Efficacy of a Nucleoside-sparing Regimen of Darunavir/Ritonavir Plus Raltegravir in Treatment-Naïve HIV-1-infected Patients (ACTG A5262)

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Objective: To explore darunavir/ritonavir (DRV/r) plus raltegravir (RAL) combination therapy in antiretroviral-naïve patients.

Design: Phase 2b, single-arm, open-label, multicenter study

Methods: 112 antiretroviral-naïve, HIV-1-infected patients received DRV/r 800/100 mg once-daily and RAL 400 mg twice-daily. Primary endpoint was virologic failure (VF) by week 24. Virologic failure was defined as confirmed viral load (VL) $\geq$ 1000 copies/mL (c/ml) at week 12, or a > 0.5 $\log_{10}$ c/ml VL increase from week 4 to 12, or > 50 c/ml at or after week 24. Protease and integrase genes were sequenced in patients experiencing VF.

Results: VF rate was 16% (95% CI: [10%,24%]) by week 24 and 26% [19%,36%] by week 48 in an intent-to-treat analysis. Viral load at VF was 51–200 c/ml in 17/28 failures. Adjusting for age and sex, VF was associated with baseline VL >100,000 c/ml (HR 3.76, 95% CI [1.52, 9.31], $p = 0.004$) and lower CD4 cell count (0.77 per 100 cells/mm^3 increase, [0.61, 0.98], $p = 0.037$). When trough RAL concentrations were included as a time-varying covariate in the analysis, VF remained associated with baseline VL $>$ 100,000 c/ml (HR = 4.67 [1.93, 11.25], $p < 0.001$) while RAL level below detection limit in plasma at one or more previous visits was associated with increased hazard (HR = 3.42 [1.41, 8.26], $p = 0.006$). All 5 participants with integrase mutations during VF had baseline VL >100,000 c/ml.

Conclusion: DRV/r plus RAL was effective and well tolerated in most patients, but VF and integrase resistance were common, particularly in patients with baseline VL $>$ 100,000 copies/mL

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Introduction

Reverse transcriptase inhibitor (RTI)-sparing antiretroviral (ARV) regimens are needed given potential toxicities of some nucleos(t)ide RTIs (NRTI) [1] and the frequency of transmitted NRTI and non-nucleoside RTI (NNRTI) resistance [2]. Two fully active ARV drugs may be sufficient to suppress HIV-1 replication in treatment-naïve patients [3]. Darunavir (DRV) is a potent and well-tolerated protease inhibitor (PI) with no observed resistance during virologic failure (VF) when combined with two NRTI in treatment-naïve patients [4]. Raltegravir (RAL), an integrase inhibitor, is potent and well tolerated for initial therapy in combination with two NRTI, but RAL resistance emerges in approximately one-third of patients with VF [5]. AIDS Clinical Trials Group (ACTG) Study A5262 was designed to explore a two-drug, RTI-sparing regimen of darunavir/ritonavir (DRV/r) plus RAL for initial ARV therapy.

Methods

Study participants

A5262 participants were treatment-naïve, HIV-1-infected adults (≥18 years old) with plasma HIV-1 RNA concentration (viral load, VL) ≥5,000 copies/mL (c/ml). Exclusion criteria included active hepatitis B, renal failure requiring dialysis and protocol-specified abnormal laboratory values. Patients with more than 1 DRV resistance associated mutation (RAM) [V11I, V32I, L33F, I47 V, I50 V, I54L, I54 M, T74P, I84 V, and L89 V], L76 V alone, or known major integrase RAM (N155H, Q148H/R/K, Y143C/R, and G140S) were also excluded. Ethics review committees at each research site approved the study. Each participant provided a written informed consent.

Study design and interventions

Study participants received open-label DRV 800 mg (two 400 mg tablets) and ritonavir 100 mg (one capsule) daily plus RAL 400 mg (one tablet) twice daily. The site investigator was permitted to construct a new regimen if a patient experienced VF or treatment-limiting adverse effects.

Procedures and assessments

Study participants had screening, pre-entry and entry visits (day 0). Subsequent evaluations occurred at weeks 1, 4, 12, 24, 36, 48 and 52. At entry and weeks 4, 12, 24, 36, and 48, VL, hematology, liver function tests and blood chemistries were analyzed. VL was also determined at week 1 in all participants and at week 52 in participants with suspected VF at week 48. CD4+ and CD8+ T-cell counts were determined at entry and weeks 12, 24, 36 and 48. Fasting lipid levels were measured at entry and weeks 24 and 48. Adherence was assessed at weeks 1, 4, 12, 24 and 48 by self-report (number of missed doses over a 4-day recall) [6]. Plasma samples for trough concentrations (C_{trough}) of RAL and DRV were stored at each visit that had an adherence assessment. Participants with suspected VF were asked to return for a failure confirmation visit within 7–35 days of collecting the initial failure sample. At the failure confirmation visit, adherence was assessed and samples collected for VL, protease and integrase genotype, T-cell counts, and C_{trough}.

VL was measured centrally using the Abbott RealTime HIV-1 Test on the m2000 system (Abbott RT/m2000 assay) [Abbott Molecular Inc. Des Plaines, Illinois, USA]. Population sequencing of the integrase and protease genes was performed during VF. Genotypic mutations according to the 2009 International AIDS Society (IAS)-USA [7] plus G140S for integrase were considered. DRV and RAL plasma concentrations were determined using internally and externally validated mass spectrometry (RAL) and ultra-performance liquid chromatography (DRV) methods. All inter- and intraday variability was <10%; the lower limits of detection for RAL and DRV were 10 and 50 ng/mL, respectively.

An independent Study Monitoring Committee reviewed study conduct, safety and efficacy approximately 24 weeks after enrollment of the 40th participant. To assess the impact of assay variation on VL determinations, stored plasma samples from the first ten patients with low-level viremia [VL 51- 200 c/ml] during VF were retested post-hoc using the Roche Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostic Systems, Branchburg, New Jersey, USA).

Outcome measures

The primary endpoint was VF prior to or at week 24. VF was defined as confirmed VL ≥1000 c/ml at week 12, or a >0.5 $\log_{10}$ c/ml increase in VL from week 4 to week 12 (including rebound to >50 c/ml from week 4 to week 12 for patients with week 4 value ≤50 c/ml), or confirmed VL >50 c/ml at or after week 24. Secondary outcome measures included VF through week 48, VL < 50 c/ml or < 200 c/ml at weeks 24 and 48, incidence of adverse events that were at least Grade 3 (severe) or any grade if it led to permanent drug discontinuation, changes in fasting lipid concentrations, protease or integrase inhibitor resistance during VF, adherence to study treatment, C_{trough} of RAL and DRV, and changes in CD4+ T cell counts (CD4 count).

Statistical analysis

For the primary analysis, the cumulative proportion of participants experiencing VF at or before week 24 and corresponding two-sided 95% confidence interval (CI) were estimated using the Kaplan-Meier method and Greenwood's formula [8]. The primary efficacy analysis used the intent-to-treat (ITT) approach, including all treated patients regardless of study treatment modification

and censoring follow-up if a participant was lost to follow-up or died without previously meeting the definition of VF. Enrolled participants who never started the study treatment were excluded. Log-rank test and Cox proportional hazards regression were used to assess predictors of VF.

Acceptable VF rate was estimated using week 24 VF rates observed in large phase III trials of current preferred regimens: efavirenz plus two NRTI arm of ACTG A5142 (22%) [3]; efavirenz plus two NRTI arm of ACTG A5095 (17%) [9]; and tenofovir/emtricitabine (TDF/FTC) plus RAL (10%-15%) [10]. It was pre-specified that RAL plus DRV/r would be considered satisfactory if the upper bound of the CI for cumulative VF at week 24 was <35%. With a sample size of 100 evaluable participants, the study was estimated to have 91% probability that the upper bound of the two-sided 95% CI for the VF rate would be <35% if the underlying true failure rate was 20%. The planned sample size was increased by 10% to 111 participants to account for loss to follow-up.

Proportions of participants with VL < 200 c/ml and < 50 c/ml at weeks 24 and 48 were estimated using ITT (missing/off study without VF ignored) and modified ITT (missing/off treatment considered failure) approaches.

Adherence was classified as perfect (zero reported missed doses) or imperfect (any missed doses of DRV/r or RAL, failure to answer the 4-day recall question, or missed the study visit). Pharmacokinetic (PK) analyses included geometric mean of trough concentrations ($C_{trough(avg)}$) obtained within a defined window (within 9–15 and 20–28 hours after the last dose for RAL and DRV, respectively). Only C_{trough} up to and including the time of VF confirmation visit was included for participants with VF. C_{trough} below the assay detection limits was replaced with half of the corresponding lower limit of quantification. Sensitivity analysis examined $C_{trough(avg)}$ using all available C_{trough} values. Adverse events were graded according to the severity scale of the Division of AIDS, National Institutes of Health [11]. Safety analyses used as-treated approach, including all patients who initiated study treatment and censoring follow-up at treatment discontinuation.

Baseline VL level and CD4 count were calculated as the geometric and arithmetic means, respectively, of pre-entry and entry evaluations. Changes in continuous measures from baseline were assessed by Wilcoxon signed rank test. Comparisons between participants with and without VF used Wilcoxon rank sum test for continuous measures, Fisher's exact test for binary measures and Cochran-Armitage test for ordered categorical measures.

All p-values and CIs presented were two-sided and nominal, unadjusted for interim analysis and multiple comparisons. Analyses were done by using SAS, version 9.2 (SAS Institute, Cary, North Carolina), StatXact 8 PROCs (Cytel, Cambridge, Massachusetts) and Splus, version 6 (Insightful, Seattle, Washington).

Results

Study participants

A total of 113 patients were enrolled at 22 sites in the US. One participant did not initiate study drugs and was taken off study. The 112 participants who initiated DRV/r plus RAL had a median age of 36 years, 88% were male, and 44% were white non-Hispanic. Median CD4 count and VL were 271 cells/mm^3 and 4.87 $\log_{10}$ c/ml, respectively (Table 1). Forty-nine (44%) participants had baseline VL > 100,000 c/ml including 6 (5%) with levels > 750,000 c/ml. Pretreatment ARV drug resistance was detected in 21 (19%) participants: 9 (8%) NNRTI, 8 (7%) NRTI, 2 (2%) PI, 1 (1%) NRTI plus NNRTI, and 1 (1%) NRTI plus NNRTI and PI mutations. No participant had a DRV RAM. Ninety-seven (87%) participants completed 52 weeks follow-up. Fifteen (13%) participants discontinued participation due to inability to get to clinic (7), inability of study staff to reach participant (4), withdrawal of consent (2), unwillingness to adhere to study requirements (1), and death (1).

Efficacy

Seventeen participants (16%, 95% CI: [10%, 24%]) experienced VF by week 24: 11 failed to suppress VL (one with >1000 c/ml at week 12; 10 with >50 c/ml at week 24) and 6 due to viral rebound. Eleven participants experienced VF (due to VL rebound to > 50 c/ml) after week 24. Thus, VF occurred in 28 participants by week 48 (Table 2); VF rate by week 48 was 26% [19%, 36%]. Three participants with VF subsequently attained VL <50 c/ml without changing therapy. In ITT analysis, VL was < 50 c/ml in 79% [70%,86%] of participants at week 24 and in 71% [61%,79%] at week 48; using modified ITT analysis, VL was < 50 c/ml in 74% [66%,82%] of participants at week 24 and in 61% [52%,70%] at week 48 (Fig. 1). VL < 200 c/ml was achieved by 93% [87%,97%] at week 24 and 86% [78%,92%] at week 48 in ITT analysis and by 88% [81%,94%] and 73% [65%,81%] at week 24 and 48 in the modified ITT analysis.

Participants with VF had higher baseline VL (median 5.22 vs. 4.70 $\log_{10}$ c/ml, $p = 0.002$) and lower baseline CD4 count (192 vs. 322 cells/mm^3, $p = 0.007$) compared to those who did not experience VF (Table 1). Of the 28 participants with VF, 21 had baseline VL >100,000 c/ml and these patients had more rapid time to VF ($p<0.001$) (Fig. 2). Multivariable model for time to VF adjusting for age and sex, demonstrated that VF was associated with baseline VL > 100,000 c/ml (hazard ratio (HR) = 3.76, 95% CI: [1.52, 9.31], $p = 0.004$) and lower CD4 count

Table 1. Baseline Characteristics of participants who initiated DRV/r plus RAL (n = 112) by virologic failure status.

Characteristic	Total	Virologic Failure (VF)Status		P-Value
		With VF	Without VF	
Age (in years)				
N	112	28	84	0.835*
Mean (s.d.)	37 (11)	37 (13)	37 (11)	
Median (Q1, Q3)	36 (27, 45)	35 (26, 44)	36 (28, 45)	
18–29	33 (29%)	9 (32%)	24 (29%)	0.760***
30–39	38 (34%)	7 (25%)	31 (37%)	
40–49	25 (22%)	7 (25%)	18 (21%)	
50–59	12 (11%)	4 (14%)	8 (10%)	
≥60	4 (4%)	1 (4%)	3 (4%)	
Sex				
Male	98 (88%)	25 (89%)	73 (87%)	1.000**
Female	14 (13%)	3 (11%)	11 (13%)	
Race/Ethnicity				
White Non-Hispanic	49 (44%)	10 (36%)	39 (46%)	0.618**
Black Non-Hispanic	45 (40%)	14 (50%)	31 (37%)	
Hispanic (Regardless of Race)	16 (14%)	4 (14%)	12 (14%)	
Asian, Pacific Islander	2 (2%)	0 (0%)	2 (2%)	
IV drug history				
Never	102 (91%)	26 (93%)	76 (90%)	0.194**
Currently	1 (1%)	1 (4%)	0 (0%)	
Previously	9 (8%)	1 (4%)	8 (10%)	
Baseline CD4 (cells/mm^3)				
Mean (s.d.)	284 (199)	199 (167)	312 (202)	0.007*
Median (Q1, Q3)	271 (107, 419)	192 (51, 310)	322 (147, 442)	
< 200	40 (36%)	15 (54%)	25 (30%)	0.018***
200 – < 350	32 (29%)	8 (29%)	24 (29%)	
350 – < 500	26 (23%)	3 (11%)	23 (27%)	
≥500	14 (13%)	2 (7%)	12 (14%)	
Baseline HIV-1 RNA (log10 c/mL)				
Mean (s.d.)	4.83 (0.60)	5.14 (0.61)	4.73 (0.57)	0.002*
Median (Q1, Q3)	4.87 (4.33, 5.31)	5.22 (4.85, 5.60)	4.70 (4.31, 5.16)	
Baseline HIV-1 RNA (c/mL)				
≤5000	3 (3%)	1 (4%)	2 (2%)	0.006***
5001 - ≤10000	5 (4%)	1 (4%)	4 (5%)	
10001 - ≤100000	55 (49%)	5 (18%)	50 (60%)	
100001 - ≤750000	43 (38%)	18 (64%)	25 (30%)	
>750000	6 (5%)	3 (11%)	3 (4%)	

*Exact Wilcoxon Test **Fisher's Exact Test ***Cochran-Armitage Test

(HR = 0.77 [0.61, 0.98] per 100 cell/mm^3 increase, p = 0.037). Seventeen (15%) participants were classified as having imperfect adherence. No association was detected between VF and adherence, age, sex, intravenous drug use, race/ethnicity, or presence of any mutation at baseline (all $p > 0.10$).

Seventeen participants with VF (61%) had VL of 51–200 c/ml at the first VL determination during VF, 4 (14%) had 201–1000 c/ml and 7 (25%) had >1000 c/ml. VL levels in the first ten patients with low-level viremia during VF were similar when determined using the Roche HIV Monitor v1.5 and Abbott RT/m2000 assays. Baseline CD4 count, VL, resistance, adherence, and detection of mutations at time of VF were not significantly different between participants who failed with VL >200 c/ml versus 51–200 c/ml (all $p \geq 0.40$). Participants with VF as a result of failure to suppress viremia versus viral rebounders were similar with respect to baseline characteristics (age, sex, race/ethnicity, IV drug use, CD4 count and VL) and RAL and DRV trough concentrations/detectability, adherence (all $p > 0.10$).

HIV-1 drug resistance

Integrase resistance testing was successful in 25 of the 28 virologic failures. All 5 participants with evidence of integrase RAMs [N155H (1), N155H/N (2), Q148Q/R and N155H/N (1), Q148K/Q and N155H/N (1)] had baseline VL > 100,000 c/ml. None of these patients had documented treatment interruption. No new PI RAMs were detected in the 23 participants with successful protease sequencing following VF.

Pharmacokinetics

Considering the defined trough period, median (Quartile1, Quartile3) $C_{trough(avg)}$ was 1218 (789, 1809) ng/mL for DRV and 117 (52, 250) ng/mL for RAL (Supplemental Table 1). DRV and RAL $C_{trough(avg)}$ values within the defined trough period were not significantly different for patients with and without VF, perfect versus imperfect

Table 2. Description of Participants who Experienced Virologic Failure on DRV/r plus RAL.

Initial VF HIV-1 RNA	ID Number	Age	Sex	Baseline HIV-1 RNA (copies/mL)	Baseline CD4 count (cells/mm^3)	Baseline Mutation(s)	Week of initial VF	Initial VF HIV-1 RNA (copies/mL)	Confirmed HIV-1 RNA (copies/mL)	Integrase Mutation(s) Detected at or after VF*
≤ 200 copies/mL	1	52	M	230,627	28		12	173	259	Q148Q/R, N155H/N
	2	25	M	28,879	262		23	161	142	amplification failure
	3	48	M	194,888	47		23	160	258	WT
	4	42	M	50,309	327	D67G, K219Q, T69ins	24	81	88	WT
	5	27	M	514,062	208	K103N	24	93	101	WT
	6	32	M	19,115	240		24	79	26,849	WT
	7	58	M	17,593	455		24	53	71	WT
	8	43	M	419,417	202		24	190	158	WT
	9	57	M	147,076	113	M41L	24	59	89	N155H/N
	10	26	M	143,422	74		24	108	73	WT
	11	21	M	215,391	334		35	189	830	WT
	12	31	M	378,540	185		36	53	88	amplification failure
	13	40	M	100,866	280		36	157	250	WT
	14	42	M	140,031	36		48	134	1,664	WT
	15	38	M	184,212	86		49	62	89	Q148K/Q, N155H/N
	16	23	M	137,659	415		50	65	.	WT
	17	63	F	851,765	198		50	66	89	amplification failure
> 200 copies/mL	18	25	F	313,848	60		13	80,073	72,137	WT
	19	32	M	4,682	564		13	3,141	.	WT
	20	44	F	825,792	98		22	584,879	2,147	WT
	21	59	M	911,043	46		24	3,893	685	N155H
	22	35	M	630,065	9		24	243	113	WT
	23	34	M	246,270	18		24	231	188	N155H/N
	24	34	M	429,729	20	T69D, K103N	24	465	411	WT
	25	44	M	133,415	55		36	178,867	3,485	WT
	26	19	M	8,576	575		36	8,466	73	WT
	27	24	M	124,901	355		43	649	.	WT
	28	21	M	29,677	293		46	1,364	.	WT

*WT, Wild Type.

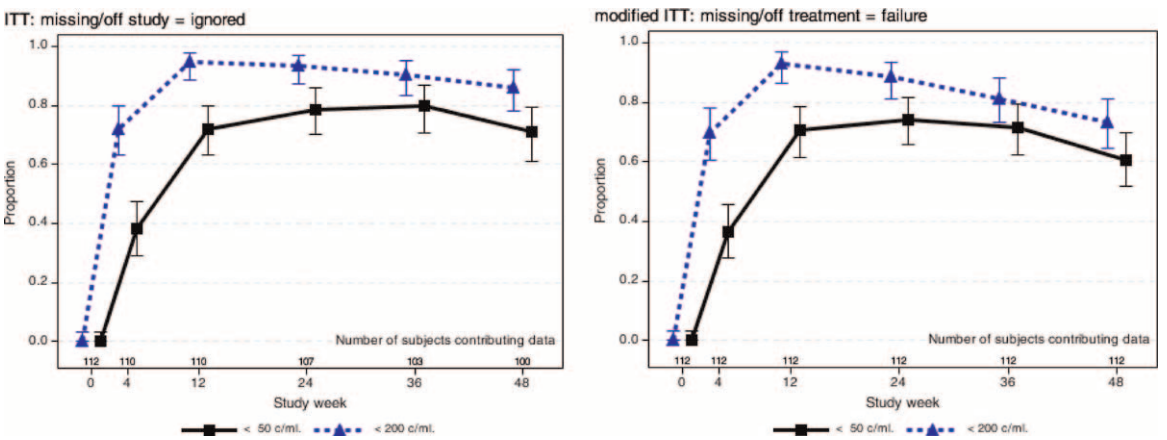

Fig. 1. Proportion of Participants with HIV-1 RNA level < 50 and < 200 copies/ml.

adherence, VL >200 versus 51 to 200 c/ml at time of VF, and presence or absence of resistance mutations at VF (all $p > 0.10$). Similar results were obtained in sensitivity analyses that included all available DRV and RAL C_{trough} except for DRV $C_{trough(avg)}$ which was lower in those with VF (1042 vs. 1649 ng/mL, $p = 0.017$). For RAL, at least one C_{trough} below detection limits (BDL) occurred in 10/27 (37%) participants with VF compared to 7/76 (9%) non VF participants ($p = 0.002$); for DRV, 5/21 (24%) VF participants had levels BDL compared to 2/62 (3%) non VF participants ($p = 0.01$) (Table 3). When adjusted for DRV or RAL C_{trough} (either a continuous or a categorical variable [BDL or not]) as a time-varying covariate with one-study-visit lag (at the visit immediately before or at all previous visits) in Cox PH models, baseline VL > 100,000 c/ml remained significantly associated with hazard of VF ($p < 0.05$). More specifically, when RAL C_{trough} was evaluated as a categorical variable, having RAL C_{trough} BDL within the defined trough period at the visit immediately before was associated with increased hazard of VF (HR = 5.25 [1.41, 19.58], $p = 0.014$) and the HR was 5.05 [1.64, 15.56] ($p = 0.005$) for baseline VL; the HR for having RAL C_{trough} BDL at one or more previous visits was 3.42 [1.41, 8.26] ($p = 0.006$) and 4.67 [1.93, 11.25] ($p < 0.001$) for baseline VL. Marginally significant association was found

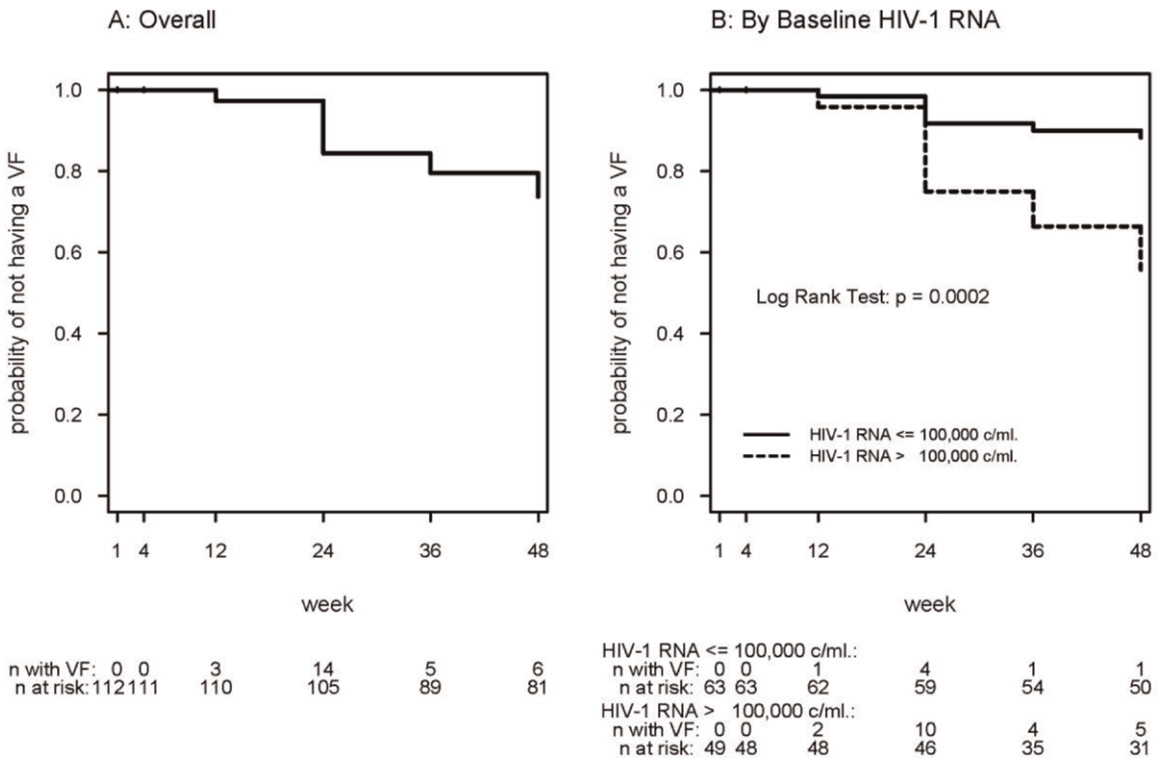

Fig. 2. Kaplan-Meier Plots of Time to Virologic Failure Using ITT Approach.

Table 3. Trough Concentration (Categorical) by Virologic Failure Status.∗∗

Drug	Virologic Failure (VF) Status		Total	P-Value∗
	With VF	Without VF		
RAL (Within True Trough Time)				
All Above Detection	17 (63%)	69 (91%)	86 (83%)	0.002
With ≥1 conc. BDL ∗∗∗	10 (37%)	7 (9%)	17 (17%)	
Missing	1	8	9	
RAL (All Available)				
All Above Detection	15 (54%)	70 (83%)	85 (76%)	0.004
With ≥1 conc. BDL	13 (46%)	14 (17%)	27 (24%)	
DRV (Within True Trough Time)				
All Above Detection	16 (76%)	60 (97%)	76 (92%)	0.010
With ≥1 conc. BDL	5 (24%)	2 (3%)	7 (8%)	
Missing	7	22	29	
DRV (All Available)				
All Above Detection	19 (68%)	77 (92%)	96 (86%)	0.004
With ≥1 conc. BDL	9 (32%)	7 (8%)	16 (14%)	

∗Fisher's Exact Test. ∗∗ For VF subjects, only those concentrations on or before VF confirmation were considered. ∗∗∗BDL, Below Detection Limit.

between VF and a DRV C_{trough} BDL within the defined trough window at the visit immediately before (HR = 4.28 [0.92, 20.04], p = 0.065; baseline VL HR = 3.75 [1.13, 12.41], p = 0.030). The association became significant when all DRV C_{tough} were included (HR = 3.89 [1.32, 11.49], p = 0.014; baseline VL HR = 4.34 [1.73, 10.86], p = 0.002).

Immunological outcomes

The median CD4 count increase from baseline was 142 (80, 196) cells/mm^3 at week 24 and 200 (114, 318) cells/mm^3 at week 48 (all $p < 0.001$) and were similar at week 24 in patients with baseline VL ≤ or >100,000 c/ml ($p > 0.1$). At week 48 the median increase was 233 cells/mm^3 in patients with baseline VL >100,000 c/ml versus 180 cells/mm^3 in those with ≤ 100,000 c/ml ($p = 0.044$).

Safety and tolerability

Twenty-one participants (19%) reported at least one Grade 3 (severe) or higher clinical or laboratory adverse events, 5 of which were classified as possibly related to study treatment: dyslipidemia (3), diabetes mellitus (1) and elevated aspartate transaminase /alanine transaminase (1). No events were considered probably or definitely related to study treatment. One participant permanently discontinued study treatment due to Grade 2 (moderate) maculopapular rash and abdominal pain. Death occurred in one patient at week 9 from cryptosporidiosis.

Median increases in fasting high-density lipoprotein (HDL), low-density lipoprotein, total cholesterol and triglycerides from baseline to week 48 were 9, 17, 30 and 23 mg/dL, respectively ($p < 0.001$, except triglycerides $p = 0.006$). Fasting total cholesterol:HDL ratio did not change significantly from baseline (0.40 at weeks 24 and 48).

Discussion

In HIV-1-infected treatment-naïve participants enrolled in A5262, an RTI-free, two-drug regimen comprising DRV/r plus RAL met the protocol definition of acceptable virologic efficacy (at week 24), but only 71% and 61% of participants had VL < 50 c/ml (86% and 73% < 200 c/ml) at week 48 in ITT and modified ITT analyses, respectively. Baseline VL >100,000 c/ml was associated with increased risk of VF. Baseline CD4 count per 100 cell increase was associated with reduced risk of VF. In multivariable models fitted with DRV or RAL C_{trough} BDL, baseline VL >100,000 c/ml remained strongly associated with increased risk of VF. Having RAL C_{trough} BDL at the visit immediately before or at one or more previous visits was also associated with an increased hazard of failure.

Potential explanations for our findings were explored. Self-reported adherence (4-day recall) was not significantly different between those with and without VF, or between those with baseline VL ≤ or >100,000 c/ml. However, having one or more DRV and RAL plasma concentrations below detection limits was significantly more common in those with VF, possibly related to unreported suboptimal adherence. Other investigators have demonstrated discordance between self-reported adherence and objectively measured adherence [12]. Adverse effects of therapy are unlikely to have been the major determinant of adherence or virologic efficacy as RAL and DRV were well tolerated. An alternative hypothesis is that asymmetrical dosing of DRV/r (once-daily) and RAL (twice-daily) predisposed to suboptimal adherence and VF, but such association has not emerged as a concern with RAL twice-daily plus TDF/FTC, a similarly asymmetrically dosed regimen [13]. It was suggested recently that RAL-DRV interactions may lower plasma concentrations of DRV [14], but DRV

C_{trough} observed in this study (Supplemental Table 1) are within the range reported in an intensive PK study of DRV 800/100 mg daily [15]. Finally, since over half of the patients who experienced VF had low-level viremia (51-200 c/ml) at the time of failure, we considered VF artifacts due to assay variability [16]. This possibility was excluded because VL determinations during low-level viremia were similar with the Abbott m2000 and the Roche Amplicor Ultrasensitive Assays in the first 10 participants with low-level VF.

An association between efficacy and baseline VL has been demonstrated with other ARV regimens. In some but not all studies of two NRTIs plus a third preferred agent, smaller proportions of patients with baseline VL > 100,000 c/ml achieved HIV RNA < 50 c/mL at 48 weeks [5,13,17–21] but these differences tend to be small, are in part related to tolerability and associated low CD4 count [16], and may not be synonymous with subsequent VF [22]. In our RTI-sparing study in which we specifically examined VF, as opposed to a combined endpoint, the differences in virologic outcomes between the high and low VL strata were striking and the results were consistent or even more evident in multivariable analyses that included baseline CD4 count or assessments of drug concentrations. A pilot study evaluating twice-daily atazanavir (ATV) plus RAL was prematurely terminated at week 24 due in part to adverse events and frequent RAL resistance in those with VF [23]. A larger randomized study, however, found no significant difference in VL < 40 c/ml at week 48 in patients treated with RAL plus lopinavir/ritonavir (LPV/r) compared to TDF/FTC plus LPV/r [24]. The mean baseline VL in the latter study was 4.25 $\log_{10}$ c/ml, which is lower than the 4.9 $\log_{10}$ c/ml in the twice-daily RAL plus ATV study and 4.83 $\log_{10}$ c/ml in our study. Our study is also the first to report virologic outcomes by baseline VL $\leq$ or > 100,000 c/ml separately from non-virologic treatment discontinuations, further limiting cross-study comparisons. The mechanisms underlying the poorer virologic outcomes in some patients with high baseline VL, as observed in this study, are uncertain. One possibility is that high baseline VL may be associated with more extensive reservoir of infected cells and prolonged viral decay time to levels below 50 c/mL. However, only one of the 28 virologic failures in the current study had viral dynamics that may be explained solely by this specific hypothesis. Another possibility is that high baseline VL may predispose to greater diversity of HIV-1 quasispecies and an increased opportunity to select drug resistant mutants. Q148R minority variants were detected at very low levels (median 0.46%) in 86% of treatment-naïve patients in one study [25]. The effect of pre-therapy RAL-resistant minority variants on virologic outcome in treatment-experienced patients has not been clearly demonstrated [25,26] and to our knowledge has not yet been reported in treatment-naïve patients.

Baseline VL > 100,000 c/ml appears to increase the risk of RAL resistance in patients receiving DRV/r plus RAL. All the patients with evidence of RAL resistance mutations at VF (twenty percent of those genotyped) had baseline VL > 100,000 c/ml. None of these patients had documented treatment interruption, and no significant difference in RAL C_{trough} was observed between those with or without RAL resistance. VL at the time of integrase genotyping in the 5 participants ranged from 62 to 685 c/ml. Notably, a participant who achieved HIV RNA < 50 c/ml at week 12 and had no subsequent documentation of VL level > 100 c/ml experienced VF at week 48 with detection of Q148K/Q and N155H/N. Thus like NNRTI and NRTI resistance mutations [27], RAL resistance mutations may be present during low-level viremia [28] an important observation since recent guidelines state that VL > 200 c/ml can be considered the threshold for VF in clinical practice [1]. Protease inhibitor resistance was not detected in any participant experiencing VF, consistent with evidence that boosted PI resistance seldom develops early in VF [29].

Interpretation of this study should take into account its single-arm design as a randomized trial could have reduced the potential impact of patient characteristics and other variables. Also, patients were not screened for pretreatment RAL resistance but primary mutations that confer resistance to RAL are uncommon in RAL-naïve patents [30]. Despite these limitations, the results of A5262 raise important issues that should be examined carefully in future clinical trials evaluating DRV/r plus RAL and perhaps in all RTI-sparing two-drug regimen trials. We urge caution in patients with baseline VL >100,000 c/ml and emphasize a need to further elucidate the implications of low-level viremia in patients receiving the regimen.

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A5262 is registered with ClinicalTrials.gov (NCT00830804).

Conflicts of interest

B.T. has served as an advisor and received research support and honoraria from Tibotec. E.P.A. has served as a consultant to Tibotec and Merck. D.R.K. is a consultant to Merck and has received honoraria and research support from the company. J.J.E is a consultant to Abbott, GlaxoSmithKline, Merck, ViiV and Tibotec, and has received research support (to UNC) from GlaxoSmithKline and Merck.

Author Contribution(s): B.T. and J.J.E. conceived the study. D.R.K was the protocol virologist and EPA the protocol pharmacologist. Substantial contributions to study design and interpretation of the data were made by B.T., L.Z., R.M.M., D.R.K., C.C.W., E.P.A., P.S.K., and J.J.E. L.Z., and R.M.M. analyzed the data. S.G. performed viral resistance assays. B.I.B. was the Field Representative and B.B. was the Clinical Trials Specialist. All authors had full access to the data and vouch for the accuracy and completeness of the data and analyses. Initial drafts of the manuscript written by B.T., L.Z., R.M.M. and J.J.E. were reviewed, edited and approved by all of the authors.

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