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Virologic and Immunologic Effectiveness at 48 Weeks of Darunavir–Ritonavir-Based Regimens in Treatment-Experienced Persons Living with HIV-1 Infection in Clinical Practice: A Multicenter Brazilian Cohort

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Abstract

Introduction: Published data addressing the effectiveness of darunavir–ritonavir (DRV/r)-based therapy for multiexperienced patients in developing countries are scarce. This study evaluated the 48-week virologic and immunologic effectiveness of salvage therapy based on DRV/r for the treatment of multidrug-experienced HIV-1-infected adults in Brazil. **Materials and Methods:** A multicenter retrospective cohort study was carried out with multidrug-experienced adults who were on a failing antiretroviral therapy and started a DRV/r-based salvage therapy between 2008 and 2010. The primary effectiveness end point was the proportion of patients with virologic success (plasma HIV-1 RNA <50 copies/mL at week 48). **Results:** At 48 weeks, 73% of the patients had HIV-RNA <50 copies/mL and a mean increase of 108 CD4 cells/mm^3. Higher baseline viral load, lower baseline CD4 count, younger age, and 3 or more DRV/r-associated resistance mutations were significantly predictive of virologic failure. Concomitant use of raltegravir was strongly associated with virologic success. **Conclusion:** The use of DRV/r-based regimens for salvage therapy is an effective strategy in the clinical care setting of a developing country.

Keywords

HIV, darunavir, resistance, antiretroviral

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Introduction

Combined antiretroviral therapy (cART) aims at achieving long-term suppression of plasma HIV-1 RNA at levels <50 copies/mL.[1] Viral suppression reduces morbidity and mortality, even in patients with current or previous virologic failure.[2,3] It also decreases inflammation and immune activation, thus lessening the risk of cardiovascular diseases and other end-organ damage reported in HIV-infected cohorts and minimizes the emergence and accumulation of resistance-associated mutations (RAMs).[4-6]

Newer antiretroviral (ARV) medications allow long-term virologic suppression even in multiexperienced patients, by targeting novel sites (eg, enfuvirtide, raltegravir, and maraviroc) or by maintaining antiviral activity despite resistance to first-generation ARV agents (eg, darunavir [DRV], tipranavir, and etravirine). Ritonavir-boosted DRV (DRV/r) was safe and effective for multidrug-experienced HIV-1-infected patients in clinical trials.[7-9] However, the evidence of effectiveness in the clinical practice is scarce and comes only from developed countries.[10,11] In 2008, Brazil made DRV/r available for salvage therapy based on studies conducted in developed countries. According to the Brazilian Ministry of Health, as of August 2012, about 7300 people living with HIV/AIDS were on a DRV/r-based regimen (unpublished). However, no large-scale studies evaluated the effectiveness of DRV/r-based regimens for salvage therapy in Brazil or in other developing countries.

This study evaluated the 48-week virologic and immunologic effectiveness of salvage therapy based on DRV/r for the treatment of multidrug-experienced HIV-1-infected adults in a Brazilian clinical practice setting. It also analyzed risk factors for virologic failure.

Materials and Methods

Patients and Setting

Patients were recruited for HIV treatment from 14 referral centers of 7 states in Southern, Southeastern, and Northeastern Brazil. Eligible patients were HIV-1-infected multidrug-experienced adults (≥18 years old) on routine outpatient follow-up, who were on a failing cART and started a DRV/r-based salvage therapy between 2008 and 2010. Failing cART was defined as a patient on regular cART and plasma HIV-1 RNA >50 copies/mL for at least 3 months documented by 2 consecutive viral loads prior to the salvage therapy. To be considered multidrug-experienced, patients had to meet at least 1 of the following clinical/genotyping criteria for DRV/r use: presence of ≥1 major protease inhibitors (PIs) RAM[12] or previous use of unboosted PI. Patients without a genotypic resistance test performed up to 18 months before DRV/r-based therapy initiation were excluded from analysis as well as those with previous or current use of maraviroc, vicriviroc, or tipranavir, since these drugs were not available in the Brazilian Public Health Care System (the latter was approved only for pediatric use). Ethical approval for the study was obtained from the National Commission of Ethics in Research.

Study Design

We conducted a multicenter retrospective cohort study. All data were retrieved through clinical chart review using standardized data extraction forms that were provided to all participating centers. Demographic information, ARV and genotyping history, clinical features, and laboratory data (viral load and CD4 counts) before and up to 48 weeks after initiation of salvage therapy based on DRV/r were extracted. A validated genotypic susceptibility score (GSS) was used to estimate the number of active drugs in the ARV regimens.[13,14] According to the Brazilian genotypic interpretation algorithm,[15] the assigned drug-specific GSS was 1.0 for ARV regimens for which HIV-1 was predicted to be fully susceptible, 0 for ARV regimens predicted to be fully inactive, and 0.5 was assigned to ARVs predicted to have intermediate levels of antiviral activity. A drug-specific GSS of 1.0 was assigned to enfuvirtide and raltegravir for patients who were naive to these drugs. The regimen GSS (rGSS) was calculated by adding up all the drug-specific GSS of the baseline regimen.

Study End Points

The primary effectiveness end point was the proportion of patients with virologic success defined as a plasma HIV-1 RNA level <50 copies/mL at week 48 (±4) after DRV/r-based regimen initiation. Secondary end points were changes from baseline in CD4 counts, the proportion of patients who experienced AIDS-defining clinical events or death, and the proportion of patients who discontinued the DRV/r-based salvage therapy. Analyses were conducted on an intent-to-treat basis applied to observational studies.[16] According to this approach, the following situations were considered treatment failures whenever they occurred before week 52: patient death for any cause; DRV/r withdrawal for any cause; or changes in the optimized backbone therapy resulting in higher antiviral activity. For the virologic end point, these patients were counted as virologic failures. For the immunologic end point, these patients had their pretreatment CD4 count carried forward, which amounted to considering the change from baseline CD4 count as being 0. For all other situations with missing viral load and/or CD4 count at week 48 (±4), 2 strategies for data analysis were adopted. According to strategy 1, the first available information after week 52 was carried backward and, when the latter was unavailable, the last observation before week 44 was carried forward. In strategy 2, the last observation before week 44 was carried forward, and any available information after week 52 was ignored.

Statistical Analysis

In the descriptive analysis, values of qualitative variables were expressed in terms of absolute and relative frequencies, and values of numerical variables were expressed as medians and

interquartile ranges. Differences in the distribution of numerical variables between baseline and week 48 (± 4) were analyzed by the Wilcoxon test. In univariate analysis of unmatched groups, categorical data were compared using analysis of contingency tables and the chi-square test; numerical variables were compared using the Mann-Whitney *U* test. Multiple analyses of factors associated with virologic failure were conducted using logistic regression models with backward manual elimination of variables. Candidate variables were those that showed an association with virologic failure with a *P* value $\leq .3$ in the univariate analysis as well as those with high clinical relevance, irrespective of their statistical significance. Analysis took into account the possibility of data clustering within study centers, which would violate the assumption of independence between observations. Accordingly, we relaxed the independence assumption using a clustered robust estimator of the standard error of the β coefficients that allows for intragroup correlation, with each center being a cluster. Because of concerns about the sparse nature of our data, goodness-of-fit testing of logistic regression models was conducted using the Goflogit macro.[17] All *P* values were 2-sided, with values less than .05 considered statistically significant. Statistical analyses were performed with Stata for Windows (version 11.2, StataCorp, College Station, Texas).

Table 1. Demographic, Clinical, and Immunovirologic Features of Patients at Baseline.

Characteristic	Values[a]
Age, y	44.1 (39.3-49.5)
Male sex, N (%)	350 (72.8)
Time since diagnosis of HIV infection, y	12.1 (10.1-14.3)
CDC HIV infection clinical stage, N (%)	
A	65 (13.5)
B	82 (17.1)
C	334 (69.4)
Number of prior antiretroviral regimens	6 (4-8)
Length of prior antiretroviral therapy, y	10.6 (8.8-12.3)
Prior use of zidovudine monotherapy, N (%)	95 (19.8)
Prior use of dual therapy, N (%)	245 (50.9)
Use of unboosted PI-based therapy, N (%)	436 (90.6)
Length of unboosted PI-based therapy, y	3.1 (1.7-4.8)
Length of PI-based therapy, y	8.6 (6.2-10.5)
Time since last genotyping test, months	4.1 (1.8-7.1)
CD4 count nadir, cells/mm^3	80 (27-173)
Time since CD4 count nadir, y	5.5 (1.0-10.3)
Highest HIV-1 plasma viral load, log10 copies/mL	5.3 (4.8-5.6)
Time since highest HIV-1 plasma viral load, y	5.2 (1.7-8.6)
Baseline CD4 count, cells/mm^3	207 (84-362)
Baseline HIV-1 plasma viral load, log10 copies/mL	4.3 (3.8-4.9)

Abbreviations: CDC, US Centers for Disease Control and Prevention; PI, protease inhibitor.
[a] Values are medians (interquartile range), unless indicated otherwise.

Results

Patients

A total of 536 multidrug-experienced patients who started a DRV/r-based salvage therapy between 2008 and 2010 were identified; 39 were excluded because they did not meet inclusion criteria for DRV/r use; 15 were not on a failing cART; and 1 was excluded due to previous use of maraviroc. Baseline characteristics of the remaining 481 patients eligible for analysis are shown in Table 1. Most were men (72.8%), and the median age was 44.1 years. Median baseline CD4 count was 207 cells/mm^3. Previous use of zidovudine (ZDV) monotherapy, dual therapy (mostly with ZDV plus didanosine [ddI]), and unboosted PI was common.

Table 2 reports the genotypic profile of patients and characteristics of DRV/r-containing regimens at baseline. The median of major PI-RAMs was 3, and about one-third had at least 2 RAMs to DRV/r. The DRV/r-containing regimens included ≥ 2 nucleoside reverse transcriptase inhibitors (97.7%), first-generation nonnucleoside reverse transcriptase inhibitors (13.9%), etravirine (7.9%), enfuvirtide (36.4%), and raltegravir (52.2%).

Study End Points

The proportions of patients with virologic success (plasma HIV-1 RNA <50 copies/mL at week 48 $\pm$ 4) were, respectively, 72.6% (95% confidence interval [CI] = 68.3%-76.5%) and 73.2% (95% CI = 69.0%-77.1%) according to strategies 1 and 2 for handling of missing data. For strategy 1, 42% of the patients had a viral load available after week 52 carried backward and 17% had a viral load available before week 44 carried forward; 50% of the latter were from week 36 or after. For strategy 2, 57% of the patients had a viral load available before week 44 carried forward; 72% of them were from week 36 or after. Over time, the proportion of patients with plasma HIV-1 RNA <50 copies/mL remained stable at about 60% to 70% (not shown).

Patients experienced a significant gain in CD4 count. At week 48, mean increases from baseline were, respectively, 121 (95% CI = 108-133) and 108 (95% CI = 96-120) cells/mm^3, according to the aforementioned strategies 1 and 2 (both *P* values <.001).

Overall, 14 (2.9%) patients were diagnosed with 18 AIDS-defining events before week 52: *Candida* esophagitis (n = 5), *Pneumocystis* pneumonia (n = 4), *Cryptococcus* meningoencephalitis (n = 2), and 1 case each of *Cytomegalovirus* retinitis, *Toxoplasma gondii* encephalitis, non-Hodgkin lymphoma, recurrent pneumonia, pulmonary tuberculosis, extrapulmonary tuberculosis, and disseminated tuberculosis. Non-AIDS-defining conditions included bacterial pneumonia (n = 3) and visceral leishmaniasis (n = 1). Up to week 52, 3 (0.6%) patients died and 4 (0.8%) patients withdrew DRV/r: 1 to avoid drug interaction with rifampin prescribed for confirmed extrapulmonary tuberculosis and 3 due to intolerance or adverse effects.

Table 3 shows the results of multiple logistic regression models for virologic failure at week 48. Higher baseline viral load, lower baseline CD4 count, younger age, and 3 or more DRV/r-RAMs were significantly predictive of virologic failure. Patients

Table 2. Genotypic Features of Patients at Baseline and Characteristics of Darunavir/Ritonavir-Containing Regimens.

Characteristic	Values[a]
Number of NRTI resistance mutations, median (IQR)	5 (4-5)
Number of TAMs, median (IQR)	3 (2-4)
Number of primary PI resistance mutations, median (IQR)	3 (2-4)
Number of darunavir resistance mutations	
0-1	325 (67.6)
2	92 (19.1)
≥3	64 (13.1)
rGSS (including DRV/r)[b]	
≤ 2	126 (26.2)
>2 and ≤3	282 (58.6)
> 3	73 (15.2)
rGSS (optimized background therapy)[b]	
≤1	105 (21.8)
>1 and ≤2	288 (59.9)
>2	88 (18.3)
Number of antiretroviral drugs[b]	
3	27 (5.6)
4	268 (55.7)
5 or 6	186 (38.7)
Number of NRTI[b]	
0 or 1	11 (2.3)
2	326 (67.8)
3	144 (29.9)
Use of first-generation NNRTI[b]	67 (13.9)
Use of etravirine[b]	38 (7.9)
Use of enfuvirtide[b]	175 (36.4)
Use of raltegravir[b]	251 (52.2)

Abbreviations: NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; rGSS, regimen genotypic susceptibility score; TAM, thymidine analog mutation; IQR, interquartile range; DRV/r, darunavir/ritonavir.
[a] Values are numbers (%), unless indicated otherwise.
[b] In DRV/r-containing regimen.

with a raltegravir-containing regimen had roughly 60% less chance of virologic failure. Longer time since CD4 count nadir and longer time since diagnosis of HIV infection were also associated with virologic failure, although less consistently.

Discussion

Darunavir/r was approved for clinical use by the Food and Drug Administration in June 2006. International studies have shown that DRV/r-based ART improves life expectancy and reduces the rate of disease progression compared with currently available PI-based therapy, leading to higher quality-adjusted life expectancy and cost savings in non-ARV-related cost categories.[18-21] In Brazil, it has been available for salvage therapy of treatment-experienced patients since 2008, based on efficacy data from clinical trials conducted in developed countries. The current study is the first Brazilian multicenter cohort to evaluate the effectiveness of DRV/r-based regimen for salvage therapy in diverse clinical settings.

Our patients were highly exposed to ARV regimens, including the use of unboosted PIs, AZT monotherapy, and/or dual therapy, the factors highly associated with virologic failure in previous studies.[22,23] When compared to the data reported in the Performance Of TMC114/r When evaluated in treatment-Experienced patients with PI Resistance (POWER) trials, our patients had more advanced disease (US Centers for Disease Control and Prevention group C 69.4% versus 36%), a similar proportion of 3 or more primary PI-RAMs (around 50%), and a lower proportion of 3 or more DRV-RAMs (13.1% versus 22%).[24] In contrast to the frequent use of raltegravir in the optimized backbone therapy (52.2%), enfuvirtide and etravirine were prescribed less frequently in our study population (36.4% and 7.9%, respectively). The use of enfuvirtide is declining in Brazil since the Brazilian government issued a recommendation for switching from enfuvirtide to raltegravir in virologically suppressed multidrug-resistant HIV-1-infected patients, based on the data from the open-label Efficacy and Tolerance of the Switch From Enfuvirtine to Raltegravir in Antiretroviral Therapy Regimen in HIV Patients With Undetectable Viral Load (EASIER) Agence Nationale de Recherche sur le Sida et les Hepatites Virales (or in english: French National Agency for Research on AIDS and Viral Hepatitis) (ANRS) 138 trial.[25] Etravirine, on the other hand, became available in Brazil in 2010, so its use is still not widespread.

The rate of virologic success was around 73% at week 48, similar to the efficacy reported in clinical trials (POWER and Blocking integrase in treatment Experienced patients with a Novel Compound against HIV: MeRcK [BENCHMRK])[9,26] as well as to the effectiveness observed in some cohorts in developed countries.[10,11] In agreement with the high rate of virologic success, immunologic response was also significant, with a mean CD4 count gain from baseline of 121 cells/mm^3 (95% CI = 108-133; strategy 1) and 108 cells/mm^3 (95% CI = 96-120; strategy 2), similar to the POWER trials (102 cells/mm^3).[23]

As expected, in the multiple logistic regression analysis, concurrent use of raltegravir was strongly associated with a favorable virologic outcome. A synergistic effect of novel ARV agents was shown in the BENCHMRK clinical trials, where coadministration of raltegravir and DRV/r improved virologic outcomes. In this trial, HIV-1 RNA levels <50 copies/mL were achieved at 48 weeks in 47% of recipients of DRV/r plus optimized backbone therapy, as compared to 69% of patients who received raltegravir and DRV/r plus optimized backbone therapy.[26] Interestingly, the aforementioned effect was not observed with concurrent use of enfuvirtide in our study, as was shown in the BENCHMRK study.[26]

Elderly individuals are more likely to have a longer duration of HIV infection and more comorbidities when compared to younger patients. Despite this, it is remarkable that, in our multiple logistic regression models, older patients exhibited lower risk of virologic failure than younger patients. The more likely explanation for this finding is a higher treatment adherence among the elderly individuals. Some authors showed that older patients exhibit better adherence to cART than younger patients and have a lower risk of virologic rebound.[27] On the other hand, in line with the results of the pooled analysis of POWER 1, 2, and 3,[28] higher baseline viral load and 3 or more

Table 3. Multiple Logistic Regression Analysis of Factors Associated with Virologic Failure at Week 48.[a]

Variable	Strategy 1[b]		Strategy 2[b]	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Baseline viral load (ref: ≤50 000 copies/mL)				
>50 000 and ≤100 000 copies/mL	1.45 (0.73-2.89)	.290	0.99 (0.50-1.98)	.982
>100 000 copies/mL	2.46 (1.12-5.43)	.026	2.65 (1.49-4.70)	.001
Baseline CD4 count >100 cells/mm^3	0.42 (0.30-0.60)	<.001	0.50 (0.34-0.73)	<.001
Number of darunavir resistance mutations (ref: 0 or 1)				
2	0.84 (0.51-1.39)	.495	1.08 (0.64-1.85)	.766
≥3	1.70 (1.03-2.81)	.039	1.97 (1.25-3.13)	.004
Raltegravir-containing regimen	0.42 (0.27-0.67)	<.001	0.38 (0.24-0.60)	<.001
Age > 60 y	0.37 (0.16-0.88)	.024	NA	NA
Age (per year)	NA	NA	0.97 (0.95-0.99)	.009
Time since CD4 count nadir >5 y	1.32 (1.03-1.71)	.031	NA	NA
Time since diagnosis of HIV infection (per year)	1.04 (1.00-1.09)	.045	NA	NA
Intercept		.009		.098

Abbreviations: OR, odds ratio; CI, confidence interval; NA, not applicable.
[a] *P* values for goodness-of-fit tests (strategies 1 and 2, respectively): IM diagonal = 0.73/0.59; Copas = 0.95/0.93; deviance = 0.23/0.17; Osius & Rojek = 0.84/0.76; McCullagh = 0.70/0.51; Farrington = 0.73/0.33.
[b] Strategies for handling of missing data (see Methods section for definitions).

DRV/r-RAMs were factors associated with treatment failure in our final models.

The results of this study must be interpreted within the context of its limitations. As an observational study, some unmeasured confounders related to or not related to ARV regimen selection may have affected the results of the study. Furthermore, the results may only identify associations, not causality. One of the main limitations of our study was the lack of some end point data (ie, viral load and CD4 count at week 48). Missing outcome data is a common problem in clinical research, even in randomized controlled trials and especially in observational studies with extended follow-up.[29] In studies in which all efforts to avoid missing data have failed, 4 different types of adjustment methods for analysis may be used: complete case analysis, single imputation methods, estimating equation methods, and methods based on a statistical model.[29,30] However, there is no consensus about the best method for handling missing data in clinical studies.[30,31] This is because all these methods are based on assumptions that, although may be scientifically plausible, are always empirically unverifiable.[30] Thus, in the presence of missing data, there is consensus that sensitivity analyses should be conducted to assess the robustness of the findings to plausible alternative assumptions about the missing data mechanism.[30,32] In our study, we conducted sensitivity analysis by comparing 2 strategies for handling missing data (see Methods section) and found the virologic success rates, mean change in CD4 count, and risk factors for virologic failure to be quite robust. We confidently conclude, therefore, that our conclusions were not highly sensitive to the missing data. Another limitation of this study was that, unfortunately, we could not assess adherence to ARV regimens in our patient population.

This study suggests that the use of DRV/r-based regimens for salvage therapy is an effective strategy in the clinical care setting of a developing country. Our results reinforce the position of DRV/r, preferentially associated with raltegravir, as a pivotal ARV regimen for salvage therapies in highly experienced HIV-infected patients. Likewise, this study strengthens the efforts of the Brazilian Ministry of Health to continuously expand access to newer ARV regimens for patients with multidrug-resistant HIV-1 infection.

Declaration of Conflicting Interests

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