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Lopinavir/ritonavir + tenofovir Dual Therapy *versus* Lopinavir/ritonavir-Based Triple Therapy in HIV-Infected Antiretroviral Naïve Subjects: The Kalead Study

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Abstract

Purpose: With reference to the clinical need for simple, potent and safe antiretroviral regimens, Lopinavir/ritonavir + tenofovir (LPV/r+TDF) two-drug initial regimen was studied for efficacy and safety in HIV-infected patients. **Methods:** Kalead was a prospective, randomized, open-label, 72-week trial comparing LPV/r+TDF versus LPV/r + two (non-TDF) NRTIs in HIV-infected adults with HIV-RNA >400 copies/mL and any CD4 count. Primary endpoint was the proportion of subjects with HIV-RNA <50 copies/mL at week 72.

Results: 152 subjects were randomized. Eleven (15.3%) subjects in the dual therapy arm and seven (8.8%) in the triple therapy arm who did not achieve HIV-RNA <50 copies/mL at least twice prior to and including week 24 were discontinued per protocol (p=0.21). Overall discontinuations were 41.7% and 43.8% in the dual therapy and triple therapyarms. At week 72, 51.4% and 52.5% of subjects in the dual therapy and triple therapy arms had HIV-RNA <50 copies/mL (p=0.89, ITT, NC=F). In an on-treatment analysis, 87.2% and 93.0% of subjects in the dual therapy and triple therapy arms had a HIV-RNA <50 copies/mL (p=0.47). Over 72 weeks of therapy, mean CD4 count increases were greater in the dual therapy arm (+332 cells/mm^3 vs +234 cells/mm^3, p=0.01). Adherence, overall incidence of adverse events, drug-related adverse events, and Grade I-IV laboratory abnormalities were comparable between the two arms.

Conclusions: A two-drug regimen of LPV/r+TDF suggests sufficient safety and efficacy warranting further investigation. However, high discontinuation rate and study design limitations restrict overall interpretation.

Keywords: Lopinavir; Tenofovir; NRTI; Dual therapy; Protease inhibitors; ARV therapy

Introduction

Use of combination antiretroviral (ARV) therapy (cART), also referred to as highly active ARV therapy (HAART), has resulted in a marked improvement in the prognosis of HIV disease [1-3]. In HIV-infected patients naïve to ARV therapy, treatment guidelines recommend three drug regimens, most often including a boosted protease inhibitor (PI/r) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) combined with two nucleoside reverse transcriptase inhibitors (NRTIs) [4-6]. Current therapies require lifelong treatment which can be associated with significant toxicity and economic cost. In some instances, the use of cART may be restricted by contraindications, drug resistance, or limited access. There is a need for simple treatment options which provide sustained potency, limited toxicity, and a high genetic barrier to development of resistance. Additionally, options which have the potential to reduce cost of treatment are needed.

Lopinavir/ritonavir (LPV/r) taken once or twice daily (BID) in combination with two NRTIs is a recommended option for initial treatment of HIV, according to many treatment guidelines [4-6]. Kalead is a study of the combination of LPV/r and tenofovir disoproxyl fumarate (TDF). TDF was chosen as a dual therapy therapy partner for LPV/r due to its good tolerability, once-daily dosing (QD), and low level of resistance development in subjects receiving their initial cART regimen [7].

Materials and Methods

Subjects

Subjects were recruited from 16 clinics in Italy. Inclusion criteria included HIV-1 infection diagnosis, age ≥ 18 years, no previous ARV therapy, HIV-1 RNA >400 copies/mL, and need to start cART in accordance with existing treatment guidelines and treating physician recommendation. Exclusion criteria included acute illness, history of psychiatric illness, medications incompatible with study drugs, acute HIV seroconversion, pregnancy, breast-feeding, recent substance abuse history, and selected hematology or blood chemistry abnormalities.

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The study protocol was approved by the institutional ethics committee of each participating site. All subjects provided written informed consent prior to any study-related procedure. The study is registered at https://oss-sper-clin.agenziafarmaco.it/ (EudraCT number 2004-000786-35) and at www.clinicaltrials.gov Database (NCT number 00234910). The study was sponsored by Abbott Laboratories.

Randomization and study design

Subjects were randomly assigned (1:1) to LPV/r soft-gel capsules (SGC) 400/100 mg BID + TDF 300 mg QD (dual therapy arm) or LPV/r SGC 400/100 mg BID + 2 investigator-chosen NRTIs (triple therapy arm) as SOC. As TDF had not yet been accepted in the Italian treatment guidelines as a standard component of initial cART regimens at the start of this study, it was not allowed in the triple therapy arm. Subjects were stratified at randomization by baseline HIV-1 RNA (>100,000 or ≤ 100,000 copies/mL). The first 24 weeks of the study aimed to ascertain initial virologic potency of the two-drug-arm. In order to continue in the trial beyond week 24, subjects in both arms were required to be virologically suppressed defined as 2 consecutive plasma HIV-1 RNA values <50 copies/mL prior to or including week 24 (i.e., at weeks 4 and 12, or at weeks 12 and 24). Subjects who did not meet the definition of viral suppression by week 24 were discontinued from the trial at week 24.

Subjects were evaluated at screening, baseline, weeks 4, 12, and 24, and every 8 weeks thereafter. All subjects were monitored for adverse events (AE) at each visit. Additional laboratory tests including; quantitative plasma HIV-1 RNA, CD4 cell count, complete blood count, and blood chemistry (fasting) were performed using each site's individual clinical laboratory. Adherence was assessed by pill count at each visit and by a validated Self-Administered Questionnaire developed by Antinori et al. [8-9] at weeks 4, 24, 32, 48 and 72. Quality of life was assessed by the Medical Outcomes Study HIV (MOS-HIV) questionnaire at baseline and weeks 24, 48 and 72.

Study endpoints

The primary efficacy endpoint was the proportion of subjects with plasma HIV-1 RNA levels <50 copies/mL at the end of the study (week 72) by intent-to-treat, non-completer = failure (ITT NC=F) analysis. Subjects who discontinued before week 72, or those with missing HIV-1 RNA values at week 72, were considered virologic failures.

Immunological efficacy was assessed through evaluation of CD4 cell count change from baseline values. Safety was assessed by AE monitoring, and routine assessment of hematology, clinical chemistry, urinalysis, vital signs and physical examinations. Specific safety criteria were included to assess metabolic (fasting total cholesterol, HDL/LDL-cholesterol, triglycerides, and glucose levels) and renal toxicity (creatinine, calculated creatinine clearance by Cockroft-Gault formula, and serum phosphate levels). Baseline HIV-1 resistance testing was not performed.

Further laboratory analyses

Sites equipped to store samples performed additional collection of plasma and PMBC's at all visits. Further, centralized laboratory testing was performed on these samples for subjects with protocol-defined virologic failure or low-level viremia. LPV/r single-point concentration in plasma was measured in random samples drawn prior to morning dose administration of study drugs by an Elisa assay (http://www.biostrands.com/); and detection of genotypic resistance mutations in protease and reverse transcriptase were obtained by viral population sequencing for resistance testing. Plasma samples with low viremia (HIV-1 RNA between 200-1000 copies/mL) were concentrated by centrifugation in order to increase the PCR amplification sensitivity.

Sample size

At least 49 evaluable subjects per treatment arm were needed to test the non-inferiority assumption of the dual therapy arm versus the triple therapy arm, with a Type I (alpha) error rate of 5% (two-sided) and 80% power. Treatment regimens were compared by calculation of the difference in proportions and 95% confidence interval (CI) of subjects with plasma HIV-1 RNA < 50 copies/mL at week 72, with the lower limit of a two-sided 95% CI above -10% (clinically non-inferior), and a conservative estimate of 85% versus 75% as the efficacy proportion for two- and three-drug regimens, respectively [10-11].

Based on prior data, approximately 20% of enrolled subjects were assumed to be discontinued at week 24, due to failure to achieve virologic suppression (HIV-1 RNA < 50 copies/mL). It was expected that an additional 15% of subjects would discontinue for various reasons during the study period, including AEs [12]. Therefore, at least 73 subjects were to be enrolled per treatment arm.

Statistical analyses

For the primary efficacy endpoint the estimate of the proportion of subjects with HIV-1 RNA levels <50 copies/mL was provided for each treatment arm, with the corresponding two-sided 95% CI for the difference in proportions (two-drug-arm minus three-drug-arm), based on the normal approximation to the binomial distribution. If the lower limit of the confidence interval was above -10%, the two-drug arm was considered non-inferior to the three-drug arm. Differences between randomized treatment arms were assessed using Chi-Square test.

Differences in CD4 cell count changes from baseline between treatment arms were assessed by ANCOVA. Differences in CD4 cell count changes from baseline between subgroups of subjects with baseline CD4 cell count >200 cells/mm^3 or ≤200 cells/mm^3 were assessed by ANOVA. The slopes of HIV-1 RNA decrease and CD4 cell count increase were analyzed by restricted maximum likelihood (REML)-based repeated measures approach. The repeated measures ANCOVA model included the HIV-1 RNA values or CD4 cell count at each visit as the response variable, treatment and visit as the fixed factors, and subject (within-treatment) as the random factor and the interactions terms. This model also included the continuous fixed covariate of HIV-1 RNA or CD4 cell count at baseline. No other adjustments for covariates were used in any analyses.

The significance level for all the statistical analyses was set at 0.05 (two-tailed). Imputation of missing data was restricted to the analysis of the primary outcome (i.e., ITT NC=F).

Populations analyzed

All efficacy and safety analyses were based on the intention-to-treat population comprising all those subjects who received at least one dose of study drug (ITT, NC=F "noncompleter = failure" analysis). The primary efficacy variable was also analyzed for the on-treatment (Per-Protocol – Completers-Compliers) population. The OT (PP-CC) population, defined prior to formal analysis, included subjects who completed the study without significant protocol violations/deviations.

Results

Subject disposition and baseline characteristics

167 subjects were screened for inclusion in the study between January 2005 and January 2006. 72 subjects were randomized to the dual therapy arm and 80 to the triple therapy arm (Figure 1); the slight imbalance is due to randomization in blocks of four. The treatment arms were balanced with regard to demographic and baseline disease characteristics with the exception of CD4 cell count. Randomization with regards to absolute CD4 count was somewhat imbalanced with the dual therapy arm having a higher mean CD4 cell count compared to the triple therapy arm. However, there was no difference between the arms with respect to the proportion of subjects with CD4 cell count ≤ 200/mm^3 (Table 1).

The investigator-selected (non-TDF) NRTIs in the triple therapy arm were lamivudine/zidovudine (3TC/AZT; 60% [48/80]), lamivudine/abacavir (3TC/ABC; 27.5% [22/80]), and other dual combination NRTIs (12.5% [10/80]). As a whole, 97.5% (78/80) of the subjects in the triple therapy arm received 3TC or FTC.

The overall discontinuation rates were 41.7% (95% CI [30.31, 53.09]) and 43.8% (95% CI [32.93, 54.67]) in the dual therapy and triple therapy arms, respectively (Figure 1). Eleven (15.3%, 95%CI [6.98; 23.62]) subjects in the dual therapy arm and 17 (21.3%, 95%CI [12.33, 30.27]) in the triple therapy arm discontinued the study during the first 24 weeks: the reasons for discontinuation are detailed in Figure 1. Additionally, 11 (15.3%, 95% CI [6.98, 23.62]) subjects in the dual therapy arm and seven (8.8%, 95% CI [2.59, 15.01]) subjects in the triple therapy arm were not allowed to continue past week 24, having not achieved the study defined definition of viral suppression which was required to continue in the trial past week 24 (two HIV-1 RNA values <50 copies/mL prior to week 24). From week 24 through week 72, 8 (11.1%, 95% CI [3.84; 18.36]) subjects in the dual therapy arm and 11 (13.8%, 95%CI [6.24, 21.63]) in the triple therapy arm discontinued the study: the reasons for discontinuation are detailed in Figure 1.

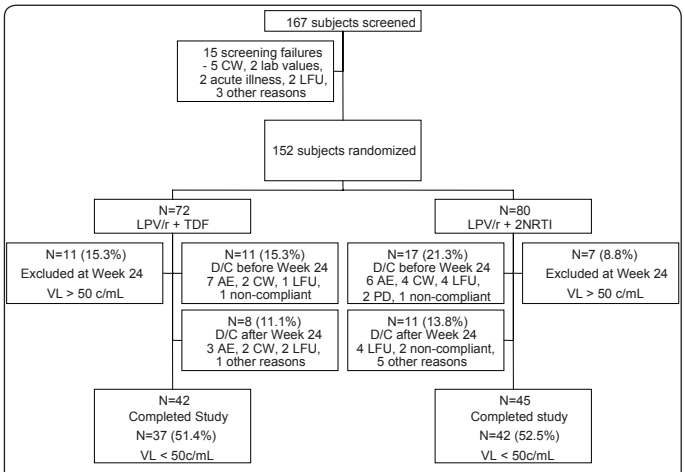

Figure 1: Subject disposition.
AE, Adverse Event; c/mL, copies/milliliter; CW, Consent Withdrawal; D/C, Discontinued; LPV/r, Lopinavir/ritonavir; LFU, Lost to Follow-Up; NRTI, Nucleoside Reverse Transcriptase Inhibitor; PD, Protocol Deviation; TDF, Tenofovir Disoproxyl Fumarate; VL, Viral Load (HIV-1 RNA).

	LPV/r + TDF (n=72)	LPV/r + 2 NRTIs (n=80)
Male[A]	63 (87.5%)	61 (76.3%)
Caucasian[A]	62 (86.1%)	70 (88.6%)
Age, years[B]	39.92 (10.0)	40.44 (9.9)
Karnofsky score (100)[A]	61 (84.7%)	68 (85.0%)
CD4 cell count, /mm^3[B]	244.77 (123.7)	200.74 (117)
CD4 cell count, /mm^3[C]	231 (2-580)	199.5 (3-639)
Subjects with CD4 cell count ≤ 200/mm^3[A]	29 (40.3%)	41 (51.3%)
HIV-1 RNA (log10)[B]	4.81 (0.7)	4.91 (0.7)
Subjects with HIV-1 RNA >100.000 copies/mL[A]	35 (48.6%)	41 (51.3%)
Duration of infection, months[B]	49.2 (74.0)	30.0 (54.7)

[A]number (%)
[B]mean (s.d.)
[C]median (range)

Table 1: Baseline demographics and disease characteristics.

Antiviral efficacy

The proportion of subjects with HIV-1 RNA <50 copies/mL at week 72 was 51.4% (95%CI [39.86, 62.94]) in the dual therapy and 52.5% (95%CI [41.56, 63.44]) in the triple therapy arm (ITT). The between-arm difference in the proportion of subjects with HIV-1 RNA <50 copies/mL was not statistically significant (ITT, NC=F; p=0.89, 95% CI [-18.34; 16.11]) (Figure 2A) however the dual therapy arm did not demonstrate statistical non-inferiority to the triple therapy arm. In an on-treatment analysis (OT (PP-CC)), 87.2% and 93.0% of subjects in the dual therapy and triple therapy arm, respectively, had HIV-1 RNA <50 copies/mL (p=0.49, 95%CI [-21.25; 9.56]) at week 72, again statistical non-inferiority to the triple therapy arm was not established for the dual therapy arm.

Fifty (69.4%) subjects in the dual therapy arm and 56 (70.0%) in the triple therapy arm achieved at least two HIV-1 RNA values <50 copies/mL within the first 24 weeks of therapy. In the dual therapy arm, the proportion of subjects achieving 2 HIV-1 RNA values <50 copies/mL within the first 24 weeks was higher among those who entered the trial with a baseline HIV-1 RNA ≤100.000 copies/mL (30/37 subjects (81%)) versus those who entered with a baseline HIV-1 RNA >100.000 copies/mL (20/35 subjects (57%); p=0.05). Conversely, this difference in response was not significant across viral load strata in the triple therapy arm although the numerical trend was similar: 30/39 subjects (77%) with baseline HIV-1 RNA ≤100.000 copies/mL versus 26/41 (63%) subjects with baseline HIV-1 RNA >100.000 copies/mL (p=0.28) (Figure 2B).

At 24 weeks, 11/72 subjects (15.3%, 95%CI [6.98, 23.62]) in the dual therapy arm and 7/80 subjects (8.8%, 95%CI [2.59, 15.01]) in the triple therapy arm (p=0.32) had failed to achieve 2 HIV-1 RNA values <50 copies/mL, and were excluded from continuing in the study as per protocol design. Of these, 10/11 (90.9%) in the dual therapy arm and 7/7 (100%) in the triple therapy arm had a baseline HIV-1 RNA >100.000 copies/mL. The exclusionary HIV-1 RNA values at week 24 ranged from 85 to 1000 copies/mL for the subjects in the dual therapy arm and from 58 to 490 copies/mL in the triple therapy arm.

Low-level viremia (HIV-1 RNA between 50-1000 copies/mL after reaching a value <50 copies/mL) was detected at week 48 in five (6.9%) subjects in the dual therapy arm and 3 (3.8%) in the triple therapy arm, while five (6.9%) subjects in the dual therapy arm and 2 (2.5%) in the triple therapy arm had low-level viremia at week 72 (Figure 2A). The low level viremia values ranged from 51 to 920 copies/mL in the dual therapy arm and from 72 to 1000 copies/mL in the triple therapy arm; one subject in the triple therapy arm had a viral rebound of 8100 copies/mL at week 72. Three of the subjects with low-level viremia at week 48 in the dual therapy arm and one in the triple therapy arm had an undetectable HIV-1 RNA at week 72, whereas the rest either remained detectable at week 72 or did not have follow-up data (discontinuation of subject or study completion).

Development of resistance

For subjects discontinued at week 24 as "protocol-defined viral failures", week 24 samples (HIV-1 RNA ≥200 copies/mL) and paired baseline samples for genotype analyses were available for 4/11 subjects in the dual therapy arm (3 samples not available, 4 samples with VL <200 copies/mL) and 2/7 subjects in the triple therapy arm (3 samples not available, 2 samples with VL <200 copies/mL). No mutations in protease nor K65R mutations in reverse transcriptase were detected; however, one subject in the triple therapy arm had selected a M184V mutation in reverse transcriptase by week 24.

For subjects with low-level viremia at further study visits (weeks 48 – 72), samples collected at these visits (HIV-1 RNA ≥200 copies/mL) and paired baseline samples for genotype analyses were available for 3/10 subjects in the dual therapy arm (3 samples not available, 4 samples with VL <200 copies/mL) and 1/5 subjects in the triple therapy arm (2 samples not available, 2 samples with VL <200 copies/mL). At week 72, one subject in the dual therapy arm had developed a single mutation in protease (I54V). At week 72, one subject in the triple therapy arm had developed a mutation in reverse transcriptase (M184V) with viral load of 8100 copies/mL and an undetectable LPV plasma concentration, suggesting poor treatment adherence as the likely cause of rebound viremia. No K65R mutation in was detected in either treatment arm.

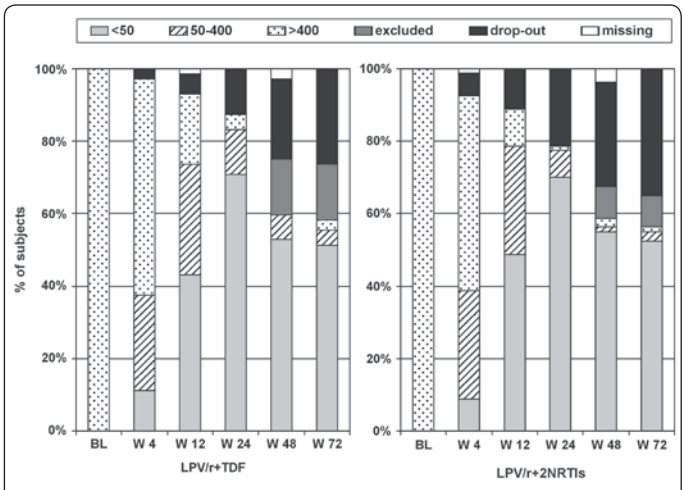

Figure 2a: Treatment response throughout the study – ITT.
Treatment response from baseline is shown as proportion of subjects with HIV-1 RNA <50 copies/mL (light gray), 50-400 copies/mL (diagonal stripes) or >400 copies/mL (dotted). Subjects excluded at week 24 (dark grey) as per study design, drop-outs for other reasons (black) and missing values (white) are also shown for each time point. ITT, Intention-to-treat analysis; LPV/r, Lopinavir/ritonavir; NRTI, Nucleoside Reverse Transcriptase Inhibitor; TDF, Tenofovir Disoproxyl Fumarate; BL, Baseline; W, treatment week.

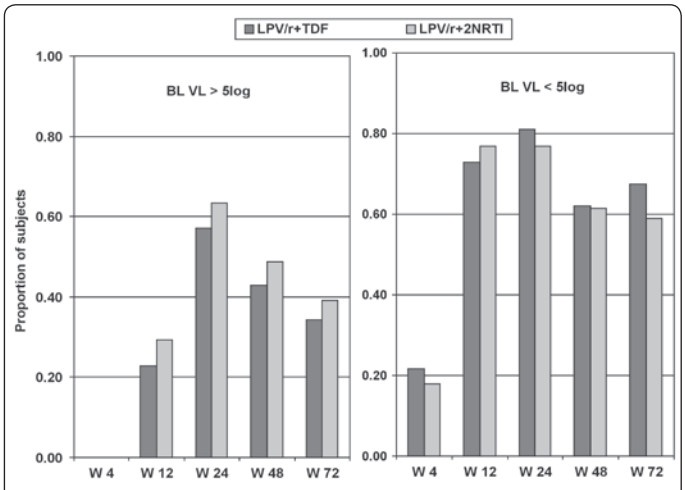

Figure 2b: Achieving HIV-1 RNA < 50 copies/mL – ITT.
The proportion of subjects achieving an HIV-1 RNA <50 copies/mL at each study visit. Subjects with baseline HIV-1 RNA > 100.000 copies /mL (left-hand panel) or ≤ 100.000 copies/mL (right-hand panel). ITT, Intention-to-treat analysis; LPV/r, Lopinavir/ritonavir; NRTI, Nucleoside Reverse Transcriptase Inhibitor; TDF, Tenofovir Disoproxyl Fumarate; BL, Baseline, W, treatment week.

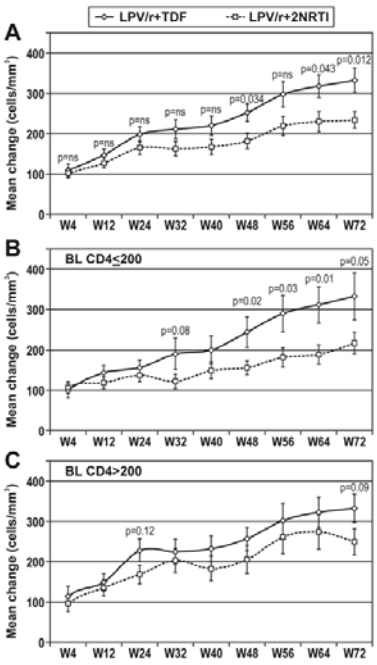

Figure 3: CD4 cell count changes from baseline.
CD4 cell count changes from baseline to each visit are shown (mean +/- SE). Panel A, all subjects; panel B, subjects with baseline CD4 cell count ≤ 200 cells/mm³; panel C, subjects with baseline CD4 cell count > 200 cells/mm³. LPV/r, Lopinavir/ritonavir; NRTI, Nucleoside Reverse Transcriptase Inhibitor; TDF, Tenofovir Disoproxyl Fumarate; W, treatment week; BL, Baseline.

Immunological efficacy

The mean CD4 cell count increase from baseline to week 72 was significantly higher in the dual therapy arm compared to the triple therapy arm (332 vs. 234 cells/mm³; p=0.01) (Figure 3). Between-arm differences favoring the dual therapy arm compared to the triple therapy arm were also observed with respect to the change from baseline to week 72 in CD4 percentage (p=0.001) (data not shown).

CD4 cell count changes from baseline to each visit were also analyzed within subgroups defined according to baseline CD4 cell counts (Figure 3). In subjects with baseline CD4 count ≤200 cells/mm³ the increase in mean CD4 cell counts from baseline to week 72 was 332 cells/mm³ in the dual therapy arm and 216 cells/mm³ in the triple therapy arm (p=0.05). In subjects with baseline CD4 count >200 the increase from baseline to week 72 was 333 cells/mm³ in the dual therapy arm and 250 cells/mm³ in the triple therapy arm (p=0.09). Similar between-arm differences were observed with respect to CD4 percentages (data not shown). Significantly greater CD4 cell count increases from baseline to week 72 were observed in the dual therapy arm compared to the triple therapy arm by repeated measures ANCOVA (p=0.03).

QoL

No between-arm differences were observed with respect to the overall score or any dimension score of the MOS-HIV Health Survey questionnaire. However conclusions from the QoL data should be interpreted with caution due to the low answering rate (59% of subjects) during follow-up.

Adherence

A significantly higher adherence was observed in the dual therapy arm (fewer missed doses of any drug: 15.4% vs 36.1% in the dual therapy vs triple therapy arm; p=0.04) at the last study visit. There were no considerable differences between the two arms in treatment adherence, as assessed by the self-administered Adherence Questionnaire at other time points of follow-up. However, as with the QoL data adherence conclusions should be interpreted with caution due to the low answering rate (57% of subjects) at the last study visit.

Adverse events

The two arms were comparable for overall incidence of adverse events of any severity and any relationship to the study drugs (Table 2) and for adverse events leading to discontinuation (Table 3). Differences between treatment arms in terms of distribution of adverse events by SOC (System Organ Class; MedDRA classification) or event severity as judged by the investigator were not significant.

Metabolic toxicity

All laboratory tests were performed at each site's individual clinical laboratory. The proportions of subjects with fasting total cholesterol, triglycerides, and blood glucose values of toxicology Grade III-IV were compared between the two arms considering the worst grade achieved throughout the study. The dual therapy and triple therapy arms were comparable for Grade III-IV lipid abnormalities (Table 2) as well as for mean change from baseline in total cholesterol, triglycerides, and blood glucose values (data not shown). Subjects in the triple therapy arm had fasting total cholesterol values more frequently above the upper limit of normal range of each site's clinical laboratory than subjects in the dual therapy arm (52.4% vs 27.4%; p=0.004). The proportions of subjects with new lipid-lowering therapy prescribed during the study were similar with 6 (8.6%) and 11 (13.8%) subjects in the dual therapy and triple therapy arms, respectively (p>0.05).

	LPV/r + TDF (n=72)	LPV/r + 2 NRTIs (n=80)
treatment exposure (weeks)	54.33	54.00
any AE	61 (84.7%)	67 (83.8%)
any drug-related AE	39 (54.2%)	52 (65.0%)
any serious AE	10 (13.9%)	7 (8.8%)
any drug-related serious AE	0	2 (2.5%)
any AE causing D/C	9 (12.5%)	6 (7.5%)
any AE causing death Dyspnea and chest pain Suspect lymphoproliferative disorder	2 (2.8%) 1 1	0
GI Disorders	37 (51.4%)	33 (41.3%)
Diarrhoea	28 (38.9%)	23 (28.8%)
Nausea	6 (8.3%)	6 (7.5%)
Vomiting	7 (9.7%)	6 (7.5%)
General disorders	10 (13.9%)	20 (25%)
Fever	6 (8.3%)	12 (15.0%)
Asthenia	3 (4.2%)	8 (10.0%)
Other		
Headache	3 (4.2%)	6 (7.5%)
Cough	5 (6.9%)	7 (8.8%)
METABOLIC DISORDERS	14 (19.4%)	23 (28.8%)
Dyslipidemia	1 (1.4%)	4 (5.0%)
Hypertriglyceridemia	9 (12.5%)	14 (17.5%)
Total cholesterol Grade III-IV	3 (4.2%)	7 (8.8%)
Triglycerides Grade III-IV	5 (6.9%)	9 (11.3%)

AE, Adverse Event; D/C, discontinuation; LPV/r, Lopinavir/ritonavir; NRTI, Nucleoside Reverse Transcriptase Inhibitor; TDF, Tenofovir Disoproxyl Fumarate.

Table 2: Summary of safety. Adverse events with incidence ≥5% in either treatment arm, Drug-related Serious Adverse events and Adverse events causing death are presented.

	LPV/r + TDF (n=72)	LPV/r + 2 NRTIs (n=80)
D/C before Week 24	**8 (11.1%)**	**6 (7.5%)**
	Fever, headache, rash	Diarrhoea
	Headache, diarrhea	Nausea, vomiting
	Nausea, vomiting, GI pain	Headache, neck pain
	Diarrhoea	Fever, lymphadenopathy
	Diarrhoea, vomiting	Asthenia, nausea
	Nausea, vomiting	Vomiting
	Dyspnea, chest pain (death)	
D/C after Week 24	**3 (4.2%)**	**0**
	Increase in transaminases	
	Suspect lymphoproliferative disorder (death)	
	Dizziness, nausea, vomiting	

D/C, discontinuation, LPV/r, Lopinavir/ritonavir; NRTI, Nucleoside Reverse Transcriptase Inhibitor; TDF, Tenofovir Disoproxyl Fumarate.

Table 3: Adverse events leading to discontinuation. Data on Adverse events given as primary reason for discontinuation are presented.

There were no Grade III-IV glucose alterations in either of the treatment arms.

Renal toxicity

Renal laboratory tests were performed at each site's individual clinical laboratory. The subjects were monitored for serum creatinine, calculated creatinine clearance and serum phosphate values at all visits. There were no Grade III-IV serum creatinine or phosphate value alterations in either the dual therapy or triple therapy arm. Mean changes from baseline through week 72 in serum creatinine were +0.04 mg/dL (95%CI [0, 0.07]) in the dual therapy arm and –0,0 mg/dL (95%CI [–0.04, 0.03]) in the triple therapy arm. Mean change in calculated creatinine clearance from baseline through week 72 was –2,9 mL/min (95%CI [–10.9, 23.6]) in the dual therapy arm and +4.68 mL/min (95%CI [–1.3, 10.6]) in the triple therapy arm, and in serum phosphate –0.13 (95%CI [–0.4, 0.1]) in the dual therapy arm and –0,07 mg/dL (95%CI [–0.4, 0.2]) in the triple therapy arm. No significant differences between treatment arms were noted in any of these renal related parameters.

Discussion

Kalead was the first study to compare a boosted PI/r-NRTI-based dual agent therapy with standard-of-care triple therapy in HIV-infected adults naïve to ART. The results suggest that the tolerability and antiviral efficacy of dual ART with LPV/r + TDF when compared to three-drug HAART warrants additional investigation. While the results suggest that the treatment arms were comparable for the primary endpoint (proportion of subjects with undetectable plasma HIV-1 RNA at 72 weeks), the dual therapy arm was not established to be statistically non-inferior to the triple therapy arm, likely due to lower than anticipated statistical power (66% according to a post-hoc analysis) as a result of a too conservative sample size calculation and the higher than expected discontinuation rates in both study arms. A higher proportion of subjects in the dual therapy vs. the triple therapy arm were discontinued from the study at week 24 as they did not meet early virologic suppression criteria (15% vs. 9%). However, the study demonstrated a significant advantage in CD4 cell recovery favoring the dual therapy arm over the triple therapy arm.

This study has a series of limitations that present challenges to interpretation. The statistical power for this study, which was limited to detect small differences between the two arms, was further reduced by the unexpectedly high rate of discontinuations: less than 60% of the subjects completed the study, which makes the interpretation of results challenging. Additional limitations of the Kalead study include the lack of a central laboratory and thus consistent assays for chemistry, CBC, CD4, and HIV RNA. Furthermore, with hindsight, it might have been advisable not to exclude TDF from the triple standard-of-care arm; however, the study design warranted a comparison between the novel dual strategy and regimens that were accepted as representing standard-of-care at the initiation of the trial. At that time, abacavir (ABC) or zidovudine (AZT) based NRTI combinations were considered SOC in Italy. Also, QD dosing of LPV/r was not permitted in either of the treatment arms, due to the lack of QD registration in the EU at the time. Obviously the attractiveness of the dual therapy approach would be enhanced if the LPV/r could be dosed QD, thereby making the entire regimen QD. Furthermore, the formulation of LPV/r (soft gelatin capsule) utilized in the Kalead trial has been removed from the market, replaced with a tablet formulation.

NRTI exposure can be associated with metabolic, mitochondrial and myelotoxicity, lactic acidosis and lipoatrophy. Failing NRTIs can create NRTI cross class resistance and therefore reduce future therapeutic options [4]. Eliminating one NRTI from the current treatment paradigm without markedly compromising the control of HIV replication or the generation of incremental resistance is a noteworthy observation of this study that warrants further investigation.

Not surprisingly, our data did demonstrate a difference in the time to achieve undetectable (<50 copies/mL) HIV-1 RNA. Subjects treated with three drugs arrived at <50 copies/mL in less time than was observed in subjects treated with two drugs. However at this time, there is no identifiable clinical benefit associated with a shorter time to <50 copies/mL. An important limitation of our study design was that subjects who were responding, but had not yet reached 2 HIV-1 RNA values below 50 copies by week 24 were classified as protocol defined virological failures and were precluded from continuing beyond week 24 in the trial. This design may have disadvantaged the dual therapy arm as 11 (15%) subjects were eliminated from the dual therapy arm at week 24 whereas only 7 (9%) subjects were discontinued from the triple therapy arm at this time point. While reflecting the delayed antiviral activity of this dual regimen compared to the triple regimen, there potentially could be no difference in ultimate suppression rates or the duration of suppression with LPV/r + TDF when compared to LPV/r + 2 NRTI's. Unfortunately, since subjects were not followed after discontinuation from the trial, this result cannot be confirmed. Also, 17/18 of these subjects who were proactively discontinued at week 24 started with a high baseline HIV burden (>100.000 copies/mL). In this situation, high viral load, boosted protease inhibitor based dual or triple therapy simply may have needed additional time to achieve < 50 copies/mL.

The total discontinuation rate in the present study was surprisingly high, exceeding 42%. Particularly worrisome were the high discontinuation rates for non-safety or non-virological reasons during the first 24 weeks of therapy (15% vs 21% in the dual therapy vs triple therapy arm); however, this phenomenon can in part be attributed to subjects who either withdrew consent or were lost to follow-up. Globally, while the two arms were comparable with regard to overall drop-out rates and discontinuations due to adverse events or withdrawal of consent, drop-outs due to loss to follow-up or lack of compliance were more frequent in the triple therapy arm (13.8%) compared to the dual therapy arm (4.2%) (Figure 1), which could reflect a bias of open label studies in favor of the regimens that are perceived as more novel or convenient. Also, while not observed as a reason listed for discontinuation, the introduction and availability of the emtricitabine (FTC)/tenofovir (Truvada) combination tablet in Italy during our trial period may have motivated or influenced some discontinuations from the triple therapy arm.

The positive effect of LPV/r treatment on CD4 cell recovery has been documented in large randomized trials [13-15]. We noted a statistically significant difference favoring the dual therapy in CD4 cell recovery of naïve subjects. The advantage of the dual therapy arm was more marked in subjects with baseline CD4 cell counts ≤200 cells/mm^3. There was a slight imbalance in the baseline CD4 cell counts between the two treatment arms. The higher initial CD4 cell count in the dual therapy arm could at least in part account for the significant increase of CD4 cell in the dual therapy arm compared to the triple therapy arm. However in a clinical setting, subjects with a lower initial CD4 cell count would more probably have demonstrated greater increases in CD4 cell counts than those subjects with a higher initial CD4 cell count.

A possible explanation for the difference in CD4 cell recovery could be the absence in the dual therapy arm of thymidine nucleoside analogues, which previously have been observed to exert a negative effect on CD4 cell recovery, probably through a moderate myelotoxic effect. We attempted to verify this through further analyses in the triple therapy arm; however, the small size of the subgroups may have precluded the detection of differences between the thymidine analogue containing- and non-containing regimens. Clinical relevance related to the observed difference in CD4 gain between the dual and triple arms has not been established.

Co-administration of TDF with LPV/r leads to increased exposures of tenofovir [4]. However, no renal adverse events or discontinuations due to renal adverse events were observed in this trial.

Conclusion

While rates of discontinuations in the present study affected our ability to rigorously compare the efficacy of the dual therapy versus triple therapy regimens, no gross efficacy differences were observed, the safety profiles of the two arms appear to be similar, and a greater increase in absolute CD4 cell count was observed with dual therapy compared to triple therapy in this trial. Initial virologic suppression on the two-drug regimen may have been delayed, particularly in patients with higher baseline viral loads. Larger studies with longer follow-up are needed to appropriately evaluate the safety and efficacy of dual therapy with LPV/r + TDF compared to conventional triple LPV/r-based therapy regimens.

In conclusion, based on these initial data LPV/r + TDF warrants additional investigation as initial therapy for HIV-1 infected individuals.

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