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Sex-Based Outcomes of Darunavir–Ritonavir Therapy: The GRACE (Gender, Race, and Clinical Experience) Study

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Abstract

Background—Women account for an increasing proportion of patients with HIV-1 but remain underrepresented in antiretroviral clinical trials.

Objective—To evaluate sex-based differences in efficacy and adverse events in treatment-experienced HIV-positive women and men receiving darunavir–ritonavir-based therapy over 48 weeks.

Design—Multicenter, open-label, phase IIIb study designed to enroll a high proportion of women, with sample size determined on the basis of a noninferiority design with a maximum allowable difference of 15% in virologic response favoring men. (Clinicaltrials.gov registration number: NCT00381303)

Setting—65 sites in the United States, Puerto Rico, and Canada.

Patients—287 women and 142 men.

Intervention—Patients received darunavir–ritonavir, 600/100 mg twice daily, plus an investigator-selected optimized background regimen.

Measurements—Virologic response (HIV-1 RNA <50 copies/mL using a time-to-loss of virologic response [TLOVR] algorithm) and adverse events were assessed over 48 weeks.

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Reproducible Research Statement: *Study protocol, statistical code, and data set:* Not publically available; however, additional information on the design and results of this study are available at www.clinicaltrials.gov (ID number NCT00381303).

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Results—67% of patients were women; 84% of patients were black or Hispanic. A higher proportion of women discontinued treatment than men (32.8% vs. 23.2%; $P = 0.042$); more women than men discontinued for reasons other than virologic failure. Response rates in women and men at week 48 were 50.9% and 58.5%, respectively (intention-to-treat TLOVR), and 73.0% and 73.5%, respectively (TLOVR-censored for patients who withdrew for reasons other than virologic failure). The absolute difference in response, based on logistic regression and adjusted for baseline \log_{10} viral load and $CD4^+$ cell count, was -9.6 percentage points (95% CI, -19.9% to 0.7 percentage points; $P = 0.067$) for intention-to-treat TLOVR and -3.9 percentage points (CI, -13.9% to 6.0 percentage points; $P = 0.438$) for TLOVR-population that censored patients who withdrew for reasons other than virologic failure. Adverse events were similar between the sexes. The most common grade 2 to 4 adverse events that were considered at least possibly treatment-related in women and men were nausea (5.2% and 2.8%, respectively), diarrhea (4.5% and 4.9%, respectively), and rash (2.1% and 2.8%, respectively).

Limitation—Baseline characteristics differed between women and men.

Conclusion—Nonsignificant, sex-based differences in response were found during the 48 weeks of the GRACE study; however, these differences were probably due to higher discontinuation rates in women, suggesting that additional efforts are needed to retain women in clinical trials.

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In 2006, an estimated 1 106 400 adults and adolescents in the United States were living with HIV or AIDS (1). Of the new cases of HIV reported in 2006, 27% were women, and 45% were black (2). Since the beginning of the epidemic, women have accounted for an increasing proportion of persons with the disease (3). Furthermore, women of color are disproportionately affected: 64% and 15% of women living with HIV/AIDS in the United States in 2005 were black and Hispanic, respectively, yet these groups collectively represent only approximately 25% of the U.S. female population (3,4).

Women, and particularly women of color, have been underrepresented in antiretroviral clinical trials for treatment-experienced patients (5). This may reflect challenges in both recruiting and retaining women in clinical trials as a whole and highlights barriers to continuous medical care for this population, such as family commitments, job-related time constraints, or other socioeconomic factors. These observations have been noted in clinical trials of several other therapeutic areas (6–9), including cardiovascular, lung, and cancer research, suggesting that the challenges of recruiting women and persons of color are not specific to trials of antiretroviral therapy. An analysis (6) of the enrollment of women in cardiovascular clinical trials funded by the National Heart, Lung, and Blood Institute showed that studies of congestive heart failure enrolled only 26% women, although women account for 43% of patients with congestive heart failure. Likewise, examination of high-impact cancer studies published in 2006 (10) showed that women were underrepresented in studies of non-sex-specific types of cancer, averaging only 38.8% of enrolled participants. This has resulted in a paucity of clinical data for diverse populations. Although sex-based analyses are required for registration trials (11), studies with less than 25% female enrollment generally have limited statistical power to assess sex differences, thereby limiting sex-based conclusions in most trials (12). The recruitment and retention of women in clinical trials are essential to provide accurate information on the efficacy and safety of therapeutic agents in all patients and, ultimately, to guide treatment decisions for women (13,14).

Darunavir, a protease inhibitor, combined with a low dose of ritonavir, was initially introduced as a therapeutic option (600/100 mg twice daily) for treatment-experienced patients with HIV (15). Subsequently, darunavir–ritonavir was approved for use in treatment-naïve adults (800/100 mg once daily) and treatment-experienced children (aged 6

to 17 years; weight-dependent twice-daily dosing) (15). In the POWER (Performance Of TMC114/r When evaluated in treatment-Experienced patients with PI [protease inhibitor] Resistance) and TITAN (TMC114/r In Treatment-experienced pAtients Naive to Lopinavir) trials, which assessed the efficacy and safety of darunavir–ritonavir, the number of women enrolled was approximately 10% to 21% of the overall population (16,17). In the ARTEMIS (AntiRetroviral Therapy with TMC114 ExaMined In naïve Subjects) trial, treatment-naïve women accounted for 30% of patients in the darunavir–ritonavir group, with results showing similar safety and efficacy between the sexes at week 96 and no differences in discontinuation rates due to adverse events (18).

To expand the clinical data available for darunavir–ritonavir in treatment-experienced women, the GRACE (Gender, Race, And Clinical Experience) trial, conducted from 6 October 2006 to 19 December 2008, was specifically designed and powered to assess sex-based differences in efficacy and to evaluate the incidence of adverse events (5). The study was also designed to enroll a high proportion of treatment-experienced North American women to reflect the distribution and demographic characteristics of women with HIV in the United States. We report the efficacy outcomes and incidence of adverse events in women and men who received darunavir–ritonavir-based therapy over 48 weeks during the GRACE study; data on outcomes by race and ethnicity will be presented elsewhere.

Methods

Study Design

The GRACE trial was an open-label, single-group, phase IIIb study done at 65 sites in the United States, Puerto Rico, and Canada (Appendix, available at www.annals.org). The goal of the study was to compare the efficacy, incidence of adverse events, and tolerability of darunavir–ritonavir combined with an investigator-selected optimized background regimen in antiretroviral-experienced women and men. Study sites were selected to correspond with the geographic distribution of women with HIV; most sites were located in the northeastern (16 sites) and southeastern (29 sites) United States (2).

An interactive voice-response system was used during enrollment. Study sites were initially required to enroll 3 women before enrolling 1 man, and thereafter each site was required to maintain at least 70% female enrollment. The interactive voice-response system would only allow a man to be enrolled if his addition did not compromise the female-enrollment quota; there were no limitations on the number of women who could be enrolled. The enrollment plan was extremely successful: Midway through recruitment, men comprised only 10% of the study population. Therefore, male-enrollment restrictions were lifted to ensure that the study had a sufficient number of men. The percentage of men who were white was monitored and limited to less than 25% of the originally planned number of men.

The trial was conducted from 6 October 2006 to 19 December 2008. The research protocol was reviewed and approved by institutional review boards for all 65 study sites, and all participants provided written informed consent. Details of the study design were registered at Clinicaltrials.gov (ID number: NCT00381303).

Study Population

Eligible patients were aged 18 years or older, had documented HIV-1 infection and a plasma viral load of 1000 HIV-RNA copies/mL or greater, and had been treated with a protease inhibitor– or nonnucleoside reverse transcriptase inhibitor–based regimen for at least 12 weeks before study entry. Patients whose previous treatment was interrupted for at least 4 weeks were also eligible for the trial. Exclusion criteria included previous use of darunavir, etravirine, tipranavir, or enfuvirtide; current use of any other investigational antiretroviral,

including raltegravir and maraviroc; use of any nonantiretroviral investigational drug within 90 days of study entry; use of disallowed medication, as specified in the investigator's brochure for darunavir (and etravirine, if applicable); or, in the opinion of the investigator, the need for enfuvirtide for a viable optimized background regimen. Patients were also excluded if they had active Category C conditions according to the Centers for Disease Control and Prevention classification, with the exception of stable cutaneous Kaposi sarcoma or HIV-associated wasting syndrome; acute viral hepatitis; grade 3 or 4 laboratory abnormalities besides asymptomatic glucose elevations and asymptomatic triglyceride or cholesterol elevations; a life expectancy of less than 6 months according to the investigator's judgment; active clinically significant disease during screening of medical history or physical examination that was not resolved or stabilized for 30 days before screening; any condition that, in the investigator's opinion, could compromise the participant's safety or adherence during the trial; or previously demonstrated allergy or hypersensitivity to any ingredient in the investigational medications. Women of childbearing potential who were not using effective nonhormonal birth control or who were pregnant or breast-feeding were also excluded. Patients co-infected with chronic hepatitis B or C were included if they were clinically stable, aminotransferase levels were less than 5 times the upper limit of normal, and they were not expected to require treatment during the trial. In total, 602 patients were screened, and 429 patients were allocated to treatment (Figure 1).

Treatment

Patients received darunavir–ritonavir, 600/100mg twice daily, plus an optimized background regimen for 48 weeks (treatment period), which consisted of commercially available nucleoside–nucleotide reverse transcriptase inhibitors and nonnucleoside reverse transcriptase inhibitors. Etravirine (not commercially available at the start of the GRACE trial), tenofovir, emtricitabine, the fixed-dose combination of tenofovir plus emtricitabine, and zidovudine were provided by the study for use as part of the optimized background regimen, if chosen by the investigator. Each patient's optimized background regimen was based on previous antiretroviral treatments received and on resistance testing performed during screening by using the virco® TYPE HIV-1 system (Virco BVBA, Mechelen, Belgium). Modifications to the optimized background regimen were not permitted during the treatment period, with the exception of either single within-class antiretroviral substitutions or temporary interruption of all antiretroviral agents due to a treatment-related adverse event or serious adverse event. Adherence was assessed via 4-day patient recall at weeks 4, 8, 12, 16, 24, and 48 and was defined as taking 95% or more of expected doses of darunavir–ritonavir during treatment ($[\text{total doses expected} - \text{total doses missed}] / \text{total doses expected}$; based on the time points for which each patient had data). Study visits were performed at screening; at baseline; and at weeks 4, 8, 12, 16, 24, 36, and 48. A follow-up visit was performed 4 weeks after either study completion (week 52) or the date of premature withdrawal. Blood samples were collected at each visit for safety assessments, immunology, and determination of plasma viral load. The HIV samples taken at screening and at virologic failure were also analyzed for antiretroviral resistance. Reasons for discontinuation were determined by the investigator and recorded on the case-report form.

Efficacy Evaluations

The primary efficacy end point was virologic response (HIV-1 RNA <50 copies/mL), confirmed by 2 consecutive assessments at least 14 days apart. Virologic response is reported for intention-to-treat populations (ITT) as well as for the population that censored patients who withdrew for reasons other than virologic failure by using the time-to-loss of virologic response (TLOVR) algorithm. The primary objective of the study was to evaluate sex-based differences in virologic response rates.

Secondary efficacy end points included change in CD4⁺ cell count from baseline to week 48, rates of virologic failure, and development of new resistance mutations on virologic failure. Virologic failure was defined as a viral load greater than 50 copies/mL from week 24 onward, confirmed by 2 consecutive assessments 14 or more days apart. Plasma viral load was measured by quantitative polymerase chain reaction (Amplicor® HIV-1 monitor, version 1.5, Roche Diagnostics, Branchburg, New Jersey).

Adverse Event and Laboratory Evaluations

The incidence and type of all adverse events, serious adverse events, and study discontinuations due to adverse events were recorded. All adverse events were recorded from the signing of informed consent forms through completion of the last study-related procedure. The diagnosis of adverse events and their relationship to the study therapy (not related, doubtful, possible, probable, or very likely) were determined by the investigators and recorded on the case-report form. Serious adverse events, including deaths, were recorded on a serious adverse event form. Adverse events of interest were specified a priori and included rash and gastrointestinal-, AIDS-, liver-, lipid-, glucose-, pancreas-, and hematologic-related adverse events.

Serum chemistries were performed on samples collected at all study visits. Fasting for at least 8 hours was required for glucose and lipid evaluations at baseline, week 24, and week 48. Samples for hematology were taken at the start of the study, week 24, and week 48. For clinical laboratory tests, abnormalities were determined according to the sponsor-enhanced, Division of AIDS Severity Grading Scale (National Institute of Allergy and Infectious Diseases, National Institutes of Health)

Statistical Analysis

The investigators aimed to enroll 420 patients (approximately 70% female, 30% male). This sample size was determined on the basis of a noninferiority model that assumed a response rate of 65% and a maximum allowable difference for women versus men of 15% or less with a 1-sided significance level of $\alpha = 0.025$ and 80% power. This allowed for the exclusion of 10% of enrolled patients. A difference in response rates of 15% was considered a priori to be clinically relevant. In addition to the study being powered to detect differences between women and men, it was also designed so that women were overrepresented to allow a greater insight into safety and tolerability of antiretroviral treatment in this population.

Efficacy and adverse event end points were analyzed for the ITT population, which was defined as all enrolled patients who received at least 1 dose of study medication. Virologic response was reported by using the TLOVR algorithm for both the ITT population and for the population that censored patients who withdrew for reasons other than virologic failure (19). In the TLOVR algorithm, the following guidelines apply: A patient is considered a responder if response is observed on at least 2 consecutive time points. Patients are listed as nonresponders at all future time points in the event of discontinuation or if they have 2 consecutive “rebound” HIV RNA values. Intermittently missing values are considered responses if the immediately preceding and following visits showed response. The introduction of any antiretroviral drug not foreseen in the study regimen is qualified as failure at the time of introduction. Virologic response was also calculated for the population that censored patients who withdrew for reasons other than virologic failure (patients were censored at the time of discontinuation). Sex-based differences in response rates were derived from logistic regression models that included sex as a covariate, as well as baseline log₁₀ HIV RNA and CD4⁺ cell count. The responses in the intent-to-treat population and the population that censored patients who withdrew for reasons other than virologic failure were also evaluated separately by using a logistic regression model with response as the

dependent variable and sex, log₁₀ HIV RNA at baseline, CD4⁺ cell count at baseline, and race (black vs. nonblack) as the independent covariates. The interaction between sex and race was not significant, and interactions between the independent covariates were not included in the model. Observed changes in CD4⁺ cell count from baseline to week 48 were evaluated by analysis of covariance, including sex, race (black vs. nonblack), baseline log₁₀ viral load, and baseline CD4⁺ cell count as factors. Observed cases included all values at a particular time point; missing values were disregarded from the analysis for that time point. A mixed-effects model, using a general, unstructured covariance matrix and assuming normality of data and missing data as noninformative withdrawals, was constructed to evaluate change in CD4⁺ cell count over time, using all available CD4⁺ cell count measurements. The model included sex, log₁₀ HIV RNA load at baseline, CD4⁺ cell count at baseline, and race (black vs. nonblack) as independent covariates. Adverse event end points and resistance determinations were analyzed descriptively by sex. A post hoc statistical analysis was done to compare rates of discontinuation by sex. Time to discontinuation was estimated by using the Kaplan–Meier method, and SAS software (version 9.1, SAS Institute, Cary, North Carolina) was used for the statistical analyses.

Role of the Funding Source

The study was funded by Tibotec Therapeutics. All authors have been involved in the study since its inception; had access to the data and results; contributed to the writing, review, and approval of this manuscript; and were involved in the decision to submit the manuscript for publication.

Results

Baseline Characteristics and Disposition

All 429 enrolled patients (287 women and 142 men) were allocated to and received treatment (Figure 1); 67.2% of women and 76.8% of men completed the trial. Baseline demographic characteristics showed some differences between groups (Table 1). There was a higher proportion of black women than black men in the study, and a higher proportion of white men than white women in the study. Weight was similar between groups, but women had a higher body mass index than men. More men had Centers for Disease Control and Prevention Class C disease than women; men also tended to have more protease inhibitor–resistance mutations and to be older (Table 1). The median treatment duration was 48.1 weeks (range, 0.1 to 57.4 weeks) for women and 48.4 weeks (range, 0.4 to 53.4 weeks) for men. Women had a higher rate of discontinuation (32.8% vs. 23.2% in men; $P = 0.042$ with a difference noted as early as week 4 and continuing until the end of the study. The primary reasons for study withdrawal in women and men were loss to follow-up (8.4% and 6.3%, respectively) and adverse events (7.7% and 4.2%, respectively) (Figure 1). Adherence of 95% or greater was reported in 64.1% of women and 69.0% of men.

Efficacy

At week 48, 50.9% of women and 58.5% of men in the ITT population had confirmed virologic responses (Figure 2, *top*); in the analysis of the population that censored patients who withdrew for reasons other than virologic failure, the response rate was 73.0% in women and 73.5% in men (Figure 2, *bottom*). The difference in response rates between women and men, based on logistic regression and adjusted for baseline log₁₀ viral load and CD4⁺ cell count, was -9.6% (95% CI, -19.9% to 0.7% ; $P = 0.067$) in the ITT population and -3.9% (CI, -13.9% to 6.0% ; $P = 0.44$) in the population that censored patients who withdrew for reasons other than virologic failure. The nonsignificant difference in response rates in the TLOVR analysis of the ITT population was because a high number of women withdrew for reasons other than virologic failure.

In the additional logistic regression analysis, which included race as an independent covariate, sex was still not a significant predictor of response in either the ITT population ($P = 0.148$) or the population that censored patients who withdrew for reasons other than virologic failure ($P = 0.61$). In this analysis, baseline \log_{10} viral load ($P < 0.001$) and race ($P = 0.016$) were significant predictors of response in the ITT population, whereas baseline \log_{10} viral load ($P = 0.010$) and CD4⁺ cell count ($P = 0.019$) were significant predictors of response in the population that censored patients who withdrew for reasons other than virologic failure.

At week 48, the mean change from baseline in observed CD4⁺ cell count was 152 cells/mm³ (SD, 153.6; $n = 188$) in women and 122 cells/mm³ (SD, 126.1; $n = 105$) in men (Figure 3). Based on an analysis of covariance, the least-squares mean difference between groups (women–men) of 32.59 cells/mm³ (CI, –1.40 to 66.57 cells/mm³) was not statistically significant ($P = 0.060$). Baseline \log_{10} viral load ($P < 0.001$) and CD4⁺ cell count ($P = 0.043$) were significant predictors of higher increases in CD4⁺ cell count in the observed population. When a mixed-effects model was used to account for missing values, sex was significantly ($P = 0.033$) associated with changes from baseline in CD4⁺ cell counts, with an estimated difference of 23.15 (CI, 1.94 to 44.36) favoring women.

Resistance

The rates of confirmed virologic failure (viral load >50 copies/mL) in this treatment-experienced population were 28.6% ($n = 82$) and 28.2% ($n = 40$) in women and men, respectively. In patients with virologic failure, 27 of 82 women (32.9%) and 17 of 40 men (42.5%) had HIV RNA greater than 1000 copies/mL at virologic failure (required for genotypic testing) and paired specimens at baseline and at virologic failure. Of these, 3 women and 4 men developed at least 1 International AIDS Society (IAS-USA) major protease inhibitor resistance-associated mutation, darunavir resistance-associated mutation, or IAS-USA nucleoside reverse transcriptase inhibitor resistance-associated mutation. A total of 2 women (7.4%) and 2 men (11.8%) developed new IAS-USA major protease inhibitor resistance-associated mutations (V32I and M46I [$n = 2$]; M46I/L, L33F, and I50V [$n = 2$]), 1 woman (3.7%) and 2 men (11.8%) developed new darunavir resistance-associated mutations (V32I, L33F, and I50V [$n = 2$]; and L89V [$n = 1$]), and 2 women (7.4%) and 2 men (11.8%) developed new IAS-USA nucleoside reverse transcriptase inhibitor resistance-associated mutations (M41L and M184V [$n = 3$]). Patients developing new IAS-USA major protease inhibitor resistance-associated mutations or new darunavir resistance-associated mutations already had substantial resistance at baseline (5 to 15 IAS-USA protease inhibitor resistance-associated mutations and 1 to 6 IAS-USA major protease inhibitor resistance-associated mutations).

Adverse Events

Overall, serious adverse events regardless of causality were reported in 47 women (16.4%) and 33 men (23.2%). A total of 2 women and 2 men died during the study (from lactic acidosis, pneumonia, multiple organ failure, and *Legionella* pneumonia); all 4 deaths were considered unrelated to darunavir–ritonavir by the investigators. In total, 259 women (90.2%) and 118 men (83.1%) had at least 1 adverse event (Table 2). The most common adverse events, regardless of severity and causality, were diarrhea, nausea, vomiting, and upper respiratory tract infection (Table 2). Although the incidence of adverse events was generally similar between the sexes, nausea and vomiting were more common in women, and diarrhea was more common in men (Table 2). A total of 78 women (27.2%) and 48 men (33.8%) had grade 3 or 4 adverse events. Overall, 134 women (46.7%) and 61 men (43.0%) had at least 1 adverse event considered by the investigators to be possibly related to darunavir–ritonavir. Of the grade 2 to 4 adverse events considered possibly related to

darunavir–ritonavir, diarrhea, nausea, and rash were the most common (Table 2). Among adverse events of interest in patients receiving darunavir–ritonavir, gastrointestinal-associated events (43.2% in women and 38.0% in men) and rash-associated events (15.3% in women and 16.9% in men) were the most common, and most were mild-to-moderate in severity.

Discontinuations due to adverse events were infrequent, and discontinuation rates did not significantly differ between women (7.7%) and men (4.2%; $P = 0.175$). (Figure 1). A total of 15 women (5.2%) and 3 men (2.1%) discontinued treatment due to an adverse event judged by the investigator to be at least possibly related to darunavir–ritonavir. There were no trends toward a specific type of adverse event driving discontinuation. Most laboratory abnormalities were grade 1 or 2, and rates of grade 3 or 4 adverse events were generally similar between sexes, except for triglyceride abnormalities, which were reported more frequently in men (Table 2).

Discussion

In the GRACE study, virologic response did not significantly differ between women and men through 48 weeks of therapy. When discontinuations were considered as failures (intent-to-treat TLOVR), there was a 9.6% difference (CI, -19.9% to 0.7% ; $P = 0.067$) in response favoring men. The lower bound of the 95% CI around this difference exceeded the prespecified 15% difference in efficacy required to claim noninferiority between men and women. Conversely, when patients who discontinued the study for reasons other than virologic failure were censored at the time of discontinuation, the response rates were similar between women and men (-3.9% [CI, -13.9% to 6.0%]; $P = 0.44$). Rates of confirmed virologic failure were nearly identical in women and men (28.6% and 28.2%, respectively). Therefore, any numeric differences in response rates between men and women in the GRACE study were probably not driven by differences in true virologic response to therapy, but rather by higher discontinuation rates in women for reasons other than virologic failure.

Increases in CD4⁺ cell count were higher in women than men (observed data); a sex-based difference in treatment outcomes that has been noted in previous assessments of antiretroviral therapy (20–22). The HEAT (HIV study with Etravirine and Truvada) trial (21) showed similar results, with women in the ITT population showing lower virologic response rates but larger increases in CD4⁺ cell counts than men. In addition, similar to the results from the mixed-effects model reported here, a study of 2229 patients starting treatment with highly active antiretroviral therapy (22) found, via multivariate analysis, that male sex was a significant predictor of lower increases in CD4⁺ cell count. All previous studies used for comparison were identified by an English-language PubMed search through September 2009.

Overall, darunavir–ritonavir therapy seemed to be well tolerated in our study, with no unexpected adverse events based on results from previous studies (16,17,23,24), and no substantial differences in the incidence of adverse events. Women had a higher prevalence of nausea and vomiting, and men had a higher prevalence of diarrhea; however, these differences were not considered clinically relevant. Differences in rates of nausea have been observed in previous antiretroviral studies, such as the CASTLE (Comparing the Antiviral Efficacy and Safety of Atazanavir/Ritonavir With Lopinavir/Ritonavir, Each in Combination With Fixed-Dose Tenofovir-Emtricitabine in HIV-1-Infected Treatment-Naïve Subjects) study (25), in which mild-to-moderate nausea was reported more frequently in treatment-naïve women than in men in both the lopinavir/ritonavir group and the atazanavir/ritonavir group. Furthermore, in other trials, diarrhea has been reported at higher rates in men (26,27).

Reports (26) have suggested that susceptibility to adverse events may differ between women and men based on differences in weight and pharmacokinetics. Although weight at baseline did not differ between women and men, preliminary pharmacokinetic analyses from the GRACE study noted a slightly higher exposure to darunavir (about 20%) and a substantially higher exposure to ritonavir (70%) in women compared with men at week 4 (28). The effect of sex-based differences in exposure on adverse events and other outcomes will be explored further in future GRACE analyses.

No individual adverse event was identified as driving discontinuations in either women or men; however, nausea, which was reported more frequently in women than men (24.4% vs. 14.1%, respectively), has been independently correlated with poor adherence (29). Although discontinuation rates due to adverse events did not significantly differ between women and men ($P = 0.175$, results from the GRACE study do reflect a trend of higher discontinuation rates in women due to adverse events, which has been observed in other antiretroviral trials (30,31). Subtle differences in tolerability that were not reported or captured may have contributed to the difference in discontinuation rates observed between sexes in the GRACE study.

The recruitment strategies used in the GRACE study took into account not only sex, race, and ethnicity but also the geographic distribution of patients with HIV in the United States. It successfully enrolled a diverse patient population that reflects current HIV demographic characteristics and case distribution in the United States. However, discontinuation rates for reasons other than virologic failure in GRACE patients were higher in women, as noted in previous antiretroviral studies (30,31). Specifically, reasons other than virologic failure, including loss to follow-up, adverse events, site closure, patient relocation, and nonadherence with study visits, drove differences in ITT response rates between men and women. Another antiretroviral study (32) designed to focus on women (50% of the overall population) and persons of color (40% black and 37% Hispanic overall) had an overall discontinuation rate of 49% ($n = 129$ of 254) over 96 weeks, with 44% ($n = 57$ of 129) resulting from loss to follow-up. Similar results have been seen in trials of other therapeutic agents in areas other than HIV. A retrospective analysis of discontinuation from trials of pharmacologic weight-loss agents (33) found that female sex was significantly related to higher levels of total discontinuation and discontinuations not related to adverse events. Another study focusing on predictors of discontinuation in a trial of an opioid antagonist (34) found that although no specific factors were associated with discontinuation in men, women with pretreatment psychiatric problems were more likely to discontinue.

These observations, along with those from the GRACE study, suggest that additional attention needs to be focused on how to retain women in clinical trials. One limitation of this trial may be that selection bias contributed to the disproportionate rate of discontinuation for reasons other than virologic failure in women; women were younger, had less treatment experience, and had fewer mutations at baseline than men; these characteristics might suggest that women were less likely to discontinue their previous therapy due to virologic failure. In future studies, collecting additional information on previous therapies could help establish whether a subset of patients tend to withdraw because of tolerability or social factors.

In conclusion, the GRACE study shows that it is possible to enroll large numbers of women into HIV treatment trials. The trial may serve as a platform for designing future studies to obtain accurate information on sex-based differences in the use of antiretroviral treatment and other therapeutic agents and to aid treatment decisions in diverse patient populations. The higher discontinuation rate in women, which was driven by reasons other than virologic failure, highlights the need for additional efforts to retain diverse populations in studies. Of

note, no sex-based statistical differences in virologic response or clinically relevant differences in adverse events were observed, suggesting that darunavir–ritonavir antiretroviral combinations have similar efficacy outcomes and incidence of adverse events in treatment-experienced women and men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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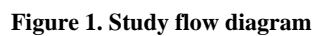
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* "Other" classification was selected by the investigator as reason for discontinuation. †

Older patient taking too many concomitant medications. ARV = antiretroviral.

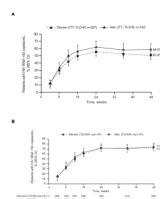


Figure 2. Confirmed virologic response in intention-to-treat population (top) and population censored for patients who withdrew for reasons other than virologic failure (bottom)
 Viral response defined as viral load less than 50 copies/mL, confirmed by 2 consecutive assessments at least 14 days apart. ITT = intent-to-treat; IQR = interquartile range; TLOVR = time-to-loss of virologic response; VF = virologic failure.

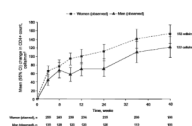


Figure 3.
Mean (95% CI) change from baseline over time in CD4⁺ cell count (observed)

Table 1

Baseline Characteristics and Demographics

Variable	Women (n = 287)	Men (n = 142)
Mean age (SD), y	41.7 (10.6)	45.2 (9.0)
Race ¹		
Black, n (%)	191 (66.6)	73 (51.4)
Hispanic/Latino, n (%)	60 (20.9)	36 (25.4)
White, n (%)	34 (11.8)	31 (21.8)
Other, n (%)	2 (0.7)	2 (1.4)
Mean weight (SD), kg	75.5 (21.45)	77.6 (15.72)
Mean BMI (SD), kg/m ²	28.2 (7.41)	25.4 (5.06)
Mean duration of infection (SD), y	10.9 (5.36)	12.2 (5.84)
Mean viral load (SD), log ₁₀ copies/mL	4.65 (0.88)	4.73 (0.86)
Median CD4 ⁺ cell count (range), cells/mm ³	210 (1 to 868)	175 (2 to 1125)
CDC Class C ² , n (%)	102 (35.5)	67 (47.2)
Entry on treatment interruption, n (%)	100 (34.8)	51 (35.9)
Recreational drug use, n (%)	39 (13.6)	22 (15.5)
Previous antiretroviral use		
≥2 PIs, n (%)	168 (58.5)	92 (64.8)
≥2 NNRTIs, n (%)	55 (19.2)	23 (16.2)
Median darunavir fold change (range) [†]	0.6 (0.3 to 128.7)	0.6 (0.4 to 148.3)
>1 major protease inhibitor mutation, n (%) [†]	77 (27.0)	57 (40.1)
≥1 Tibotec darunavir resistance-associated mutation, n (%) [†]	47 (16.5)	33 (23.2)
Background regimen		
Etravirine (TMC125), n (%)	116 (40.4)	87 (61.3)
Emtricitabine, n (%)	221 (77.0)	110 (77.5)
Lamivudine, n (%)	30 (10.5)	16 (11.3)
Tenofovir, n (%)	239 (83.3)	122 (85.9)
Zidovudine, n (%)	50 (17.4)	34 (23.9)
Mean phenotypic susceptibility score of the optimized background regimen (SD)	2.0 (0.65)	2.0 (0.81)

BMI = body mass index; CDC = Centers for Disease Control and Prevention; NNRTI = nonnucleoside reverse transcriptase inhibitor.

¹ Significantly different for women and men ($P=0.011$).

² Significantly different for women and men ($P=0.021$).

[†] By virco[®]TYPE HIV-1 system (Virco BVBA, Mechelen, Belgium).

Table 2**Summary of Adverse Events***

Adverse Events	Women (n = 287)	Men (n = 142)
Patients with ≥1 adverse event [†]	259 (90.2)	118 (83.1)
Patients with ≥1 serious adverse event [‡]	47 (16.4)	33 (23.2)
Deaths	2 (0.7)	2 (1.4)
Patients with ≥1 adverse event at least possibly related to darunavir/ritonavir [§]	134 (46.7)	61 (43.0)
Patients with ≥1 grade 2 to 4 adverse event at least possibly related to darunavir/ritonavir [§]	80 (27.9)	38 (26.8)
Patients with ≥1 grade 3 to 4 adverse event overall	78 (27.2)	48 (33.8)
Patients who discontinued due to an adverse event	22 (7.7)	6 (4.2)
Patients who discontinued due to an adverse event at least possibly related to darunavir/ritonavir [§]	15 (5.2)	3 (2.1)
Most common adverse events	259 (90.2)	118 (83.1)
Diarrhea	47 (16.4)	32 (22.5)
Nausea	70 (24.4)	20 (14.1)
Vomiting	33 (11.5)	9 (6.3)
Upper respiratory tract infection	32 (11.1)	11 (7.7)
Serious adverse events	47 (16.4)	33 (23.2)
Pneumocystis	1 (0.3)	4 (2.8)
Pneumonia	10 (3.5)	1 (0.7)
Grade 2 to 4 adverse events at least possibly related to darunavir/ritonavir ^{/§**}	80 (27.9)	38 (26.8)
Diarrhea	13 (4.5)	7 (4.9)
Nausea	15 (5.2)	4 (2.8)
Rash	6 (2.1)	4 (2.8)
Weight gain	5 (1.7)	3 (2.1)
Vomiting	4 (1.4)	3 (2.1)
Grade 3 to 4 laboratory abnormalities		
Hyperuricemia	0	4 (2.9)
Lipase	5 (1.9)	5 (3.7)
Pancreatic amylase	4 (1.5)	6 (4.4)
Plasma prothrombin time	0	4 (4.4)
Hyperglycemia	6 (2.2)	4 (2.9)
Total cholesterol (grade 3 only) level	10 (4.6)	5 (4.2)
Triglyceride level	1 (0.5)	11 (9.2)
Alanine aminotransferase	6 (2.2)	4 (2.9)
Aspartate aminotransferase	8 (3.0)	7 (5.1)

* All values are n (%).

[†] Any untoward medical occurrence, new in onset or aggravated in severity or frequency from baseline, related to study treatment or not.

[‡] Any adverse event that resulted in death, was life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, or resulted in persistent or significant disability or incapacity.

[§] As judged by the investigator.

^{//} Occurring in $\geq 10\%$ of patients in either group.

[¶] Occurring in $\geq 2\%$ of patients in either group.

^{**} Excluding laboratory abnormalities.