

Virologic efficacy of boosted double versus boosted single protease inhibitor therapy

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Objective: Although regimens containing two protease inhibitor (PI) together with ritonavir boosting are used with the aim of improving virologic response to salvage therapy, there is little evidence to support or reject this approach. We compared the probability of attaining an undetectable HIV RNA level after using either boosted double or boosted single PI regimens.

Design: Retrospective clinical cohort.

Methods: PI-experienced subjects in a Northern California-based database who initiated either a boosted single or boosted double PI salvage therapy regimen were analysed. Traditional multivariable regression and marginal structural model analyses were used to compare the effects of the two regimens on virologic suppression 12–36 weeks after initiation of salvage therapy, controlling for confounding by baseline HIV RNA level, CD4 lymphocyte count, treatment history, drug resistance, and multiple characteristics of the salvage regimen.

Results: Fifty-one percent of boosted single PI regimens (n=805) and 51.6% of boosted double PI regimens (n=183) achieved a plasma HIV RNA level of <75 copies/ml at week 12–36. In models including multiple potentially confounding variables, estimates of the relative odds of suppression on boosted double versus boosted single PI regimens ranged from 1.17 (95% CI, 0.54–2.55) to 1.33 (95% CI, 0.82–2.14).

Conclusions: We were not able to reject the null hypothesis that boosted double versus boosted single PI regimens, resulted in equivalent probabilities of virologic success.

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Introduction

HIV-infected patients frequently lose virologic suppression on antiretroviral therapy (ART) due to the emergence of resistant virus. While salvage therapy regimens are sometimes able to re-suppress the virus, the virologic efficacy of salvage therapy remains significantly below that of initial therapy regimens.

Standard protease inhibitor (PI)-based regimens combine a single PI with nucleoside reverse transcriptase inhibitors

(NRTI). A low dose of ritonavir is usually included in the regimen to boost PI blood levels by inhibition of the CYP450 enzyme system. The use of two PI in combination with ritonavir boosting has been proposed as a means of improving the efficacy of PI-based salvage therapy. There have been pharmacokinetic studies examining PI blood levels in persons receiving boosted double PI and several small case series examining their efficacy [1–3]. However, data comparing virologic response to boosted double PI regimens with that of boosted single PI regimens are extremely limited.

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We performed a retrospective analysis to examine the virologic efficacy, as shown by HIV RNA levels <75 copies/ml, of boosted double versus boosted single PI regimens in a treatment-experienced population. Subjects were from 16 clinics of a California Health Maintenance Organization (HMO). Analyses included both traditional multivariable regression and estimation of three marginal structural model estimators [4–6].

Methods

Subjects and data collection

The subjects were HIV-infected patients of Kaiser Permanente Medical Centers (KPMC), Northern California whose blood was sent to Stanford University Hospital for HIV-1 genotypic resistance testing. Plasma HIV-1 RNA levels and CD4 lymphocyte counts for these patients were obtained electronically. Plasma HIV-1 RNA levels and CD4 lymphocyte counts were performed in a single Kaiser regional laboratory using standard methodology. Plasma HIV RNA levels were assayed using the quantiplex HIV-1 RNA 3.0 bDNA assay (Chiron Diagnostics, Emeryville, California, USA) with lower limits of detection of 50 copies/ml up to November 2002, and the Versant HIV-1 RNA 3.0 bDNA assay (Bayer Diagnostics, Berkeley, California, USA) with lower limits of detection of 75 copies/ml thereafter. For consistency, the lower limit of detection of 75 copies/ml was used throughout our analyses. All drug resistance testing was done at the Stanford virology laboratory. Treatment histories were obtained for those persons who obtained their drugs from the KPMC pharmacies and were confirmed by physician chart review.

We identified all treatment change episodes (TCE) between April 1998 and November 2005 in which a PI-experienced patient initiated a new ART regimen that included either a single PI drug plus ritonavir ('boosted single PI regimen') or two PI drugs plus ritonavir ('boosted double regimen'). A TCE was defined as follows: (i) initiation of at least two antiretroviral drugs not used in the prior boosted double PI regimen; (ii) measurement of HIV RNA level and CD4 lymphocyte count within 24 weeks before initiating the new regimen (if several measurements were available, the most recent was used); (iii) measurement of at least one viral genotype prior to initiating the new regimen; (iv) availability of all antiretroviral treatments used prior to initiating the availability of data on new regimen.

Sequence data for an individual patient were summarized in two ways, based on the Stanford HIV Drug Resistance Database HIVdb algorithm (<http://hivdb.stanford.edu/pages/asi/releaseNotes/>): (i) the number of individual resistance mutations specific to each antiretroviral drug

class was summed for that class; and (ii) a susceptibility score was calculated for each non-PI drug in a subject's salvage regimen, and these scores were summed to yield a non-PI genotypic susceptibility score (GSS). The last available sequence prior to the TCE was used in the primary analyses; additional sensitivity analyses used a composite of all past genotypes. PI were excluded from the GSS because were they included and, all else, such as resistance and background regimen, being equal, a boosted double PI regimen would get a higher GSS score than a boosted single PI regimen. Thus, including PI drugs in the GSS and then controlling for the GSS score would amount to controlling for the hypothesized effect of interest, resulting in a bias part of the effect estimate towards the null.

Key confounders controlled for in analyses included baseline clinical characteristics, genotype, and treatment history. Treatment history prior to the TCE was summarized as the number of drugs that had been used in each main antiretroviral class of drugs, past use of T20, history of treatment with a mono- or dual-NRTI drug regimen, and date of earliest antiretroviral treatment. In addition, because patient treatment history and baseline genotype were generally available to inform regimen selection, we controlled in the analyses for various aspects of the salvage regimen, including the total number of drugs, number of drugs used for the first time, use of a non-nucleoside reverse transcriptase inhibitor (NNRTI) for the first time, and the GSS. A full list of confounders considered is provided in Table 1.

Consistency of treatment availability between subjects receiving boosted double versus boosted single PI regimens was addressed in three ways. First, our sample consisted of subjects who were all both residents of the same geographic region and members of the same HMO, under which all approved drugs were available to all patients. Second, data were restricted to TCE which occurred after September 1998, when boosted double PI regimens were first used in our dataset. Third, we explicitly controlled for calendar time in our analyses.

The outcome considered was the first plasma HIV RNA level measured between 12 and 36 weeks after initiating the new regimen, and before any subsequent change of regimen or interruption in treatment. The recommendations of both the International AIDS Society and the United States Department of Health and Human Services call for achieving plasma HIV RNA levels below the lower limit of assay detection as the goal of ART [7,8]. We aimed to assess the relative effectiveness of boosted single versus boosted double PI therapy in achieving this goal.

Ethics

The studies were approved by the IRBs of Stanford University, Kaiser Foundation Hospitals, and the University of California, Berkeley, California, USA.

Table 1. Sample characteristics [median (IQR)] and association with use of boosted double PI regimen^a.

	Boosted single PI	Boosted double PI	<i>P</i> value ^b
Continuous variables			
Calendar date	3/2002 (9/2000, 8/2003)	7/2002 (6/2001, 5/2003)	0.07
Peak log ₁₀ viral load (log ₁₀ copies/ml)	5.1 (4.5, 5.5)	5.2 I (4.7, 5.5)	0.39
Baseline log ₁₀ viral load (log ₁₀ copies/ml)	4.2 (3.3, 4.9)	4.2 (3.4, 4.9)	0.59
Nadir CD4 cell count (cells/μl)	90 (30, 189)	67 (19, 168)	0.03
Baseline CD4 cell count (cells/μl)	239 (117, 373)	212 (99, 356)	0.44
Time from baseline viral load to treatment change (days)	26 (13, 50)	28 (10, 46)	0.51
Time from baseline CD4 cell count to treatment change (days)	27 (13, 52)	27 (10, 47)	0.12
Time from most recent genotype to treatment change (days)	107 (24, 376)	121 (30, 352)	0.43
PI drugs experienced (prior to treatment change) (n)	2 (1, 4)	3 (2, 4)	< 0.01
NRTI drugs experienced (prior to treatment change) (n)	4 (3, 5)	4 (4, 5)	< 0.01
NNRTI drugs experienced (prior to treatment change) (n)	1 (0, 1)	1 (1, 2)	< 0.01
Major PI mutations (n)	1 (0, 3)	2 (0, 3)	0.04
NRTI mutations (n)	4 (2, 6)	5 (4, 7)	< 0.01
NNRTI mutations (n)	1 (0, 2)	1 (0, 2)	< 0.01
Date of first ART therapy	9/1995 (1/1994, 3/1997)	1/1995 (1/1994, 8/1996)	0.05
Drugs used for the first time in current regimen (n)	2 (1, 3)	2 (1, 3)	0.37
NRTI in current regimen (n)	2 (2, 3)	1 (2, 3)	0.18
Drugs switched in current regimen (n)	3 (2, 4)	3 (2, 4)	0.19
Genotypic susceptibility score for non-PI drugs in current regimen	2.0 (1.8, 2.6)	1.8 (1.5, 2.6)	0.02
Categorical variables			
History of mono/dual NRTI treatment	552 (67.2%)	124 (79.0%)	0.01
Use of T20 prior to treatment change	10 (1.9%)	3 (1.2%)	0.49
Use of T20 in current regimen	43 (5.2%)	13 (8.3%)	0.13
Use of NNRTI for the first time in current regimen	127 (15.4%)	17 (10.8%)	0.14

^aAmong 822 boosted single PI and 157 boosted double PI regimens with outcome measured.

^bRobust *P*-value for unadjusted association (odds ratio) between covariate and use of boosted double PI regimen, based on univariable logistic regression using General Estimating Equations with exchangeable working covariate matrix.

IQR, Interquartile range; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; ART, antiretroviral therapy.

Statistical analyses

Both multivariable logistic regression and marginal structural model-based analyses (which use distinct approaches to control for confounding) were used to test the null hypothesis that use of two versus one PI drugs in combination with ritonavir does not increase the probability of virologic success. In marginal structural model analyses, three estimators were used that control for confounding in different ways: G-computation, Inverse Probability of Treatment Weighted (IPTW), and Double Robust [4–6,9,10].

The G-computation estimator is closely related to standard multivariable regression. A multivariable logistic regression model was fit, regressing virologic success on confounders. This model, with coefficients refit for the two treatment groups, was used to predict two outcomes for each TCE: (i) the outcome that would have been observed had the salvage regimen used two boosted PI, and (ii) the outcome that would have been observed had the salvage regimen used one boosted PI. A logistic regression of these predicted outcomes on use of a boosted double PI regimen provided an estimate of effect.

The IPTW estimator uses a multivariable logistic model that regresses use of a boosted double PI regimen on confounders; this model is referred to as the treatment mechanism. The treatment mechanism was used to assign each TCE a weight inversely proportional to the

predicted probability of the observed treatment for that TCE. An example using a single confounder follows: because they might be more likely to receive a boosted double PI regimen, patients with extensive treatment experience might be under-represented among those who received a boosted single PI regimen, and over-represented among those who received a boosted double PI regimen. Inverse weighting would correct for this by assigning larger weights to those patients with extensive treatment experience who received a boosted single PI regimen and smaller weights to those patients with extensive treatment experience who received a boosted double PI regimen.

The Double Robust estimator uses both the treatment mechanism and the multivariable regression of outcome on confounders (refit for each treatment group). The consistency of the IPTW estimator relies on correctly estimating the treatment mechanism, while the consistency of G-computation relies on correctly estimating the regression of outcome on confounders. The Double Robust estimator, in contrast, remains consistent if either of these models is correct, and is maximally robust to model misspecification [9,10].

Not all eligible individuals had outcome HIV RNA levels measured. Missing outcomes were due to either changes in regimen made before 12 weeks or failure to measure viral load in the appropriate window (12–36 weeks).

Primary analyses were based on those individuals with a measured outcome. Secondary analyses were performed using inverse probability of censoring weights (IPCW) to account for potentially informative missing values [11]. Among all subjects meeting entry criteria, we fit a regression model predicting the probability that HIV RNA level between 12 and 36 weeks was measured, given baseline covariates and, where available, last viral load measured prior to 12 weeks while on the current regimen. This model was then used to adjust for possible bias in that subgroup of the sample with outcome measured.

Multivariable regression models were fit using the Deletion/Substitution/Addition algorithm (D/S/A) [12]. The D/S/A is an aggressive search algorithm for fitting data-adaptive regressions. Five-fold cross-validation was used to select the number of terms and order of interactions that provided the optimal tradeoff between bias and variance based on model performance on independent data. Covariates associated with the outcome ($P < 0.1$) were considered candidate confounders.

Subjects could contribute more than one TCE to the analysis. To account for repeated measures on individual subjects in estimating the significance of coefficients in single and multivariable regressions, robust P values from General Estimating Equations with an exchangeable working covariance matrix were used. Confidence intervals for marginal structural model estimators were based on 200 non-parametric bootstrap samples.

Results

Between April 1998 and November 2005, 183 eligible subjects switched to a salvage regimen containing a boosted double PI and 805 eligible subjects switched to a regimen containing a boosted single PI. Among individuals starting a boosted double PI regimen, outcome HIV RNA levels were measured for 139 individuals (157 TCE); among individuals starting a boosted single PI regimen, outcomes were measured for 664 individuals (822 TCE). Primary analyses were based on those 803 subjects with a measured outcome; secondary analyses incorporated all eligible subjects and accounted for potentially informative missing values.

Median HIV RNA prior to treatment change was 4.2 \log_{10} copies/ml. Subjects had had extensive prior ART. Over half the sample had a history of more than 6 years on ART, all were PI-experienced, 71% were NNRTI-experienced, and 69% had a history of treatment with mono- or dual-NRTI therapy. This treatment experience was reflected in the prevalence of preexisting resistance mutations; the median numbers of NRTI, NNRTI, total PI, and primary PI mutations present at baseline were 4, 1, 4, and 1, respectively (Table 1).

At week 12–36 51.6% of boosted double PI regimens and 51.0% of boosted single PI regimens achieved virologic suppression. Fifty four individuals (57 TCE) used a boosted double PI regimen containing amprenavir and lopinavir. The findings from the 54 persons who used amprenavir/lopinavir were grouped with those from all other boosted double regimens in subsequent analyses because virologic outcomes were similar: 54% of TCE using amprenavir and lopinavir were successfully suppressed versus 50% of the 100 TCE (among 93 individuals) using other boosted double PI regimens. Other frequently used boosted double PI regimens included lopinavir and saquinavir (38 TCE), lopinavir and indinavir (13 TCE) and lopinavir and atazanavir (12 TCE).

Predictors of virologic success

Univariable (unadjusted) associations between potential confounders and virologic success are shown in Table 2. Virologic suppression was significantly associated with more recent treatment date, lower baseline and peak plasma HIV RNA levels, and higher baseline and nadir CD4 lymphocyte counts. Subjects with a shorter duration between baseline genotype and treatment change, and use of T20 prior to or as part of the current regimen had a lower probability of success. A less extensive treatment history, fewer mutations, more drugs in the new regimen used for the first time, and first use of an NNRTI in the new regimen were significantly associated with virologic success. Higher GSS based on the non-PI drugs in the regimen was a non-significant predictor of virologic suppression ($P = 0.09$).

Table 3 reports adjusted odds ratios (OR) based on multivariable logistic regression of virologic success on use of a boosted double versus boosted single PI regimen and potential confounders. After adjusting for confounding in this multivariable regression, use of a boosted double PI regimen was not significantly associated with improved virologic success (OR, 1.29; $P = 0.26$). Several other covariates were significantly associated with increased probability of virologic success: more recent calendar date (OR, 1.13 per year; $P = 0.02$); higher baseline CD4 lymphocyte count (OR, 1.14 per 100 cells; $P = 0.04$); and use of an NNRTI drug in the new regimen for the first time (OR, 1.86; $P = 0.02$). Probability of success was lower for TCE with a higher baseline \log_{10} viral load (OR, 0.54 per \log_{10} ; $P < 0.01$), higher peak \log_{10} viral load (OR, 0.65 per \log_{10} ; $P < 0.01$), more NRTI drugs experienced in past regimens (OR, 0.83 per drug; $P = 0.03$); and more PI mutations (OR, 0.78 per primary mutation; $P < 0.01$). Higher GSS also significantly predicted higher success (OR, 1.35; $P = 0.02$).

Predictors of double-boosting

Univariable (unadjusted) associations between use of a boosted double PI regimen and potential confounders are

Table 2. Univariable associations between covariates and virologic success^a.

Covariate	Unadjusted OR (<i>P</i> -value)
Calendar date (per year)	1.17 (< 0.01)
Peak log ₁₀ viral load	0.35 (< 0.01)
Baseline log ₁₀ viral load	0.44 (< 0.01)
Nadir CD4 count (per log ₁₀ copies/ml)	1.49 (< 0.01)
Baseline CD4 count (per log ₁₀ copies/ml)	1.46 (< 0.01)
Time from baseline viral load to treatment change (per 4 weeks)	0.93 (0.26)
Time from baseline CD4 count to treatment change (per 4 weeks)	0.96 (0.50)
Time from most recent genotype to treatment change (per 4 weeks)	1.01 (0.04)
PI drugs experienced (prior to treatment change) (per drug)	0.67 (< 0.01)
NRTI drugs experienced (prior to treatment change) (per drug)	0.72 (< 0.01)
NNRTI drugs experienced (prior to treatment change) (per drug)	0.56 (< 0.01)
Number of major PI mutations (per mutation)	0.72 (< 0.01)
NRTI mutations (per mutation)	0.92 (< 0.01)
NNRTI mutations (per mutation)	0.80 (< 0.01)
Date of first ART (per calendar year)	1.11 (< 0.01)
Drugs used for the first time in current regimen (per drug)	1.49 (< 0.01)
NRTI used in current regimen (per drug)	0.93 (0.33)
Drugs switched in current regimen (per drug)	0.98 (0.81)
Genotypic susceptibility score for non-PI in current regimen (per unit increase)	1.17 (0.09)
History of mono/dual NRTI treatment	0.72 (0.03)
Use of T20 prior to treatment change	NA ^b
Use of T20 in current regimen	0.47 (< 0.01)
Use of NNRTI for the first time in current regimen	1.99 (< 0.01)

^aRobust *P*-value for unadjusted association (odds ratio) between covariate and virologic success, based on univariable logistic regressions, using General Estimating Equations and exchangeable working covariate matrix.

^bNo virologic successes among subjects with history of T20 use prior to current regimen.

OR, Odds ratio; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

shown in Table 1. Use of a boosted double PI regimen was more likely among subjects with lower nadir CD4 lymphocyte counts; in those with histories of mono- or dual-NRTI exposure, earlier initiation of first antiretroviral drug, and more extensive past treatment with PI, NRTI and NNRTI; and when there were more mutations in all three drug classes. An NNRTI drug was used for the first time in 15.4% of boosted single PI

regimens, and 10.8% of boosted double PI regimens. Subjects on a boosted double PI regimen were treated slightly later in calendar time (*P*=0.07).

Marginal structural model results

In implementing the three marginal structural model estimators, the D/S/A algorithm was used to select, based on the data: (i) a multivariable logistic regression of

Table 3. Multivariable associations between virologic success and covariates^a.

Covariate	Adjusted OR (<i>P</i> -value)
Use of boosted double versus boosted single PI regimen	1.29 (0.26)
Calendar date (per year)	1.13 (0.02)
Peak log ₁₀ viral load	0.65 (< 0.01)
Baseline log ₁₀ viral load	0.54 (< 0.01)
Nadir CD4 lymphocyte count (per log ₁₀ copies/ml)	1.01 (0.95)
Baseline CD4 lymphocyte count (per log ₁₀ copies/ml)	1.14 (0.04)
Time from most recent genotype to treatment change (per 4 weeks)	1.00 (> 0.99)
PI experienced (prior to treatment change) (per drug)	0.89 (0.14)
NRTI experienced (prior to treatment change) (per drug)	0.83 (0.03)
NNRTI drugs experienced (prior to treatment change) (per drug)	1.04 (0.82)
Major PI mutations (per mutation)	0.78 (< 0.01)
NRTI mutations (per mutation)	1.05 (0.23)
NNRTI mutations (per mutation)	0.97 (0.65)
Date of first ART (per calendar year)	1.05 (0.28)
Drugs used for the first time in current regimen (per drug)	1.13 (0.19)
Genotypic susceptibility score (per unit increase)	1.35 (0.02)
History of mono/dual NRTI treatment	1.44 (0.11)
Use of T20 in current regimen	1.73 (0.20)
Use of NNRTI for the first time in current regimen (per mutation)	1.86 (0.02)

^aBased on multivariable logistic regression model including all covariates as main terms, selected using the D/S/A algorithm and cross-validation. *P*-values based on non-parametric bootstrap.

OR, Odds ratio; PI, protease inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; ART, antiretroviral therapy.

Table 4. Marginal structural model estimates of effect of boosted double versus boosted single PI regimen on virologic success.

Estimator	OR for success (95% CI) ^a	Relative success probability (95% CI) ^b	Difference in success probability (95% CI) ^c
Among subjects with viral load at 12–36 weeks			
G-comp	1.23 (0.84, 1.81)	1.10 (0.92, 1.29)	0.05 (–0.04, 0.15)
IPTW	1.17 (0.54, 2.55)	1.08 (0.76, 1.40)	0.04 (–0.12, 0.19)
DR	1.33 (0.82, 2.14)	1.14 (0.91, 1.37)	0.07 (–0.04, 0.18)
Adjusted to account for missing outcome ^d			
G-comp	1.18 (0.77, 1.80)	1.09 (0.86, 1.31)	0.04 (–0.06, 0.15)
IPTW	1.04 (0.52, 2.08)	1.02 (0.69, 1.35)	0.01 (–0.15, 0.16)
DR	1.21 (0.75, 1.96)	1.10 (0.86, 1.35)	0.05 (–0.07, 0.17)

^aNull hypothesis: odds ratio (OR), 1.

^bNull hypothesis: relative success probability, 1.

^cNull hypothesis: difference in success probability, 0.

^dAdjusted Using Inverse Probability of Censoring Weights.

CI, Confidence interval; G-comp, G-computation estimator; IPTW, Inverse Probability of Treatment Weighted estimator; DR, Double Robust estimator.

virologic success on potential confounders; and (ii) a multivariable logistic regression of use of a boosted double PI regimen on potential confounders. In both cases, cross-validation selected a model including all covariates as main terms, with no two-way interactions.

Table 4 shows estimates of the causal odds ratio, relative success probability, and difference in success probability resulting from treatment with a boosted double versus boosted single PI regimen, based on the three marginal structural model estimators. Among 803 subjects with a HIV RNA level measured between 12–36 weeks while still on the salvage regimen, we estimated that use of a boosted double versus boosted single PI regimen was associated with a relative odds of success between 1.17 (95% CI, 0.54, 2.55) and 1.33 (95% CI, 0.82, 2.14), or a 4% (95% CI, –0.12, 0.19) to 7% (95% CI: –0.04, 0.18) higher absolute probability of virologic success. These estimates were higher than the crude associations (unadjusted for confounders) between use of a boosted double PI regimen and success probability (1.02 relative odds or 0.6% higher absolute probability of suppression among those receiving a boosted double PI regimen). For none of the estimates were we able to reject (at the $P=0.05$ level) the null hypothesis that use of a boosted double PI regimen resulted in an equivalent probability of virologic success to use of a boosted single PI regimen.

Table 4 reports the results of marginal structural model analyses in which IPCW were used to adjust for the possibility that the subjects for whom the outcome was measured might be unrepresentative of the larger sample meeting inclusion criteria. The resulting estimates were slightly lower than those based on the sample of subjects for whom an outcome was measured. Again, none of the estimates allowed us to reject the null hypothesis that use of a boosted double versus boosted single PI regimen resulted in equivalent probability of virologic suppression.

The following additional sensitivity analyses were implemented: (i) viral sequence data were summarized

based on a composite of all past genotypes, instead of only the most recent genotype; (ii) the inclusion criterion requiring a history of treatment with PI drugs prior to the current regimen was relaxed, resulting in the inclusion of an additional 38 subjects initiating a boosted double and 142 subjects initiating a boosted single PI regimen; (iii) number of drugs used for the first time in the current regimen was not controlled for. Marginal structural model-based point estimates of the effect of use of a boosted double versus boosted single PI regimen were comparable under all of the above sensitivity analyses, none of which rejected the null hypothesis of no significant effect.

Discussion

Boosted double PI salvage regimens are often used in clinical practice. However, sparse research evaluates the efficacy of this approach. A clinical trial showed that the virologic response to a new four-drug class salvage regimen, that included amprenavir plus a second PI, was better than the response to a similar regimen that included amprenavir without another PI [13]. However, the regimens evaluated did not include low dose ritonavir. In the only published cohort comparing boosted double versus boosted single PI regimens of which we are aware, Loutfy *et al.* used observational data to compare virologic suppression between 154 patients treated with lopinavir and low dose ritonavir and 100 patients treated with amprenavir/lopinavir and low dose ritonavir [14]. The investigators saw no evidence that the boosted double PI regimen improved virologic efficacy.

After controlling for confounding by using traditional multivariable regression analyses, as well as three distinct marginal structural model estimators, we were unable to reject the null hypothesis that use of a boosted double versus single PI regimen resulted in an equivalent probability of virologic suppression 12–36 weeks after regimen initiation. Point estimates of effect were

reasonably consistent between estimators, ranging from a 4% to 7% higher probability of success among subjects receiving a boosted double PI regimen. The upper limits of our confidence intervals suggest that a moderate-sized benefit from use of a boosted double versus single PI regimen is possible, and might prove significant if a larger cohort were available. However, our study, to the best of our knowledge, represents the largest series yet assembled of patients receiving a boosted double PI salvage regimen.

The objective of these analyses was to compare the efficacy of two general salvage strategies, not the efficacy of specific salvage regimens. We recognize that specific boosted double PI regimens might significantly improve virologic outcome, while others might not. Contrary to expectations, we did not find that amprenavir/lopinavir regimens resulted in worse virologic outcome than seen with other boosted double PI regimens.

Limitations of our analyses include inability to control for potential confounding by drug dose, demographics, and adherence because these data were unavailable. It is possible that PI in boosted single PI regimens were administered at higher doses than PI in boosted double PI regimens. As pharmacokinetic synergy is one hypothesized mechanism for improved efficacy of at least some boosted double PI regimens, variation in dosing might mask a beneficial effect of regimens containing two boosted PI. Lower adherence to boosted double PI regimens than to boosted single PI regimens, as a result of greater pill burden or increased adverse effects, could also potentially mask a beneficial effect of regimens containing two boosted PI. However, the current analyses provide insight into the effectiveness of boosted double versus single PI regimens as used in clinical practice.

As in any observational study, it is impossible to exclude unmeasured confounding. However, we controlled for obvious major confounders, including treatment history, preexisting drug resistance, baseline and nadir CD4 lymphocyte count, baseline and peak plasma HIV RNA level, and several characteristics of the salvage regimen. Use of boosted double PI regimens was associated with more extensive treatment history, lower nadir CD4 lymphocyte count, and more extensive preexisting resistance. Although non-significant, boosted double PI regimens were also less likely to include an NNRTI drug used for the first time. These factors might decrease the likelihood of virologic success, and controlling for confounding by these factors showed a larger estimated benefit for boosted double PI regimens (4–7%, versus 0.6% prior to controlling for such confounding). However, even after controlling for these and other factors, we were still unable to reject the null hypothesis that boosted double and boosted single PI regimens result in an equivalent probability of virologic suppression.

More recent calendar date was a strong predictor of virologic success. This association remained after adjusting for baseline plasma HIV RNA level and CD4 lymphocyte count, treatment history, antiretroviral resistance, and salvage regimen characteristics. The implication is that success rates for salvage antiretroviral therapy have improved over time, even after controlling for basic shifts in the patient population and numbers of new drugs used in the regimen, suggesting that newer drugs are more effective and/or that physicians have become more skillful in constructing salvage treatment regimens.

In conclusion, our analyses show no statistically significant improvement in virologic outcome resulting from a boosted double PI salvage regimen. The observational nature of the data and moderate sample size suggest that our results be interpreted with caution. However, given its increase in pill burden, expense, and potential adverse effects, the wisdom of using a boosted double PI regimen is worth contemplation.

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