

J Antimicrob Chemother 2011; **66**: 192–200
doi:10.1093/jac/dkq384 Advance Access publication 29 October 2010

Comparative evaluation of seven resistance interpretation algorithms and their derived genotypic inhibitory quotients for the prediction of 48 week virological response to darunavir-based salvage regimens

Daniel Gonzalez de Requena^{1*}, Stefano Bonora¹, Ottavia Viganò², Andrea Calcagno¹, Cristina Cometto¹, Antonio D'Avolio¹, Lorena Baietto¹, Valeria Ghisetti³, Silvia Magnani¹, Stefania Ferramosca², Paola Vitiello², Massimo Galli², Stefano Rusconi² and Giovanni Di Perri¹

¹Department of Infectious Diseases, University of Torino, Torino, Italy; ²Department of Infectious Diseases, University of Milano, Milano, Italy; ³Laboratory of Virology, Amedeo di Savoia Hospital, Torino, Italy

*Corresponding author. Tel: +39-011-4393980; Fax: +39-011-4393882; E-mail: danifarre@hotmail.com

Received 21 February 2010; returned 1 June 2010; revised 2 September 2010; accepted 14 September 2010

Background: The darunavir genotypic inhibitory quotient (gIQ) has been suggested as one of the predictors of virological response to darunavir-containing salvage regimens. Nevertheless, which resistance algorithm should be used to optimize the calculation of gIQ is still debated. The aim of our study was to compare seven different free-access resistance algorithms and their derived gIQs as predictors of 48 week virological response to darunavir-based salvage therapy in the clinical setting.

Methods: Patients placed on two nucleoside reverse transcriptase inhibitors + 600/100 mg of darunavir/ritonavir twice daily ± enfuvirtide were prospectively evaluated. Virological response was assessed at 48 weeks. Darunavir resistance interpretation was performed according to seven different algorithms, of which two were weighted algorithms. Analysis of other factors potentially associated with virological response at 48 weeks was performed.

Results: Fifty-six treatment-experienced patients were included. Overall, 35 patients (62.5%) had a virological response at 48 weeks. Receiver operator characteristic curve analysis showed that De Meyer's weighted score (WS) and its derived gIQ (gIQ WS) were the most accurate parameters defining virological response, and related cut-offs showed the best sensitivity/specificity pattern. In univariate logistic regression analysis, baseline log viral load ($P=0.028$), optimized background score ≥ 2 ($P=0.048$), WS > 5 ($P=0.001$) and WS gIQ ≥ 600 ($P<0.0001$) were independently associated with virological response. In multivariate analysis, only baseline log viral load ($P=0.008$) and WS gIQ ≥ 600 ($P<0.0001$) remained in the model.

Conclusions: In our study, although different resistance interpretation algorithms and derived gIQs were associated with virological response, gIQ WS was the most accurate predictive model for achieving a successful virological response.

Keywords: weighted scores, protease inhibitors, HIV

Introduction

Darunavir is the most recently licensed protease inhibitor with *in vitro* activity against both wild-type and protease inhibitor-resistant HIV-1 isolates. Darunavir showed efficacy in patients in whom multiple protease inhibitor-containing regimens failed.^{1,2} Modelling and crystallographic studies of the HIV-1 protease have demonstrated unusual darunavir binding characteristics that predict greater resilience to the development of resistance and greater activity against resistant viruses than

earlier protease inhibitors.^{1,3–6} *In vitro* selection of darunavir-resistant HIV-1 from wild-type strains appears to be slower and less frequent than with other protease inhibitors,^{1,5} reflecting the particularly strong binding of darunavir to the HIV protease.^{3,6}

The efficacy of ritonavir-boosted darunavir in combination with an optimized background regimen has been studied in highly treatment-experienced HIV-infected patients in two Phase IIb trials, in which virological and immunological efficacy were superior to the control group.^{2,7–10}

The number of active drugs included in the antiretroviral regimen and the degree of viral resistance to darunavir are factors associated with virological and immunological responses in patients undergoing darunavir-based salvage therapy.^{7,11–15} The usefulness of a genotypic resistance test for the prediction of virological response to darunavir has been widely studied, and several amino acid changes in the protease gene have been associated with higher rates of virological failure.¹¹ Baseline darunavir phenotypic resistance and the phenotypic inhibitory quotient (IQ) appeared to be predictive of virological outcome.^{11,14} Moreover, the darunavir virtual IQ has been shown to predict virological response to darunavir-based salvage therapy better than trough concentration (C_{trough}) or resistance mutations considered separately.¹⁶

The genotypic IQ (gIQ), calculated as the ratio between protease inhibitor C_{trough} and the number of protease inhibitor-associated mutations, is simpler to derive in the clinical setting than the virtual IQ or phenotypic IQ and has been associated with virological response to other protease inhibitors.^{17–21} Three studies consensually showed a relationship between the darunavir gIQ and virological response,^{16,22,23} although this association appeared to be mainly driven by the number of mutations. However, in our opinion, the darunavir gIQ has not been fully evaluated in the clinical setting. In previous studies, in fact, all mutations were equally weighted in the calculation of the darunavir gIQ, while the use of weighted mutation scores could optimize the predictivity and reliability of the latter.

Therefore, our aim was to explore the impact of seven different resistance interpretation algorithms and their derived gIQs on 48 week virological response to darunavir-based salvage regimens in clinical practice.

Patients and methods

Study design

Patients enrolled in the darunavir Expanded Access Program study in two different centres in northern Italy from May 2006 to August 2008, and treated with a regimen based on 600/100 mg of darunavir/ritonavir twice daily plus an optimized background regimen, were prospectively evaluated. The criteria for inclusion were baseline plasma HIV-RNA >50 copies/mL, HIV genotypic resistance test performed in the last 3 months before initiation of darunavir-based regimen, regular follow-up, availability of at least one darunavir plasma concentration measurement, and self-reported adherence of >90% in the last 7 days before each visit. Viral load and CD4+ cell count were assayed by RT-PCR (Cobas Amplicor HIV-1 monitor test v 1.5, Roche Molecular Systems, Switzerland) and flow cytometry, respectively, at baseline and at 4, 12, 24 and 48 weeks. This study was performed in the context of the Expanded Access Program for darunavir, so ethics approval was obtained from the local ethics committee in the context of patient inclusion in this study. The only test not considered for routine monitoring was the therapeutic drug monitoring. For this test, informed consent was obtained from patients at each visit.

Study endpoints

The primary virological response endpoint was reaching complete viral load suppression (<50 copies/mL). Additionally, significant antiviral activity was considered if viral load decreased by ≥ 1 log and/or the patient achieved <50 copies/mL HIV-RNA with no viral load increase >0.5 log as compared with the maximal viral load decrease. Immunological response at each timepoint was also studied. The Intention to

Treat Last Observation Carried Forward method was used. In this approach, for subjects who discontinued darunavir before week 48, the last HIV-RNA and CD4+ cell count recorded before darunavir withdrawal were considered for further analysis.

Genotypic resistance test

A genotypic resistance test was performed at baseline by using the ViroSeq HIV-1 Genotyping System (Celera Diagnostics, LCC, Alameda, CA, USA) with an automatic sequencer (ABI PRISM 3100, PE Biosystems, Foster City, CA, USA). FASTA analysis of the reverse transcriptase (amino acids 1–335) and protease (amino acids 1–99) genes was conducted using the Stanford HIVdb Program (<http://hivdb.stanford.edu/>, accessed on 24 July 2009). Specific mutations in the protease gene were obtained from the Stanford HIVdb report, and genotypic darunavir resistance interpretation was performed according to seven different algorithms: the HIVdb—weighted interpretation algorithm (<http://hivdb.stanford.edu/>, accessed on 24 July 2009); the weighted mutation score (WS) proposed by De Meyer *et al.*²⁴ (V11I, I54L/M, G73S and L89V, score 1; V32I, L33F and I47V, score 3; L76V and I84V, score 4; and I50V, score 5); the National Agency for AIDS Research resistance algorithm (ANRS) (<http://www.hivfrenchresistance.org/>, accessed on 15 October 2009); the number of darunavir resistance mutations derived from the POWER trials (POW);¹¹ the number of primary protease inhibitors resistance mutations from the last International AIDS Society (IAS) list update;²⁵ the total number of mutations associated with resistance to protease inhibitors in the IAS list;²⁵ and the genotypic score calculated in the PREDIZISTA study.²²

Calculation of the optimized background score (OBS)

The OBS was calculated by relying on the genotypic sensitivity score provided by the Stanford HIVdb Program (<http://hivdb.stanford.edu/>, accessed on 24 July 2009). The FASTA sequence was submitted and a resistance score for each companion drug was obtained. A drug resistance estimate for the latter was obtained by summing the scores of each mutation associated with resistance to such drug. A drug was considered to be not active with a score of ≥ 60 . Drugs reported to be active or partially active (<60) were scored as 1, while drugs reported to be not active (≥ 60) were scored as 0. The OBS was defined as the sum of the scores of all the drugs included in the regimen. In subjects administered with enfuvirtide, this drug was considered to be not active if previously administered to the subject and discontinued due to virological failure.

Pharmacokinetic analysis

Blood samples were collected into lithium heparin tubes before the morning dose, and plasma was separated by centrifugation at 5000 rpm, refrigerated at 4°C for 10 min and then stored at –70°C until analysis. At the time of blood sampling, patients were asked about the time of their last darunavir dose intake. Only plasma samples obtained 10–14 h post-dose were considered for C_{trough} analysis. Darunavir concentrations were determined by using a validated HPLC method with UV detection.²⁶ In subjects with more than one pharmacokinetic measurement, the mean value of all available C_{trough} values throughout the study period was considered. The gIQ values were calculated for each patient as the ratios between mean darunavir C_{trough} and the scores derived from the seven different algorithms considered.

Statistical analyses

Receiver operator characteristic (ROC) curve analysis was used to discriminate between the different interpretation algorithms and their derived gIQs. ROC curve analysis was used to determine cut-off values

for those parameters significantly associated with virological response at 48 weeks. The area under the curve (AUC) of ROC, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio positive [LHR+; calculated as sensitivity/(1 – specificity)] and likelihood ratio negative [LHR–; calculated as (1 – sensitivity)/specificity] were analysed for the selection of the best resistance algorithm and gIQ. Overall, the best predictors were those with the highest LHR+ and the lowest LHR–. Once identified, it was included in a logistic regression model with all the other baseline characteristics potentially correlated with virological response at 48 weeks. Variables showing *P* values of <0.05 in univariate analysis were considered for multivariate analysis (forward conditional method). Survival analysis was performed by Kaplan–Meier analysis with the log rank test as the comparison factor.

The χ^2 test was used to analyse the association between categorical variables. Statistical significance was considered at *P*<0.05. Statistical analysis was performed using SPSS software (2004, version 13.0, SPSS Inc., Chicago, IL, USA).

Results

Baseline patient characteristics

Baseline characteristics of the 56 patients included in this study are reported in Table 1. Median (range) numbers of previous nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors were 5 (2–6) and 1 (0–2), respectively. Subjects had previous failures to a median (range) of 4 (1–7) protease inhibitor-based regimens.

The nucleoside reverse transcriptase inhibitors most frequently co-administered with darunavir/ritonavir were tenofovir (*n*=38, 67.9%), emtricitabine (*n*=29, 51.8%) and lamivudine (*n*=20, 35.7%). Tenofovir and emtricitabine were co-administered in 23 (41.1%) subjects. Other antiretroviral drugs co-administered with darunavir/ritonavir were zidovudine (*n*=15, 26.8%), abacavir (*n*=8, 14.3%), stavudine (*n*=8, 14.3%), didanosine (*n*=5, 8.9%), zalcitabine (*n*=2, 3.6%) and etravirine (*n*=7, 12.5%). Enfuvirtide was administered to 18 (32.1%) subjects, of whom only 2 were enfuvirtide-experienced and had previous virological failure with a regimen including this drug.

The optimized background regimen included a median (range) of 3 (2–4) drugs, of which 2 (0–3) were considered active. Seven subjects (12.5%) had no active drugs in the optimized background regimen, 18 (32.1%) had one active drug and 31 (55.4%) had two or more active drugs.

Virological and immunological outcomes

The median [interquartile range (IQR)] log HIV-RNA and CD4+ cells at baseline were 3.95 (2.95–4.93) log and 265 (124–388) cells/mm³. The median (IQR) CD4+ cell count increases were 36 (7–99), 52 (–2–139), 90 (15–140) and 83 cells/mm³ (33–181) at 4, 12, 24 and 48 weeks, respectively. Median (IQR) plasma HIV-RNA decrease was –1.92 (–2.53 to –1), –1.88 log (–2.66 to –0.89), –1.71 log (–2.69 to –0.65) and –1.38 log (–2.69 to –0.62) at 4, 12, 24 and 48 weeks, respectively. Complete viral suppression below 50 copies/mL was observed in 26 (46.4%), 29 (51.8%), 34 (60.7%) and 35 (62.5%) subjects at 4, 12, 24 and 48 weeks, respectively. Significant antiviral activity was observed in 49 (87.5%), 43 (76.8%), 38 (67.9%) and 35 (62.5%) subjects

Table 1. Demographics and baseline characteristics of the study population

	Total population
Total number of patients	56
Sex (male) ^a	44 (78.6)
Age (years) ^b	45 (42–50)
Weight (kg) ^b	69 (58–75)
Height (cm) ^b	175 (168–180)
HCV co-infection ^a	17 (30.4)
Clinical status (CDC 1993 classification) ^a	
A	29 (51.8)
B	9 (16.1)
C	18 (32.1)
Pharmacological history	
no. of previous PIs ^c	5 (2–7)
no. of PIs with virological failure	4 (1–7)
no. of previous NRTIs ^c	5 (2–6)
no. of NRTIs with virological failure	5 (0–6)
previous TDF ^a	54 (96.43)
TDF with virological failure	25/54 (46.3)
no. of previous NNRTIs ^c	1 (0–2)
no. of NNRTIs with virological failure	1 (0–2)
previous enfuvirtide ^a	28 (50)
OB regimen	
no. of drugs in OB regimen ^c	3 (2–4)
OB regimen with enfuvirtide ^a	18 (32.14)
no. of subjects with previous virological failure to enfuvirtide	2/18 (11.1)
no. of drugs in OB considered to be active (OBS) ^c distribution of OBS ^a	2 (0–3)
0 ^a	7 (12.5)
1 ^a	18 (32.1)
2 ^a	24 (49.2)
3 ^a	7 (12.5)
Baseline immuno-virology	
log HIV-RNA ^b	3.95 (2.95–4.93)
CD4+ cells/mL ^b	265 (124–388)
CD4+ ^b	15.1 (14–22)

NRTIs, nucleoside reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; TDF, tenofovir disoproxil fumarate; OB, optimized background; OBS, optimized background score; IQR, interquartile range; HCV, hepatitis C virus.

^aValues are expressed as number of subjects (%).

^bValues are expressed as median (IQR).

^cValues are expressed as median (range).

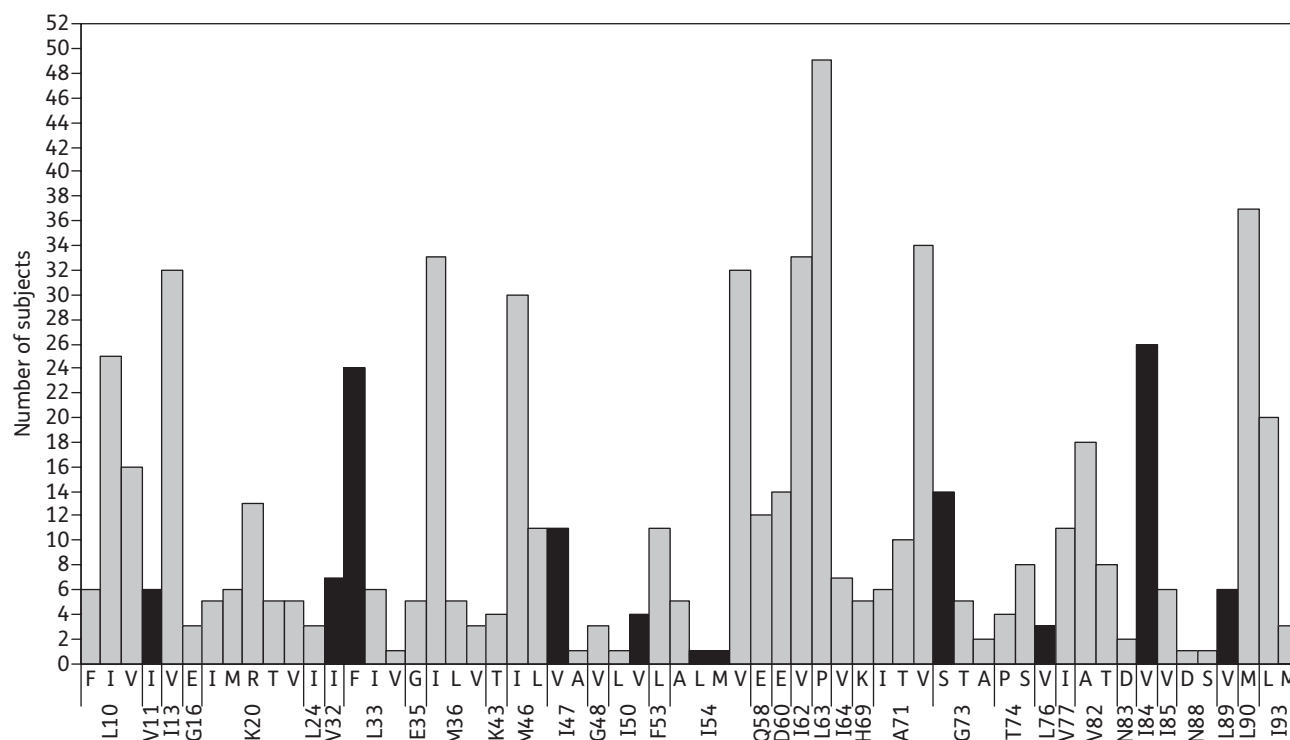


Figure 1. Frequency of specific amino acid changes at specific codons in the protease gene. Black bars represent amino acid changes associated with darunavir resistance mutations in the POWER studies.

at 4, 12, 24 and 48 weeks, respectively. Three (5.35%) subjects never had significant antiviral activity and 18 (32.14%) subjects had significant antiviral activity during the follow-up period, but this was not sustained. According to the OBS, complete viral suppression at 48 weeks was observed in 2/7 (28.5%), 10/18 (55.5%) and 23/31 (74.2%) of subjects with an OBS of 0, 1 and ≥ 2 , respectively ($\chi^2=5.6$, $P=0.06$). No differences in virological response or viral load decrease at 48 weeks were observed according to enfuvirtide administration. In fact, virological response at 48 weeks was observed in 12/18 subjects treated with enfuvirtide, whereas it was observed in 23/38 without enfuvirtide ($\chi^2=0.196$, $P=0.658$). Viral load decrease in subjects administered with enfuvirtide was -1.92 ± 1.32 versus -1.52 ± 1.35 without enfuvirtide ($P=0.658$). No differences in the number of darunavir-associated resistance mutations or gIQs were observed, but a higher number of active drugs were administered in the enfuvirtide arm (2 versus 1, $P=0.001$). Nevertheless, the increase in the CD4+ cell count and percentage at this timepoint was higher in the enfuvirtide group [88 ± 156 versus 165 ± 139 cells/mm³ ($P=0.08$) and 2.6 ± 3.47 versus 4.4 ± 4.1 cells/mm³ ($P=0.15$), respectively], although this difference was not statistically significant.

Pharmacokinetic analysis

A total of 188 plasma samples were collected for pharmacokinetic analysis. A median (IQR) of 4 (2–4) samples were obtained from each subject. The mean (\pm SD) darunavir C_{trough} was 4720 ± 2380 ng/mL ($n=43$), 3666 ± 1987 ng/mL ($n=41$), 4836 ± 2249 ng/mL ($n=41$) and 4421 ± 2182 ng/mL ($n=28$) at

4, 12, 24 and 48 weeks, respectively. The overall mean (\pm SD) of all available darunavir C_{trough} measurements through 48 weeks was $4561 (\pm 1933)$ ng/mL. The intra- and inter-individual variability (CV%, SD/mean $\times 100$) of the darunavir C_{trough} were 29.6% and 47.5%, respectively. Although the darunavir C_{trough} was higher in the virological response group (4913 ± 1990 versus 3974 ± 1722 ng/mL), this difference was not statistically significant ($P=0.078$).

Analysis of resistance interpretation algorithms and their derived gIQs for the prediction of 48 week virological response

Specific amino acid changes in the protease gene are presented in Figure 1. In ROC curve analysis, WS and WS gIQ were the most accurate parameters defining virological response (AUC=0.797 and AUC=0.848, respectively), and calculated cut-offs showed that these resistance interpretation algorithms had the best sensitivity/specificity profile (WS >5, sensitivity 77.1%/specificity 81%; and WS gIQ ≥ 600 , sensitivity 82.9%/specificity 85.7%), the highest LHR+ (4.06 and 5.79, respectively) and the lowest LHR– (0.28 and 0.19). Results of the different HIV-1 resistance algorithms and their derived gIQs are presented in Table 2. Twenty-seven out of 31 (87.1%) subjects with WS ≤ 5 showed virological response, whereas it was achieved in only 8/25 (32%) with WS >5 ($\chi^2=17.9$, $P=0.001$). In the same way, 29/32 (90.65%) subjects with a darunavir WS gIQ ≥ 600 achieved virological response, whereas it was recorded in only 6/24

Table 2. ROC curve analysis of the seven resistance interpretation algorithms

	Median value (IQR)	ROC curve analysis								
		AUC	P value	cut-off	sensitivity (%)	specificity (%)	PPV (%)	NPV (%)	LHR+	LHR-
Mutation score										
WS	4.5 (3–7)	0.797	0.001	>5	77.1	81	87.1	68	4.06	0.28
ST	33 (28–47)	0.763	0.001	>37	71.42	71.47	80.64	60	2.5	0.39
POW	2 (1–3)	0.783	<0.0001	>2	88.6	57.1	77.5	75	2.06	0.2
ANRS	1.5 (1–3)	0.783	<0.0001	>2	88.57	47.6	73.8	71.4	1.69	0.2
PRIMs	4 (3–5)	0.655	0.054	—	—	—	—	—	—	—
TOTIAS	12 (11–13)	0.543	0.594	—	—	—	—	—	—	—
PRED	4 (2–4)	0.544	0.588	—	—	—	—	—	—	—
gIQ										
WS	888 (473–1728)	0.848	<0.0001	≥600	82.9	85.7	90.62	75	5.79	0.19
STAN	113 (82.5–204)	0.809	<0.0001	>106	80	85.7	90.32	72	5.59	0.23
POW	2584 (1355–4350)	0.826	<0.0001	>2100	80	85.7	87.8	73.9	4.35	0.21
ANRS	2681 (1473–4402)	0.824	<0.0001	>2200	82.9	85.7	90.6	75	5.79	0.19
PRIMs	1128 (795–1967)	0.738	0.003	>1100	68.6	76.4	82.75	59.25	2.88	0.41
TOTIAS	349 (272–493)	0.684	0.022	>350	60	71.4	77.7	51.7	2.09	0.56
PRED	1440 (920–2375)	0.614	0.158	—	—	—	—	—	—	—

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; LHR+, likelihood ratio positive; LHR-, likelihood ratio negative; gIQ, genotypic inhibitory quotient.

WS, weighted score (V11I, I54L/M, G73S and L89V, score 1; V32I, L33F and I47V, score 3; L76V and I84V, score 4; and I50V, score 5).

ST, HIVdb—Stanford University genotypic resistance interpretation algorithm (<http://hivdb.stanford.edu/>).

POW, power mutation score (V11I, V32I, L33F, I47V, I50V, I54L/M, G73S, L76V, I84V and L89V).

ANRS, National Agency for AIDS Research resistance algorithm (V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V and L89V).

PRIMs, protease inhibitor primary resistance mutations from the IAS list [30N, 32I, 33F, 46 I/L, 47 V/A, 48V, 54 L/M, 58E, 74P, 82 A/T/F/S, 88S and 90M⁹].

TOTIAS, total number of protease inhibitor-associated resistance mutations from the IAS list.⁹

PRED, PREDIZISTA study score (I13V, V32I, L33F/I/V, E35D, M361/L/V, I47V, F53L and I62V).

(25%) subjects with a darunavir WS gIQ <600 ($\chi^2=25.2$, $P<0.0001$).

Determinants of virological response at 48 weeks

Baseline factors potentially associated with virological response to darunavir-based regimens were analysed by using logistic regression analysis. In univariate logistic regression analysis, baseline viral load ($P=0.028$), OBS ≥ 2 ($P=0.048$), WS >5 ($P=0.001$) and WS gIQ ≥ 600 ($P<0.0001$) were shown to be independently associated with virological response. In multivariate analysis, baseline viral load ($P=0.008$) and WS gIQ ≥ 600 ($P<0.0001$) remained in the model as predictors of virological response at 48 weeks. The results of logistic regression analysis are reported in Table 3. Kaplan–Meier survival analysis (Figure 2) showed that subjects with WS gIQ <600 (log rank=17.95, $P<0.0001$) and with WS >5 (log rank=10.35, $P=0.001$) were more likely to have virological failure.

Analysis of the different virological response patterns

Three different patterns of virological response were observed in our population: the null response group ($n=3$), when significant antiviral activity was not reached at any time during the study;

Table 3. Summary results of logistic regression analysis; virological response at 48 weeks was considered as a dependent variable, whereas variables listed in the first column of the table were tested as independent variables.

	Univariate analysis P value	Multivariate analysis P value
Baseline log viral load	0.028	0.008
Baseline CD4+ cell count	0.171	
OBS ≥ 2	0.048	NS
Previous no. of PIs	0.77	
ENF administration	0.658	
ETV administration	0.204	
TDF administration	0.461	
CDC stage	0.564	
Darunavir C_{trough}	0.089	
gIQ WS ≥ 600	<0.0001	<0.0001
WS >5	0.001	NS

PI, protease inhibitor; ENF, enfuvirtide; ETV, etravirine; TDF, tenofovir disoproxil fumarate; NS, not significant; OBS, optimized background score; WS, weighted score; gIQ, WS genotypic inhibitory quotient.

Table 4. Factors associated with 48 week outcome according to virological response pattern

	Null virological response (n=3)	Partial virological response (n=18)	Full virological response (n=35)	P value
Darunavir C_{trough}	3261 ± 2889	4093 ± 1549	4914 ± 1990	0.19*
Baseline log HIV-RNA	3.58 ± 1.34	4.5 ± 1.08	3.6 ± 1.12	0.033*
Δ viral load	0.16 ± 0.76	-0.64 ± 0.83	-2.3 ± 1.12	<0.0001*
POW	2.6 ± 1.5	2.6 ± 1.1	1.4 ± 1.1	0.001*
WS	7 ± 6.5	7.5 ± 2.6	3.88 ± 3	0.001*
WS >5	2 (66.6%)	15 (83.3%)	8 (22.8%)	0.001**
gIQ WS	437 ± 127	632 ± 487	2050 ± 1675	0.002*
gIQ WS ≥600	0 (0%)	3 (16.6%)	29 (82.85%)	<0.0001**
OBS	2 ± 1	1.2 ± 1.06	1.68 ± 0.7	0.12*
OBS ≥2	2 (66.6%)	6 (33.3%)	23 (65.7%)	0.074**

C_{trough} , trough concentration; POW, POWER trials; WS, weighted score; gIQ WS, WS genotypic inhibitory quotient; OBS, optimized background score. Significant differences are indicated in bold ($P < 0.05$).

*ANOVA test.

** χ^2 test.

the partial response group ($n=18$), when significant antiviral activity was reached at any time during the study, but viral load increased by >0.5 log at the end of the study; and the full response group ($n=35$), when significant antiviral activity was reached at any time during the study and was maintained at the end of the study. Differences in the main factors affecting the antiviral activity according to the virological response pattern are presented in Table 4. Overall, higher gIQ WS and lower number of darunavir-associated resistance mutations, according to the POWER study algorithm, were observed in the full response group compared with the null response and partial response groups. Moreover, the partial response group had a higher WS (7.5 ± 2.6 , 83.3% with WS >5) and baseline viral load (4.57 log), and a low OBS (33.3% OBS ≥ 2). In this group, the mean gIQ WS (632 ± 487) was around the estimated cut-off of 600, but only 16.6% had a gIQ WS ≥ 600 .

Discussion

In the clinical setting, the selection of components for a salvage regimen is a crucial issue, involving prediction of efficacy balanced with the tolerability and acceptability of treatment. As shown in previous studies, several factors have been associated with virological response to darunavir-based regimens.^{2,7,8,10,12,14,16,22}

To the best of our knowledge, this is the first study comparing the relationship between different resistance interpretation algorithms and their derived gIQs with virological response to darunavir-containing salvage regimens at 48 weeks.

Among the resistance algorithms analysed, two were weighted algorithms and five were non-weighted. Nowadays,

non-weighted algorithms are generally used for the calculation of the gIQ, but this approach does not take into account the different weight of the amino acid changes in the protease gene. To date, weighted resistance algorithms have been developed only for tipranavir^{27,28} and for etravirine,²⁹ with the exception of the Stanford HIVdb Program, which gives a different weight for all the antiretroviral drugs. It is important to note that in previous studies with tipranavir, the performance of distinct gIQs did not prove to be much more superior to that of some genotypic resistance algorithms.³⁰ Conversely, in our study, the gIQ performed better than resistance mutations alone, independent of whether they were weighted or non-weighted. In this regard, although no apparent advantage of using the weighted WS gIQ versus the ANRS non-weighted gIQ was observed (as showed by the equality in parameters such as the sensitivity, specificity, PPV, NPV, LHR+ and LHR-), the higher area under the ROC curve of the WS gIQ led us to choose it from among the different gIQs for the logistic regression analysis. This highlights the importance of integrating both pharmacological and virological data in order to obtain a more accurate prediction of virological response, but does not confirm the importance of the different impact of each protease resistance mutation on virological response. Moreover, the resistance algorithms that considered specific amino acid changes associated with darunavir resistance, and their derived gIQs, provided a more accurate prediction of virological response than non-specific algorithms. The exception is the lack of association of the resistance score proposed in the French study PREDI-ZISTA,²² which is in agreement with a recently published work.³¹

In our study, a higher rate of viral load <50 copies/mL at week 48 was observed (62.5%) when compared with the

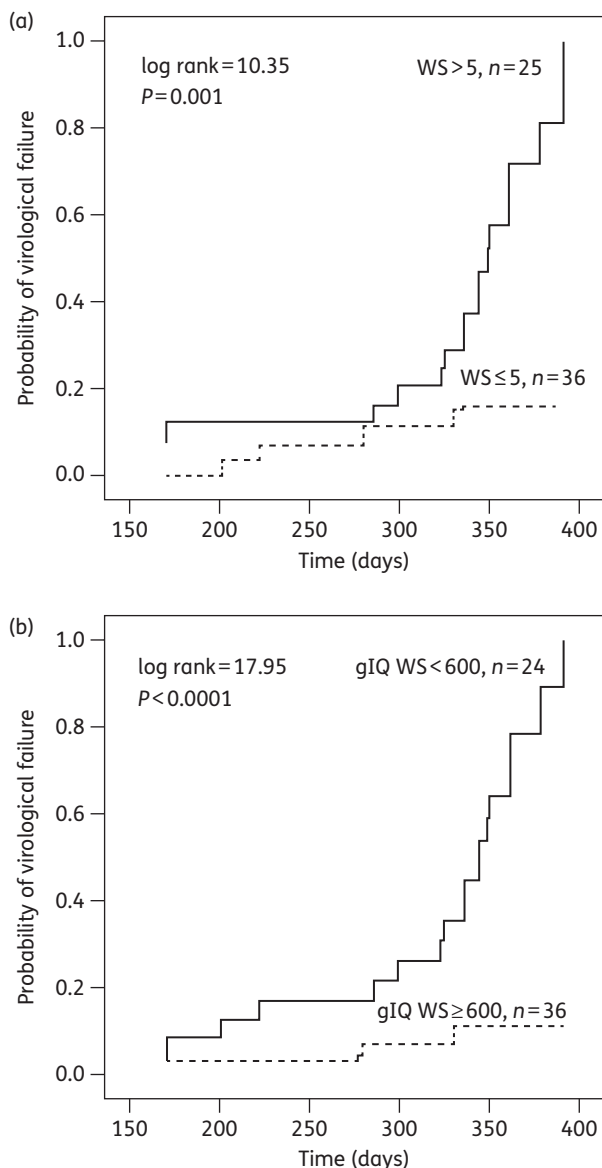


Figure 2. Kaplan–Meier curve for the time of maintenance of virological response over 48 weeks. The probability represents the proportion of patients who presented virological failure. (a) According to De Meyer's mutation weighted score (WS). Patients with darunavir WS ≤ 5 (broken line) or > 5 (continuous line). (b) According to WS genotypic inhibitory quotient (gIQ WS). Patients with darunavir gIQ WS < 600 (continuous line) or ≥ 600 (broken line).

POWER trials (40%–45%).^{2,7} Similar rates of undetectability were recorded in the clinical setting (59.5% at 48 weeks),¹⁶ but a lower proportion of subjects with undetectable viral load was recorded in the PREDIZISTA study at 12 weeks.²² A possible explanation could be that the baseline immuno-virological status of our population was better than in other studies. More than 85% of our patients had less than four POW mutations, which is the proposed cut-off of the current genotype interpretation algorithm. The low number of darunavir POW resistance mutations and the high proportion of subjects with at least one active drug in the OBS ($> 87\%$) could explain these

differences among the studies. Moreover, only 32% of the subjects in our study were classed as advanced AIDS (CDC clinical status C) versus $> 50\%$ in other studies. Due to these differences in both virological response rates and population characteristics, the use of the likelihood ratios seems to be the best option for selecting the appropriate parameter, since likelihood ratios are independent of disease prevalence.

When previous cut-offs for the resistance score and the gIQ were analysed together with all baseline factors potentially associated with virological response, baseline viral load, OBS ≥ 2 , WS > 5 and gIQ WS ≥ 600 were initially selected, but only baseline viral load and gIQ WS ≥ 600 remained in the final model. This result indicates the superior performance and usefulness of the gIQ over genotypic resistance alone. Several studies have demonstrated the superiority of the virtual IQ or the real IQ over the gIQ or the genotypic resistance profile. The disadvantage of the virtual IQ or the phenotypic IQ is that its applicability in daily clinical practice is seriously compromised, since it is not a free-access parameter and this could compromise its applicability in some outpatient clinics with low economic resources.

In our population, the darunavir C_{trough} showed only a trend toward significance ($P=0.089$) as a predictor of virological response, probably due to the low number of darunavir-specific resistance mutations (median, 2), and a better overall immuno-virological status, confirming previous findings from POWER trials.¹⁴

Moreover, although significant antiviral activity was initially reached in a high number of subjects (87.5% at 4 weeks), in $> 32\%$ of subjects viral replication was not fully controlled and a viral load rebound of more than 0.5 log occurred. The proportion of subjects with significant antiviral activity gradually decreased, being 87.5% at 4 weeks, 76.8% at 12 weeks, 67.9% at 24 weeks and 62.5% at 48 weeks. In contrast, the proportion of subjects with virological response (viral load < 50 copies/mL) increased through follow-up, being 46.4%, 51.8%, 60.7% and 62.5% at 4, 12, 24 and 48 weeks, respectively. Although the low number of subjects with null response ($n=3$) made an accurate statistical analysis impossible, a gradual increase in the gIQ WS and the proportion of subjects above the proposed cut-off of 600 was observed among null response, partial response and full response groups, with no differences in OBS. We can hypothesize that although significant antiviral activity could be initially reached with a given therapy, long-term efficacy of the treatment could be maintained only at higher gIQ values, in which maximal pharmacological pressure in relation to the specific genotypic resistance profile is reached. In this regard, the role of persistent low gIQ values on the development of new protease-inhibitor resistance mutations under darunavir/ritonavir regimens deserves further attention, although the high genetic barrier of darunavir could overcome the effect of suboptimal pharmacological levels. However, despite a similar OBS in the three virological response patterns, the overall effect of the background regimen over virological response should not be underestimated. In fact, the interplay between the less favourable situation in the four principal factors affecting virological response in the partial response group (higher baseline viral load, WS > 5 , OBS ≥ 2 and low proportion of subjects with a gIQ WS ≥ 600) could lead to the loss of activity of the darunavir/ritonavir-based regimen over time. Therefore, patients presenting this profile at baseline with a gIQ WS close to or below the estimated gIQ cut-off (600) could theoretically benefit

under a long-term perspective by increasing the pharmacological pressure with a darunavir dose adjustment.

Although no apparent effect of enfuvirtide administration on virological response or viral load decrease at 48 weeks was observed, a higher CD4+ cell count (plus 80 cells/mm³) and CD4+ percentage increase were observed in the subset of patients administered enfuvirtide, confirming a potential benefit of the latter over immune recovery independent from virological response.³²

It is true that early therapeutic drug monitoring (between weeks 4 and 12) must be performed to allow dose adjustment, but in order to simplify the analysis and to allow the inclusion of the maximum number of subjects, mean 48 week darunavir C_{trough} was used in the analyses. As shown in the 'Pharmacokinetic analysis' results section, the darunavir C_{trough} remained stable during the 48 week study period, and the intraindividual variability of C_{trough} was ~29%, which is quite similar to that of other protease inhibitors. Moreover, when 4 and 12 week data were analysed (data not shown), results remained similar to those presented at 48 weeks, being the WS by De Meyer and its derived gIQ WS the most accurate factors predicting virological response at 48 weeks. At these time points, the cut-offs were very close to those obtained for 48 week parameters.

The main limitation of our study is the relatively low number of patients. However, although this could limit the power of statistical analysis performed in some subpopulations, as we pointed out, the sample size was sufficient to draw conclusions on the role of the different resistance algorithms and their derived gIQs.

In conclusion, this is the first study comparing different resistance interpretation algorithms and their derived gIQs for the prediction of 48 week virological response to darunavir-based salvage regimens. The gIQ WS was the parameter that predicted virological response more accurately, with a proposed cut-off of 600. Specifically, a subgroup of subjects with unfavourable baseline characteristics (high baseline viral load, WS > 5 and without an optimal background regimen) could benefit in terms of long-term virological response from attaining a gIQ WS much higher than 600.

Acknowledgements

We thank Mauro Sciandra and Michela Capasso for their helpful technical assistance. We also thank Antonio di Garbo for his expertise advice on HIV resistance interpretation.

Funding

This study was supported using internal funding.

Transparency declarations

S. R. and M. G. have received research grants and have been involved in advisory boards or educational courses supported by the following companies: Abbott; Boehringer Ingelheim; Bristol-Myers Squibb; Gilead; GlaxoSmithKline; ViiV; and Janssen-Cilag (as well as Roche for M. G.). All other authors: none to declare.

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