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A Randomized Open-Label Study of Three- versus Five-Drug Combination Antiretroviral Therapy in Newly HIV-1 Infected Individuals

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Abstract

Background—To understand whether combination antiretroviral therapy (cART) has been optimized, we asked whether 3-drug protease inhibitor (PI)-based cART intensified with raltegravir and maraviroc and initiated during early infection would improve outcomes when compared to similarly applied 3-drug PI-based cART.

Methods—40 newly HIV-1 infected patients were randomized 1:2 to receive 3-drug (N=14) or 5-drug (N=26) therapy. The primary endpoint was the percent of subjects with undetectable plasma viremia using standard RT-PCR and the single copy assay (SCA) after 48 weeks. Secondary endpoints included levels of cell-associated HIV-1 DNA and RNA and levels of infectious virus in resting CD4+ T cells at week 96 and quantitative and qualitative immunologic responses.

Results—At 48 weeks, 34 subjects remained on study and are included in the as-treated analysis. Three of 11 (27.3%) in the 3-drug arm and 9 of 21 (42.9%) in the 5-drug arm had plasma HIV-1 RNA levels below detection by both standard RT-PCR and SCA (P= 0.46, Fishers exact test). No significant differences in absolute levels of proviral DNA or changes in cell-associated RNA were seen during 96-weeks of therapy. Mean levels of infectious HIV-1 in resting CD4+ T cells at week 96 in 7 subjects treated with 3-drugs and 13 with 5-drugs were 0.67 and 0.71 IUPM respectively (P= 0.81). No differences were seen in quantitative or qualitative immunologic determinations including markers of immune activation.

Portions of these finding have been presented at the 18th Conference on Retroviruses and Opportunistic Infections, Boston, Mass. 2011 and the HIV Persistence During Therapy Workshop, St. Martin, 2011.

Conflict of interest: M.M. is a paid consultant to Merck, Gilead Sciences, and Janssen, serves on the speaker's bureau for Gilead Sciences, Bristol Myers Squibb and Janssen, and has received grant support from Merck, Pfizer, Gilead Sciences, GlaxoSmithKline, and Tobira within the past 24 months. Additional authors have no conflict of interest to report.

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Conclusions—Intensified 5-drug cART initiated during early infection fails to significantly further impact virologic or immunologic responses beyond those achieved with standard 3-drug PI-based cART.

Keywords

Intensified cART recent HIV-1 infection

Introduction

The use of effective combination antiretroviral therapy (cART) has substantially reduced HIV-1-related mortality and morbidity as the vast majority of treated individuals will experience sustained virologic suppression and associated immunologic benefit [1–3]. Nevertheless, despite apparent suppression, HIV-1 persists and when treatment is interrupted virologic rebound generally occurs [4,5].

Longitudinal studies have found that in suppressed patients levels of viremia [6] and cell-associated HIV-1 [7,8] remain relatively stable, suggesting slow to no decay. Dynamically, this can be explained by two mechanisms that are not mutually exclusive. Ongoing viral replication could account for constant replenishment of HIV-1 infected cells accompanied by a slow inherent decay rate resulting in an observed stable level of persistence. Alternatively persistence can be maintained by mechanisms that establish latency and allow for maintenance of this pool though proliferation and slow decay. Should the former be the case, it would follow that intensification would result in further suppression of low level viral replication and result in lower levels of persistence. To resolve this issue of ongoing viral replication during cART we asked whether intensified cART would result in differences in levels of persistence after one to two years of suppressive therapy. This effort was made feasible by the availability of novel antiretroviral agents that could be readily incorporated into a compact daily oral regimen.

At the time this study was designed two new potent agents became available for clinical use, maraviroc, a CCR5 antagonist that interferes with HIV-1 entry [9], and raltegravir [10,11], an inhibitor of HIV-1 integrase. To minimize pill burden we used protease inhibitor-based (PI) cART as the standard regimen to which we added raltegravir and maraviroc.

Here we report and compare the virologic and immunologic responses to 3-and 5-drug cART in a cohort of newly HIV-1 infected individuals. This study was unique in applying intensified 5-drug therapy in treatment naïve individuals and measuring not only routine virologic and immunologic responses, but also determining levels of plasma viremia with the single copy assay (SCA), measuring levels of cell associated HIV-1 DNA and RNA by PCR and directly measuring the levels of virus in the latent reservoir after approximately 2 years of suppressive therapy. We also performed comprehensive quantitative and qualitative immune responses to therapy including levels of naïve and central memory CD4+ T cells and assessed markers of immune activation prior to and during therapy.

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Materials and Methods

Study subjects—Study participants were screened at the Aaron Diamond AIDS Research Center (ADARC), Rockefeller University Hospital (RUH). Participants were defined as acutely infected with HIV-1 based on documentation of plasma HIV-1 RNA levels above 5,000 copies/ml, with a contemporaneous negative or indeterminate HIV enzyme immunoassay (EIA). Recent infections were confirmed with laboratory results using the following criteria: a positive HIV-1 EIA or Western blot and a documented negative HIV-1 EIA within the previous 6 months, or a less sensitive ("detuned") HIV-1 antibody assay with an EIA optical density (O.D) 0.5 (Vironostika LS-EIA) or its equivalent (Ortho Vitros LS-ECi). The duration of infection was estimated in 38 subjects as 14 days prior to the onset of symptoms consistent with the acute seroconversion syndrome [5]. In two subjects who were asymptomatic, we used an algorithm developed by the Acute Infection and Early Disease Research Program [12]. Post-treatment markers of immune activation were measured in treated subjects as detailed below and compared to that observed in a cohort of 13 HIV-1 uninfected healthy volunteers recruited from the general population. Written informed consent was obtained from all participants. The RUH Institutional Review Board approved the study protocol. The study was registered with ClinicalTrials.gov number NCT #00525733.

Study procedures and treatment regimen—This was a pilot, randomized, study comparing open label ritonavir-enhanced PI-based cART with a 5-drug regimen including maraviroc and raltegravir. Randomization was performed by the Rockefeller University Pharmacist using the website randomization.com. All patients were treated with a once daily combination of fixed-dose combination tenofovir difumarate (TDF) 300mg and emtricitabine (FTC) 200mg with either atazanavir 300 mg or darunavir 800 mg with ritonavir 100mg. The 5-drug regimen included maraviroc 150 mg and raltegravir 400 mg dosed twice daily. Patients were randomized 1:2 to receive either 3-drug or 5-drug therapy. Subjects were excluded if they had evidence of infection with drug resistant virus to any components of the regimen.

Participation in the study was terminated for non-adherence defined as missing more than 7 consecutive days of prescribed therapy or 2 consecutive scheduled visits. In addition, virologic failure (VF) was defined as either failure to reach a plasma HIV-1 RNA level below the detection limit (50 copies/mL) at the week 36 visit or confirmed virologic rebound, that is 2 detectable plasma viral loads above 50 copies/mL at least 2 weeks apart after achieving an undetectable viral load on at least 2 consecutive determinations. Consenting subjects underwent leukapheresis after 96 weeks of therapy so that levels of infectious HIV-1 in resting CD4+ T cells could be determined. The study was continued until the final participant completed the week 96 visit in October 2011.

Virologic assessments

Plasma HIV-1 RNA levels were measured using the Roche Amplicor version 1.5, and subsequently the Roche TaqMan assays v. 1.0 and 2.0 with lower detection limits of 50, 48 and 20 copies/mL plasma respectively (Roche Diagnositcs, Branchburg, NJ). The single

copy assay (SCA), with a lower detection limit of 0.3 HIV-1 copies/mL plasma, was used to measure low-level plasma viremia in patients in whom HIV-1 RNA levels were not detectable at week 48 per published methods [13]. Cell- associated HIV-1 RNA and DNA was measured at baseline, weeks 12, 24, 48 and 96. Frozen PBMC were thawed, washed and CD4+ cells isolated with Dynabeads FlowComp Human CD4 (Dynal). Cell-associated DNA was isolated using Qiagen DNA Blood Kit (Qiagen), and the concentration was measured by NanoDrop 1000 (Thermo Scientific). Purified DNA, 200 ng to 450 ng, was used to amplify HIV proviral DNA. Real time PCR for HIV-1 DNA was run in parallel with an HIV DNA standard, pNL4-3 [14] with 5-fold dilutions starting at 5,000, down to 0.32 copies per well as well as negative controls. All PCR reactions were performed in triplicate. The sequences of the primers/probe, RF/RR/PB, are as follows: a forward primer mix, RF-1: 5′-CGGCGACTGGTGAGTACG-3′ and RF-2: 5′-GGCGGCTGGTGAGTACG-3′, a reverse primer, RR: 5′-GACGCTCTCGCACCCAT-3′ and a dual labeled probe, RB: 6-FAM-TTTGACTAGCGGAGGCTAGAAGGAGA-BHQ-1, as published [15]. Real time PCR was performed with AmpliTaq Gold with Buffer A (Applied Biosystems) using the Stratagene Mx3000P QPCR System (SABiosciences) at the thermal cycle condition of 95°C for 10 min, followed by 50 cycles of 95°C, 15 sec. and 60°C, 50 sec.

As primer/probe sets, including RA/RR/PB described above, did not optimally cover every HIV-1 variant, we tested different primer/probe sets for their compatibility with the baseline samples of all subjects. A primer/probe set that gave the lowest Ct value, indicating its highest amplification efficiency, was selected. Accordingly, the primer set, RF/RR/PB, was used for 31 cases, and an alternative primer set, pbs.F/pbs.R/FAM.pbs, was used for the remaining 4 cases. The sequences are as follows: pbs.F: 5′-CAGTGGCGCCCGAACAGG-3′, pbs.R: 5′-GCCGCCCCTCGCCTCTTG-3′, and FAM.pbs: 6FAM-CTCGACGCAGGACTCGGCTTGCTG-BHQ-1. To estimate the amount of template DNA, CCR5 gene copy number in the DNA sample was measured by performing a real time PCR method using primers, CCR5.F: 5′-TTATACATCGGAGCCCTGCC-3′, CCR5.R: 5′-GCCCACAAAACCAAAGATGA-3′) and a dual labeled probe, FAM.CCR5: 6FAM-CGCCTCCTGCCTCCGCTCTACT-BHQ-1 at the same thermal cycle condition as of HIV-1 PCR. One tenth the amount of DNA used for the HIV-1 DNA PCR was used in triplicate with the CCR5 DNA standard, a series of dilutions of 659 bp-long PCR product starting at 50,000, down to 80 copies and negative controls. Thus, HIV-1 DNA copy number per 2 million copies of CCR5 genome was calculated and expressed as HIV-1 DNA number per million CD4+ cells.

Purified CD4+ cells as described above were used to isolate cell-associated RNA using QIAshredder and RNeasy Mini Kit (Qiagen). RNA was further treated with DNase I, Amplification Grade (Invitrogen) and re-purified using RNeasy Mini Kit (Qiagen). RNA concentration was measured using NanoDrop1000. Template RNA, 50 ng to 500 ng, was primed with random hexamers for 15 min at 25°C, and reverse-transcribed with Superscript II (Invitrogen) for 50 min at 42°C, followed by incubation for 10 min at 85°C. Twenty μl of PCR reagent mix containing primers, probe and AmpliTaq Gold was added to each of RT product (total 50 μl), and real time PCR was performed at the same conditions as used for the cell associated HIV DNA real time assay described above. Assays were performed in triplicate and a no RT control was performed for each determination. An 888-nt long in vitro

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transcribed RNA from pNL4-3 was used as a standard, with 5-fold dilutions, starting at 5,000, down to 0.32 copies per well. Primers and probes were used as detailed above. As total amount of RNA per cell is relatively constant, cell-associated HIV-1 RNA copy number was expressed as copy number per one μg of RNA in purified CD4+ cells.

Levels of infectious HIV-1 were measured in resting CD4+ T cells using a limiting dilution virus co-culture assay as per published methods at week 96 [16]. Pre-treatment genotypes were determined using TruGene (Siemens) and tropism by Trofile (Monogram Biosciences) [17] or genotypic methods [18,19].

Immunologic assessments

Routine T cell subsets were performed commercially (Quest). Additionally we examined cell surface expression of the following molecules: CD3, CD4, CD8, CD45RO, CCR7, CD27, CD38 and HLA-DR. Aliquots of 2×10^6 PBMCs were incubated with appropriate fluorochrome-conjugated antibodies: anti-CD3 (Pacific Blue™ Clone UCHT1 BD Pharmingen), anti-CD4 (Alexa Fluor® 700 Clone RPA-T4 BD Pharmingen), anti-CD8 (APC-Cy7 Clone SK1 BD Pharmingen), anti-CD45RO (PE-Cy7 Clone UCHL1 BD Pharmingen), anti-CCR7 (FITC Clone 150503 R & D Systems), anti-CD27 (PE Clone M-T271 BD Pharmingen), anti-CD38 (APC Clone HIT2 BD Pharmingen), and anti-HLADR (PerCP Clone L243 BD Pharmingen). Stained samples were examined on a LSRII flow cytometer (BD Biosciences). Analysis of flow cytometry data was performed using FlowJo software, version 9.6.4 (Tree Star Inc., Ashland, OR).

Study design and statistical considerations

This pilot translational trial was primarily designed to answer whether intensified therapy further suppresses markers of viral persistence and thus an "as treated" analysis was deemed appropriate. The primary endpoint selected was the proportion of patients with undetectable plasma viremia using both standard RT-PCR and the SCA after 48 weeks of treatment. In addition, additional virologic and immunologic assays were designated as secondary endpoints to provide a comprehensive assessment of the response to intensified versus standard 3-drug therapy. An unbalanced 2:1 randomization scheme favoring the 5-drug arm was used to promote recruitment as standard 3-drug PI based cART was readily available in the community and both raltegravir and maraviroc were experimental and not readily available at the time of study initiation. We recruited 40 subjects assuming a drop out rate of approximately 20%. This would provide the requisite 11 subjects in the 3-drug arm and 22 subjects in the 5-drug arm for primary endpoint analysis; allowing us to detect a 50% treatment effect between arms with 85% power [20]. Fisher's exact test was used to compare response to treatment, expressed as the proportion of subjects below detection by standard RT-PCR and SCA. All other comparisons were performed using the Mann-Whitney U Test and the unpaired T test. All were performed with the use of Graph Pad Prism v. 5.0.

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Results

Study subjects

Forty subjects met entry criteria as described above and were entered into the trial between October 2007 and August 2009. Twenty-six were randomized to receive 5-drugs and 14 were treated with standard 3-drug PI-based cART. Baseline characteristics of the 34 subjects included in the primary endpoint analysis were comparable between treatment groups (Table 1). Thirty-four subjects were available for primary endpoint analysis at week 48 and 27 completed 96-weeks of assigned therapy (Fig. 1). No differences in adverse events were noted between treatment arms and only one subject discontinued therapy due to an adverse reaction, that being a persistent moderate rash attributed to ritonavir exposure.

Virologic response to therapy

Patients treated with the 5-drug raltegravir-containing regimen were more likely to reach HV-1 RNA levels below detection more rapidly (Fig. 2). However, by week 16, 82% of participants in both arms had plasma HIV-1 RNA levels below the limit of detection. At week 48 all subjects in the 3-drug had plasma viral loads below detection whereas 3 subjects in the 5-drug arm met criteria for VF. One subject reached a plasma viral load below detection by week 12 and maintained that level until the week 48 visit at which time plasma viremia rebounded to 159 copies/mL and was subsequently confirmed. Treatment was discontinued and plasma HIV-1 RNA promptly rebounded. Though no changes in the HIV-1 coding regions for reverse transcriptase, protease, or integrase were seen, the rebounding viral population tested dual tropic, consistent with a change in tropism. The remaining 2 subjects were detectable at week 36 and thus met protocol-defined criteria for VF. They remained viremic at subsequent visits and were removed from study. They did however, subsequently suppress to plasma viremia levels below detection with standard 3-drug therapy. During the second year of study all 27 subjects remaining on therapy remained virologically suppressed (Fig. 2).

Thirty-four subjects were available for primary end point analysis. Low-level viremia measurements could not be determined in 2 subjects in the 5-drug arm due to primer mismatch. At week 48, 3 of 11 (27%) subjects in the 3-drug arm and 9 of 21 (43%) in the 5-drug arm were undetectable by both standard RT-PCR and SCA (OR 2.0, 95% CI, 0.41–9.74, P= 0.46) thus, failing to meet the pre-specified primary endpoint. Of those subjects who were detectable, the mean levels of viremia in both arms were approximately 3.0 HIV-1 RNA copies/mL, not different from what has been published in other treated cohorts.

Levels of cell associated HIV-1 DNA were determined in both treatment groups at baseline, weeks 12, 24, 48, and 96 (Fig 3a). Mean levels, expressed as log DNA copies/10^6 CD4+ cells, were 4.2, 3.1, 3.0, 2.8, and 2.9 in the 3-drug arm and 3.9, 3.1, 2.9, 2.8, and 2.9 in the 5-drug group respectively. There were no statistically significant differences seen at any time point between treatment arms. Similarly, cell associated HIV-1 RNA levels were measured longitudinally at the same time points (Fig 3b). Mean levels, expressed as log RNA copies/μg total CD4+ T cell RNA, were 3.7, 2.4, 2.2, 2.2, and 2.0 in the 3-drug arm and 3.4, 2.2, 2.2, 2.1, and 1.8 in the 5-drug group respectively. No statistically significant differences

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were seen at any of the time points between treatment arms with the exception of the week 96-time point (95% CI, 1.1–99.8, P= 0.01).

Twenty (74%) of the 27 subjects who completed 96-weeks of assigned therapy, 13 in the 5-drug and 7 in the 3-drug arms, agreed to leukapheresis so as to collect adequate CD4+ T cells to determine levels of infectious HIV-1 in resting cells (Fig. 3c). Expressed as infectious units per million resting CD4+ T cells (IUPM) mean levels were 0.675 in the 3-drug and 0.702 in the 5-drug arms respectively (95% CI, −1.16–1.11, P= 0.80). In summary, with the exception of absolute levels of cell associated HIV-1 RNA at week 96, there were no significant differences between treatment arms in the virologic responses to treatment.

Immune response to treatment

Levels of CD4+ T cells were measured during the course of the 96-week study (Fig 4a). There were no significant differences in absolute CD4+ T cell levels at baseline or at any point during the 96-weeks of treatment. Increases in CD4+ T cell counts were comparable in both treatment groups and similarly did not differ significantly at any time point. Among 34 evaluable subjects, mean CD4+ T cell increases at week 48 was 299 cells/mm^3 in patients treated with 3-drugs and 328 cells/mm^3 in the 5-drug arm (95% CI, −205–146, P= 0.7). Similarly at week 96 in the 27 subjects remaining on assigned therapy, mean CD4+ T cell increases were 374 cells/mm^3 and 279 cells/mm^3 in the 3-drug and 5-drug arms respectively (95% CI, −68–259, P= 0.24). In addition to the quantitative T cell response to treatment we assessed the qualitative response to therapy by longitudinal measurements of levels of naïve (CD45RO− CD27+ CCR7+) (Fig. 4b) and central memory (CD45RO+ CD27+ CCR7+) CD4+ T cells (Fig. 4c). Levels of both naïve and central memory CD4+ T cells increased significantly from baseline during the course of therapy in both treatment groups. However, at no time point during the 96-weeks were there statistically significant differences in levels of naïve or central memory CD4+ T cells between the two treatment groups (Fig. 4b and 4c).

We also measured select markers of immune activation, both cellular and soluble. We determined percent of CD8+ T cells double staining for CD38 and HLA-DR and plasma levels of sCD14, a marker of monocyte activation at baseline, weeks 48 and 96. The mean percentage of CD8+ T cells expressing CD38 and HLA-DR were markedly elevated at baseline in both treatment arms, 49.3% and 36.8% in the 3-drug and 5-drug groups respectively (95% CI, −1.4–27.0, P= 0.12). A highly statistically significant ($p<0.001$) fall in these levels was observed in both groups however, there were no differences between arms at either week 48 or 96 (7.4% in both arms at week 48 (95% CI, −2.6–2.5, P= 0.95) and 3.8% and 5.4% in the 3-drug and 5-drug arms respectively (95%CI, −4.5–1.8, P= 0.43). Levels of sCD14 were comparable between groups at all time points and did not change significantly with therapy. At baseline mean sCD14 levels were 1582 ng/mL and 1473 ng/mL in the 3-drug and 5-drug groups respectively (95% CI, −138.0–354.7, P= 0.12). At week 48 mean levels were 1681 and 1558 ng/mL (95% CI, −127.6–372.7, P= 0.26) and at week 96, 1482 and 1433 ng/mL (95% CI, −148.7–246.8, P= 0.48). Of note levels of these 2 select markers of immune activation at weeks 48 in this cohort of early treated subjects were comparable to that measured in 13 HIV-1 uninfected healthy volunteers in whom levels of

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CD8+CD38+HLADR+ T cells and sCD14 were 7.2% (95% CI, −4.4–0.64, P= 0.14) and 1412 ng/mL (95% CI, −14.7–375.6, P= 0.07) respectively.

Discussion

The use of triple cART has dramatically altered the course of HIV-1 infection. However, daily oral therapy has its challenges and shortcomings and despite long term suppression of viral replication, antiretroviral therapy alone cannot cure HIV-1 infection due in large part to the presence of the latent reservoir that is established early in the course of infection and persists [7,8]. We designed this study to understand whether we could further suppress viral replication with intensified antiviral therapy de novo, and measure an effect on viral persistence. Additionally we hypothesized that subtle differences in viral persistence could be mirrored by differences in qualitative and quantitative immune parameters, particularly markers of immune activation. Our approach differed from others at the time in that we chose to initiate 5-drug therapy from the outset rather than intensify therapy in patients already suppressed with cART. We also enrolled subjects identified as early in the course of infection, anticipating that these individuals would benefit most from a regimen with “enhanced” antiviral activity.

At the time the study was designed the effect of intensified therapy on low-level viremia had yet to be established and the nature of residual viremia was thought to be due to ongoing viral replication. Thus we hoped to show a large treatment effect- a 50% reduction in the percent of subjects found to be detectable after 48-weeks of treatment using the SCA. It has since become established that intensification has no effect on low level viremia during therapy [21–24] and the source of persistent viremia remains obscure [25]. That said, the intensified regimen failed to reveal statistically significant differences in the percent of subjects undetectable using this assay or the absolute levels in patients in whom low-level viremia was detected. That there were 3 protocol-defined virologic failures in the 5-drug arm versus none in the 3-drug arm was unexpected. Though we were unable to document non-adherence by history, we suspect this is the likely cause in the one individual with rebounding viremia as he was non-adherent to appointments and study procedures. It is possible that the remaining 2 subjects were slow responders due to high pre-treatment levels of plasma viremia, though as a group there were no significant differences in baseline levels of viremia between the subjects receiving 5- or 3-drug regimens.

Unique to this study is the measurement of infectious virus in resting CD4+ T cells in a subset of subjects after 96 weeks. Though only 20 subjects reached that time point and agreed to a leukapheresis, our finding of absolutely no difference in IUPM between arms is critical and novel. This assay remains the gold standard in measuring levels of the latent reservoir and that there were no differences between study groups supports the prevailing hypothesis that the measureable latent reservoir in peripheral blood is likely established prior to the initiation of therapy and is maintained in large part by proliferation as opposed to further suppressible ongoing viral replication [26].

Along similar lines, levels of proviral DNA fell precipitously as expected from baseline, but leveled off after 12 to 24 weeks of treatment and were not different between treatment

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groups. We found a near identical pattern when measuring cell associated HIV-1 RNA. There is a difference between mean levels of cell associated HIV-1 RNA between the 2 arms, with the 5-drug arm being approximately 0.4 log lower. However, this is likely due to non-significant differences at baseline, as subjects in the 3-drug arm had on average a 1.7 $\log_{10}$ reduction in cell associated HIV-1 RNA at week 96 and those in the 5-drug arm a 1.6 $\log_{10}$ decrease.

We also comprehensively assessed the immunologic response to intensified therapy. As detailed, no difference in immune reconstitution could be seen between arms, either quantitative, total CD4+ T cell levels or qualitative, that being levels of naïve and central memory CD4+ T cell subsets. Levels of systemic immune activation as measured by cellular co-expression of CD38 and HLA-DR and sCD14 were also comparable between arms. Of note however, we did observe that in our treated subjects measurements of activation in the CD8+ T cell population as well as sCD14 levels in plasma fell to levels comparable to that measured in a small cohort of unmatched healthy volunteers. This is in contrast to a recent report suggesting persistent immune activation in the peripheral blood despite early initiation of therapy [27]. Further investigation is underway to determine whether early therapy can indeed overcome this recognized shortcoming of cART in cohorts of HIV-1 infected individuals.

It is critical that we point out the limitations of this study. Our cohort is on average 1.5 months into the course of infection. Recent studies have suggested that therapeutic efforts to reduce reservoir size must begin much earlier [28]. Whether it is feasible to identify acutely infected subjects early enough to impact the reservoir size with current technology remains an important issue. This study is also of small size and larger studies to validate secondary endpoints such as the size of the latent reservoir may be required. Finally, all our assays are performed on peripheral blood. Some have suggested that ongoing replication may be occurring in tissue such as the terminal ileum and thus benefits of intensification may not be reflected in the parameters we have measured [29].

In summary, the addition of raltegravir and maraviroc to PI-based triple cART failed to show any substantial differences in the comprehensive panel of virologic and immunologic parameters measured during 96-weeks of therapy. Though our data cannot exclude the possibility that there remains cryptic low level viral replication during standard 3-drug therapy, we were unable to show that the addition of 2 novel antiretroviral agents have demonstrable effects on viral persistence or associated immune parameters. We believe these findings suggest that it is likely that we have reached optimal benefit with current antiretroviral agents and novel approaches will be required to advance the treatment paradigm.

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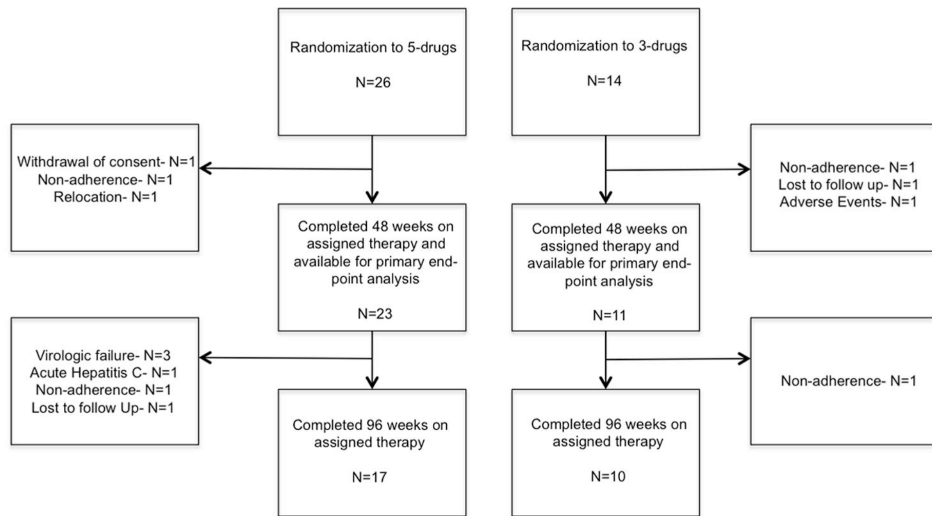

Figure 1.
Patient disposition during 96-weeks of treatment and reasons for premature discontinuation.

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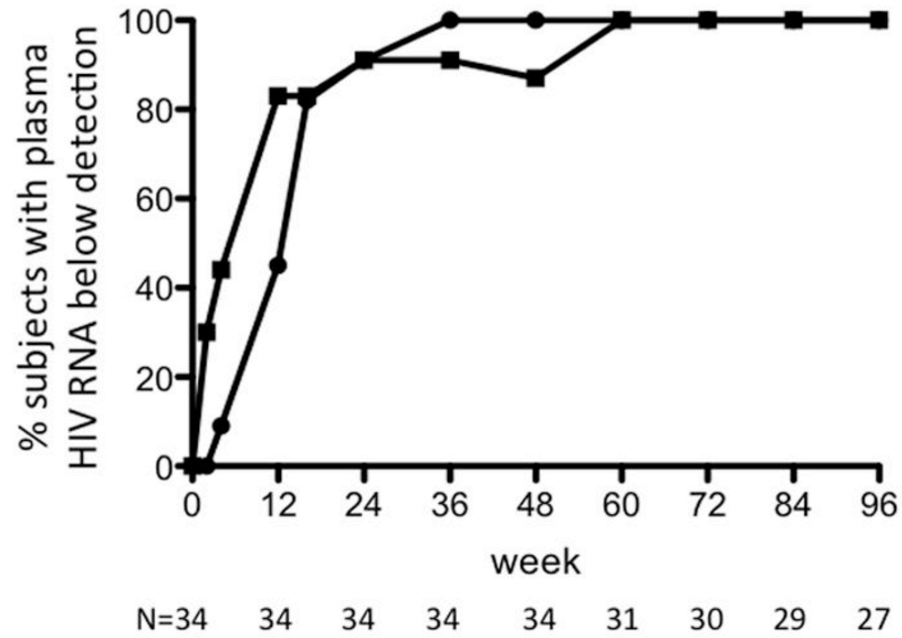

Figure 2.
The percent of subjects with plasma HIV-1 RNA levels below the level of detection during 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares). Number of subjects included in the analysis is shown below the X-axis.

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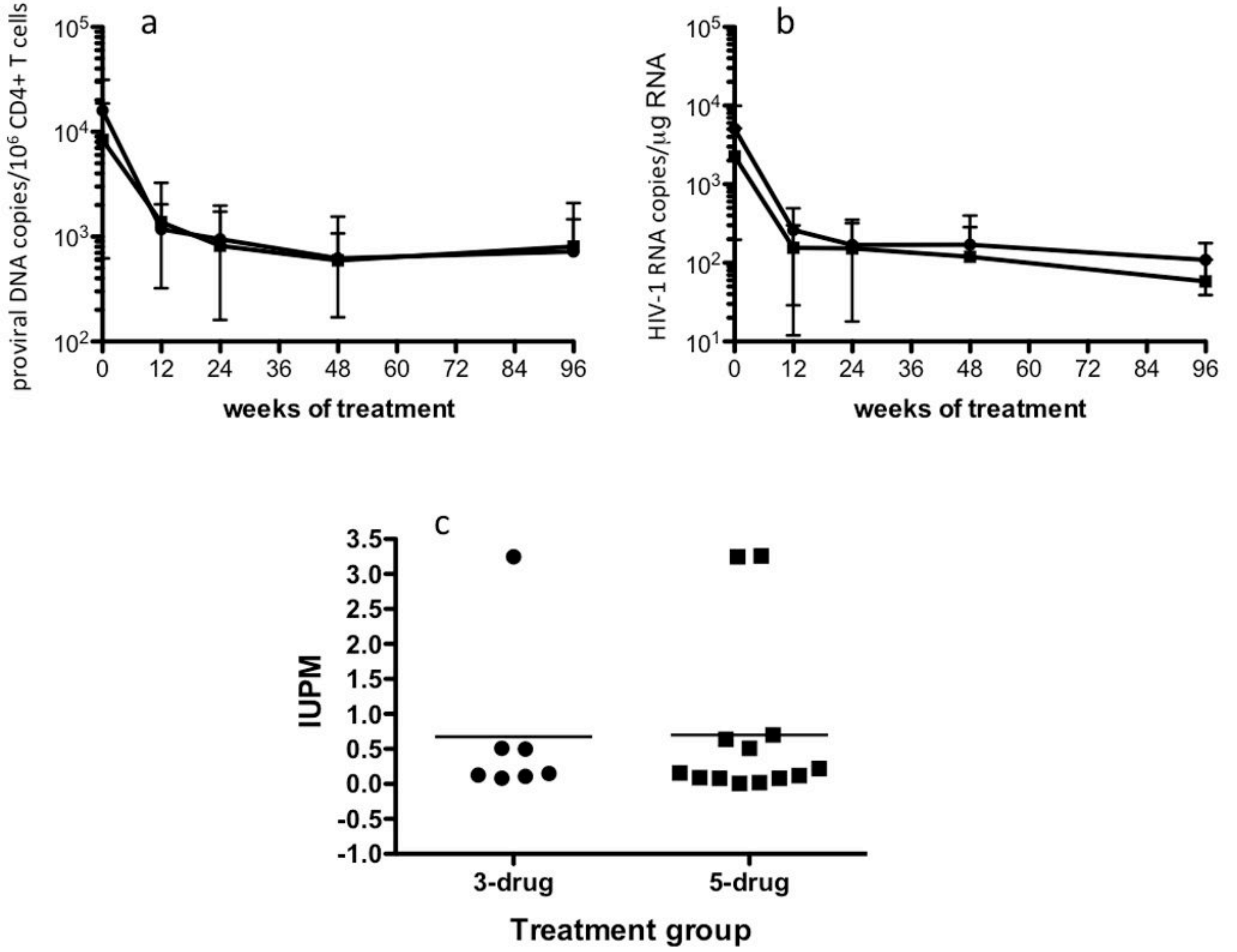

Figure 3.

Panel a. Levels of proviral DNA per 10^6 CD4+ T cells during 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares). Panel b. Levels of cell-associated HIV-1 RNA per μg total RNA during 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares). Panel c. Levels of infectious HIV-1 in resting CD4+ T cells expressed as infectious units per million cells (IUPM) in subjects after 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares).

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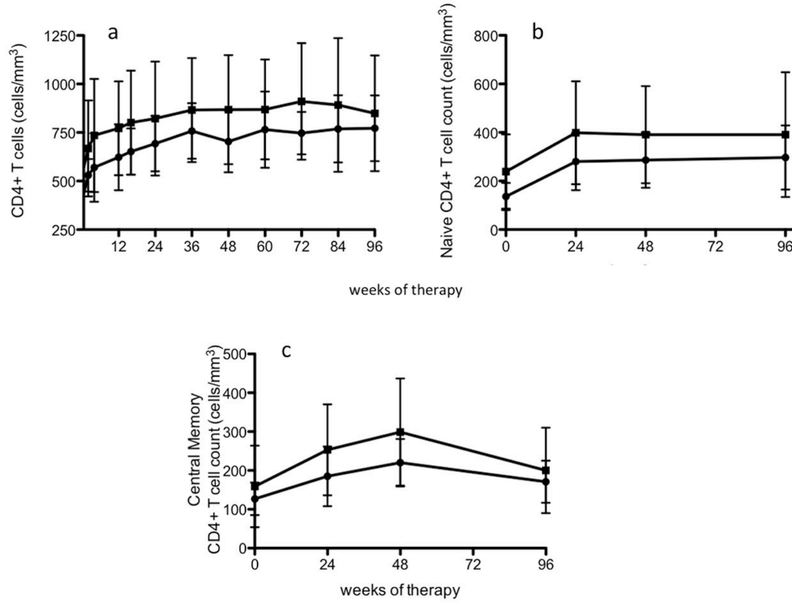

Figure 4.

Panel a. Levels of CD4+ T cells during 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares). Panel b. Levels of naïve CD4+ T cells during 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares). Panel c. Levels of central memory CD4+ T cells during 96 weeks of treatment with 3-drug therapy (circles) and 5-drug therapy (squares).

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Table 1

Baseline Characteristics

	3-drugs (N=11)	5-drugs (N=23)	P value
% Male Sex	100	100	NA
% Men who have sex with men	90.9	100	NA
Mean age (Years, range)	41 (29–69)	37 (25–48)	NA
% Symptomatic	100	91.3	NA
Mean # and duration of symptoms	4, 7d	4, 4d	NA
Mean est. duration of infection (days, range)	48 (27–77)	54(19–155)	NA
Mean log baseline HIV-1 RNA (log copies/mL, range)	6.3 (4.8–7.0)	5.6 (3.1–6.4)	0.17
Mean CD4+ T cell count (cells/mm^3, range)	405 (305–524)	539 (230–1066)	0.15