N88D+2* L89V-L10I/V-I13V-G16E-G48V-F53I/L-I62V-I66F-V77I	Susceptible: score <0; partial resistance: score 0–1; resistant: score ≥2	0.395

Total $n=132$.

of the different mutations may represent one of its strengths and may confer to it the sufficient robustness to predict response in different contexts. Interestingly, however, the rules derived from a large dataset of clinical trials (the Power and Duet studies) associating genotypic resistance with phenotypic susceptibility and virological response (the Tibotec score) did not predict virological response as well in this dataset, although they were significantly associated with the time of virological response under survival analysis. This was probably due to different distributions of follow-up times in the various cohorts that were considered for the determination of the GSS.

Prompted by this unexpected finding, we tried to use several categorizations to analyse the correlation of this score with virological response (ADL *et al.*, data not shown), but this did not improve its prediction. As a possible explanation we hypothesize that this interpretation score was derived from a different context of use of DRV, where patients were included not based on a DRV susceptibility assay result. On the contrary, since all the patients from this study were selected from the early access programme of DRV or from the post-marketing clinical practice, in most (if not all) cases DRV was selected based on the results of a genotypic resistance test with its interpretation derived from the indications of the drug manufacturer. In this context, the previously existing and already applied rules may be less predictive of subsequent response.

Further in this study, we developed a new algebraic weighted score, denominated DRV-2009, which also showed independent predictive value for virological response, even after adjusting for the Rega score. While rules derived from associations of viral genotypes with subsequent virological outcomes are certainly those most clinically relevant, we recognize that this methodology may be subject to limitations, particularly since the rarest mutations, which may indeed play a relevant role, may be missed by their low representativeness in the study datasets. In order to partially overcome this limitation, we used a combined approach to derive the new score, firstly by using genotype–outcome association data and secondly by testing the addition of mutations that have been reported to affect phenotypic susceptibility to DRV and were missed or contradicted in the previous step. Thus, the final score included protease mutations already known to confer decreased *in vitro* susceptibility or decreased *in vivo* response to DRV, such as V11I, L33F, I47V, I50V, I54M, T74P, L76V and L89V, but was also reinforced by a number of protease substitutions that are either polymorphisms (R41K, K55R and D60E) or are known to confer resistance to other PIs (N88D). Interestingly, other mutations were associated with improved response to DRV, including some associated with reduced response/susceptibility to other PIs (L10I/V, G16E, G48V and F53L), polymorphisms that are more frequent in PI-treated individuals (I13V and I62V) and other polymorphisms (I66F and

Figure 1. Proportions of patients achieving virological response according to the DRV-2009 resistance categorization in the derivation dataset and in the validation dataset

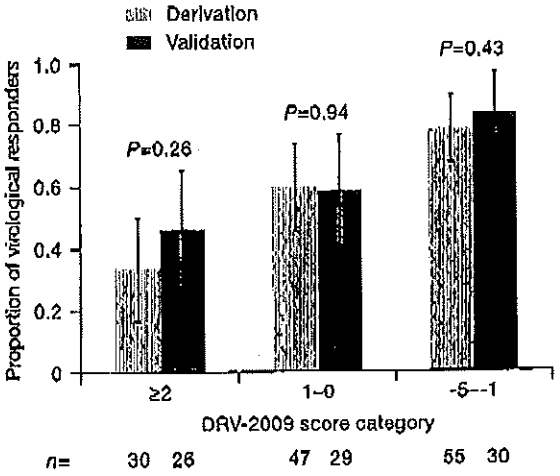

Table 5. AUROC of the different interpretations of DRV towards predicting the virological response in the validation set

Interpretation	AUROC	95% CI	*P*-value[a]
DRV-2009 score, per higher susceptibility category R, I and S	0.68	0.56–0.79	0.006
DRV ANRS score, per higher susceptibility category R, I and S	0.63	0.51–0.75	0.045
DRV HIVdb score, per higher susceptibility category R, I and S	0.63	0.51–0.76	0.043
DRV Rega score, per higher susceptibility category R, I and S	0.68	0.56–0.80	0.006
ANRS DRV-ATU score, per resistance score higher, algebraic sum	0.62	0.50–0.74	0.069
DRV Tibotec score, per resistance score higher	0.51	0.39–0.64	0.873
PREDZISTA score, per resistance score higher	0.55	0.43–0.68	0.430
Number of IAS-USA 2008 DRV resistance mutations, per 1 more	0.54	0.42–0.67	0.488

Total *n*=85. [a]*P*-value for difference from random guessing. ANRS, Agence Nationale de Recherche sur le SIDA et les hépatites virales; AUROC, area under the receiver operating characteristic curve; DRV, darunavir; I, intermediate resistant; R, resistant; S, susceptible.

V77I). A potential limitation of our newly proposed score is the absence of a further external validation, which is therefore needed to show that it may be applied to the majority of patients. In addition, only one genotype resistance test was considered, corresponding to the baseline date, whilst the inclusion of historical genotypes (for example, the consideration of a broader set of mutations) might have an important effect in augmenting the confidence in predicting the virological response [28]. However, our DRV/RTV resistance score is based on a relatively large number of patients with multiple PI resistance mutations at baseline and was validated in a separate dataset included in this study, although the patients' follow-up periods were spanning a considerable time range and we could not evaluate specific short-term or medium-term responses.

In our analysis we did not find any independent association of the usage of maraviroc or raltegravir with the virological response, probably because of the low proportion of patients that were administered DRV/RTV together with either maraviroc or raltegravir. Moreover, this patient cohort was derived from a large number of different clinics probably representing different populations and drug prescription attitudes.

In conclusion, interpretation methods (such as Rega) based upon weighting of the different mutations show a major advantage to predict response to DRV/RTV in different contexts. Weighting mutations and considering at least linear combinations of weighted mutations are necessary, but it is also important to assess with accuracy each mutation weight. The newly developed DRV resistance interpretation presented here could be usefully employed for predicting the virological response to DRV/RTV in PI-experienced patients and for revising current genotypic interpretation systems.

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Additional file

Additional file 1: A list of the members of the TMC114-C226 and ARCA study groups can be found at http://www.intmedpress.com/uploads/documents/AVT-10-OA-1672_De_Luca_Add_file1.pdf

Additional file 2: Survival analyses using multivariable Cox models and accounting for the time needed to reach a virological response can be found at http://www.intmedpress.com/uploads/documents/AVT-10-OA-1672_De_Luca_Add_file2v2.pdf

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