

Markers of HIV reservoir size and immune activation after treatment in acute HIV infection with and without raltegravir and maraviroc intensification

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Abstract

Background: It is unclear whether intensification of standard highly active antiretroviral therapy (HAART) with entry and integrase inhibitors during acute HIV infection (AHI) could yield greater benefits in reducing markers for HIV reservoir size and immune activation.

Methods: Thai patients with Fiebig I–IV AHI were prospectively enrolled and offered treatment. They were randomised 1:1 to HAART (tenofovir/emtricitabine/efavirenz, $n=31$) or megaHAART, a standard regimen intensified by raltegravir/maraviroc ($n=31$), during the first 24 weeks of therapy. Participants were monitored at weeks 0, 2, 4, 8 and 12, then every 12 weeks. Frequencies of peripheral blood mononuclear cells (PBMCs) carrying HIV DNA (total, integrated and 2-LTR episomes), plasma C-reactive protein (CRP) concentrations, and frequencies of activated T cells were measured. Flexible sigmoidoscopy was performed in willing participants ($n=25$) at baseline, weeks 24 and 96, and proviral DNA and RNA were determined.

Results: Baseline characteristics were similar in the HAART and megaHAART arms. Median age was 28 years and 95% were men. Median CD4 cell count was 388 cells/mm³. HIV RNA was 5.6 log₁₀ copies/mL. HIV RNA declined more rapidly in the first 4 weeks with megaHAART (median -3.3 log₁₀) than HAART (-2.6 log₁₀). Time to achieve HIV RNA <50 copies/mL was shorter with megaHAART (median 55 days) than HAART (83 days, $P=0.04$). Viral suppression rates after week 12 did not differ between arms, and overall, 97% achieved suppression by week 48. The frequency of cells harbouring total HIV DNA was similarly low after 96 weeks in both treatment arms (median of 7 and 4 copies/10⁶ PBMCs in the megaHAART and HAART arms, respectively, $P=0.41$). At weeks 2 and 12, frequency of cells carrying 2-LTR circles were significantly higher with megaHAART ($P=0.03$). In the sigmoid colon, total HIV DNA and HIV RNA declined after treatment, with no differences between arms. The frequencies of cells with 2-LTR circles were also higher in the sigmoid colon at week 24 with megaHAART. Plasma levels of CRP and frequencies of CD4+ and CD8+ T cells expressing CD38 and HLA-DR or Ki67 were similar between arms.

Conclusions: Intensification of standard HAART with raltegravir and maraviroc was not associated with either statistically significant reductions of markers of HIV reservoir size in blood and sigmoid colon or markers of immune activation in blood.

Keywords: Acute HIV infection, ART, raltegravir, maraviroc, reservoir, immune activation, HIV DNA

Introduction

The HIV reservoir is established very early after the onset of infection, leading to an interest in evaluating treatment during acute HIV infection (AHI) as a strategy to contain the reservoir [1,2]. Indeed, early treatment appears to be the most effective way to restrict HIV seeding [3–6], which may, in turn, favourably impact the ability to achieve HIV remission, that is control plasma viraemia to undetectable levels without antiretroviral therapy (ART) [7,8]. Evidence so far suggests that, in early treated individuals, lower frequencies of peripheral blood

mononuclear cells (PMBCs) that harbour total and integrated HIV DNA correlate with prolonged time to viral rebound following ART interruption [9], and sustained HIV remission in the VISCONTI cohort [8]. Despite early ART, however, latently infected cells can persist indefinitely and represent a major barrier to HIV cure [10].

The best practice for when to initiate ART and which antiretrovirals to use for AHI is not well understood. The initiation of ART at the earliest feasible time (seronegative AHI) is empirically justified to curb the rapid rise in viral load, reduce T cell activation [11], limit seeding of HIV deep reservoirs [12] and prevent damage to the immune system [13,14]. The rapid viral load decline observed with regimens that include integrase inhibitors, such as raltegravir, makes this drug class an attractive option for treating AHI [15]. The CCR5 antagonist, maraviroc, is an entry inhibitor that could be used during AHI to prevent new

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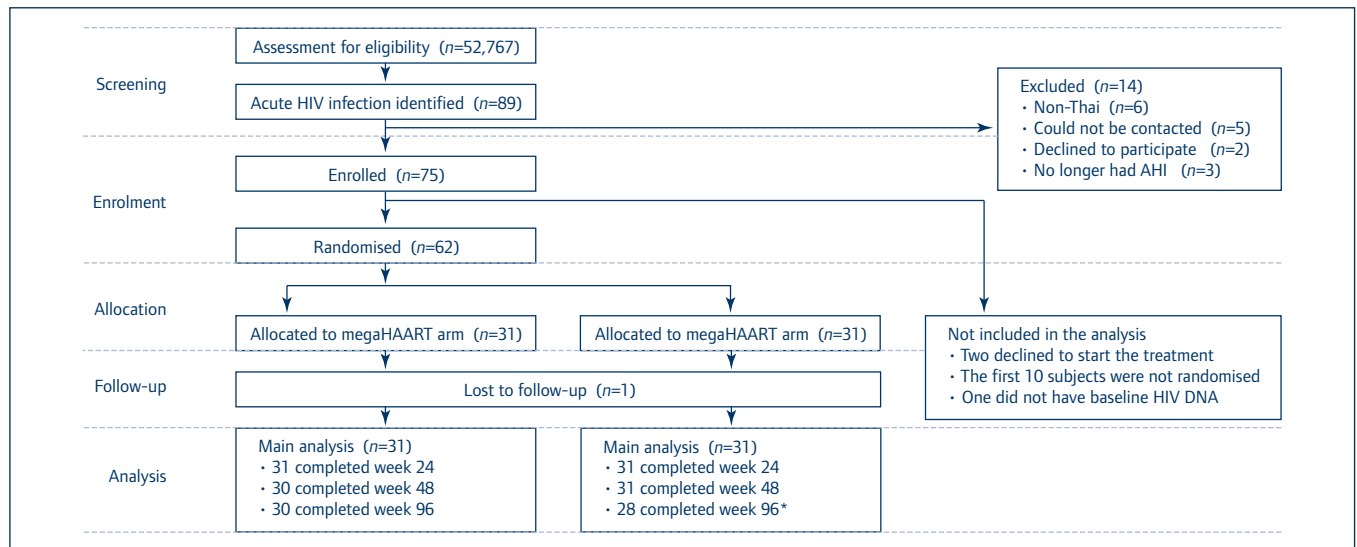


Figure 1. Study screening and accrual

*Three megaHAART patients missed their week 96 visit

cell infection [16,17]. Maraviroc may also have anti-inflammatory properties, although data are conflicting [18,19].

Studies of raltegravir and/or maraviroc intensification have mainly been conducted in chronically infected, virally suppressed individuals in an attempt to reduce the size of the HIV reservoir. However, almost all failed to show a benefit, possibly because there is little new cell infection during long-term successful ART [20–23]. To our knowledge, there are only two randomised studies that have compared raltegravir and maraviroc intensification vs standard regimens given during early/recent HIV infection, and neither has shown differences between these regimens with regard to the size of the HIV reservoir [16,17]. In this study, we investigate the intensification of a standard regimen (tenofovir, emtricitabine, efavirenz) with raltegravir and maraviroc on markers of the HIV reservoir size and immune activation in volunteers with well characterised early stage (Fiebig I–IV) AHI [24]. The primary hypothesis is that interruption of replication, integration and spread would lead to a more rapid viral load decline that could in turn lead to lower frequencies of cells that harbour HIV, and reduce immune activation. Our study uniquely contributes to this knowledge base by its inclusion of patients at the earliest stages of infection and its use of a non-nucleoside reverse transcriptase (NNRTI)-based standard regimen that is available worldwide.

Methods

The RV254/SEARCH 010 study is an ongoing prospective study in Bangkok, Thailand (clinicaltrials.gov identification NCT00796146)[3]. Thai subjects who met the AHI laboratory criteria for Fiebig stages I–IV at screening were enrolled, as described previously [24]. The mean time from screening to enrolment was 2 days (range 1–4).

ART was optional and offered as part of an accompanying local open-label protocol (clinicaltrials.gov identification NCT00796263). Treatment was started on average 2 days (range 0–5 days) after enrolment. Only the first 62 subjects who agreed to ART and who were randomly allocated to five-drug (megaHAART) vs three-drug (HAART) regimens were included in this analysis (Figure 1).

The HAART consisted of tenofovir 300 mg, once daily, emtricitabine 200 mg, once daily, and efavirenz 600 mg, once daily. The megaHAART regimen was HAART plus raltegravir

400 mg, twice daily, and maraviroc 600 mg, twice daily, for the first 24 weeks, followed by HAART thereafter. When efavirenz was discontinued for intolerance or resistance, it was replaced with raltegravir, and in subjects on maraviroc, this drug was then decreased from 600 mg to 300 mg, twice daily. Lamivudine was used interchangeably with emtricitabine depending on drug availability.

Follow-up included clinical examination, CD4+ cell count, plasma HIV RNA and safety blood tests at weeks 0, 2, 4, 8, 12 and every 12 weeks thereafter. Flexible sigmoidoscopy and biopsy was an optional procedure that was performed at weeks 0, 24 and 96. The Thai Chulalongkorn University and relevant US institutional review boards approved the studies. All subjects gave informed consent.

Laboratory methods

Diagnosis of AHI

Samples from clients who received voluntary counselling and testing for HIV at the Thai Red Cross Anonymous Clinic were screened in real time by pooled nucleic acid testing (NAT) and sequential immunoassay according to published methods [25]. Briefly, samples were first screened with HIV antigen/antibody combination detection assay. Non-reactive samples underwent pooled NAT, whereas reactive samples underwent a less sensitive immunoassay. Individuals with either positive NAT and non-reactive HIV IgM (Fiebig I/II) or reactive HIV IgM and negative or indeterminate Western blot (Fiebig III/IV) at screening were eligible for enrolment.

HIV quantification

HIV RNA in plasma was performed using Roche Amplicor v 1.5 ultrasensitive assay with a lower quantitation limit of 50 copies/mL (Roche Diagnostics, Branchburg, NJ, USA). The Siemens Quantiplex HIV-1 3.0 assay (Siemens, New York, NY, USA) was used to measure HIV RNA in gut tissue as previously described [3].

HIV DNA in PBMCs and sigmoid colon

Quantifications of total HIV DNA, integrated HIV DNA and 2-LTR circles were performed as described previously [26], using assays specifically designed for CRF01_AE and B. Briefly, a modified nested PCR was used to quantify HIV DNA and CD3 gene copy numbers. As a standard curve for both quantifications, dilutions

of ACH2 cells (NIAIDS reagent program) ranging from 36,105 to three cells were amplified together with experimental samples. HIV sequences and the CD3 gene were co-amplified for 12 cycles in triplicate wells. PCR products were diluted and HIV and CD3 copy numbers were determined in separate second amplification reactions on the Rotor-gene Q instrument (Qiagen, Valencia, CA, USA). The limit of detection of these assays was one copy per reaction tube.

Plasma levels of biomarkers and immunophenotyping of PBMC

C-reactive protein (CRP) was measured by electrochemiluminescence (Meso Scale Discovery, Gaithersburg, Maryland, USA). Immunophenotyping was performed on cryopreserved PBMCs as described earlier [13]. In brief, cells were first stained with Aqua Live/Dead dye (Invitrogen, Eugene, Oregon, USA). Subsequently samples were stained with the following antibodies to identify the different cell subsets: anti-CD3 PE-Cy7 (Invitrogen, Eugene, Oregon, USA), anti-CD4ECD (Beckman Coulter, Brea, CA, USA), anti-CD8 PerCPy5.5, anti-HLA-DR V450 and anti-CD38 APC (BD Bioscience, San Jose, CA, USA) and anti-Ki67 FITC (BD Pharmingen, San Diego, CA, USA). After staining, cells were resuspended in 1% formaldehyde and acquired within 24 hours using a custom built BD LSRII or Fortessa flow cytometer (BD, San Jose, CA, USA) and analysed using FlowJo software version 9.6.3 or higher (TreeStar, Ashland, OR, USA). At least 100,000 live cells were acquired in the lymphocyte gate.

CD4ECD (Beckman Coulter, Brea, CA, USA), anti-CD8 PerCPy5.5, anti-HLA-DR V450 and anti-CD38 APC (BD Bioscience, San Jose, CA, USA) and anti-Ki67 FITC (BD Pharmingen, San Diego, CA, USA). After staining, cells were resuspended in 1% formaldehyde and acquired within 24 hours using a custom built BD LSRII or Fortessa flow cytometer (BD, San Jose, CA, USA) and analysed using FlowJo software version 9.6.3 or higher (TreeStar, Ashland, OR, USA). At least 100,000 live cells were acquired in the lymphocyte gate.

Statistical analysis

This analysis included the first 62 subjects who were randomised to megaHAART vs HAART. The non-parametric Mann–Whitney U-test was used to compare the frequency of cells harbouring HIV DNA in PBMCs as well as other continuous variables between treatment arms. Change in HIV DNA levels from baseline was examined by Wilcoxon signed-rank test. Correlation between variables was assessed by Spearman's rank correlation coefficient. Plasma HIV RNA slope during the first 12 weeks after ART was calculated using the random effects model. Survival analysis was used to estimate the median time from baseline to undetectable plasma HIV RNA (<50 copies/mL). Area under the curve (AUC) of plasma HIV RNA between baseline and week 24 was calculated using the trapezoidal method. Statistical tests were 2 sided and P values <0.05 were considered statistically significant. Statistical analyses were performed using Prism version 5.01 software (GraphPad Software Inc.) and Stata Statistical Software: Release 12 (StataCorp LP).

Results

Baseline characteristics

Between April 2009 and April 2012, 52,767 samples were screened and 89 individuals were diagnosed with AHI. Of these, 75 enrolled in the study and 73 elected to initiate treatment. Eleven of 73 were not included in this analysis because they were enrolled prior to HAART vs megaHAART randomisation ($n=10$) or did not have baseline HIV DNA value ($n=1$). Therefore, 62

subjects were included and were randomly allocated: 31 to the megaHAART and 31 to the HAART arms (Figure 1). Table 1 shows the similarities of the baseline characteristics between arms. The subjects were mainly young MSM with a median time from HIV exposure of 17 days. The majority were in Fiebig III followed by Fiebig I acute HIV stages. The median HIV RNA was 5.6 log₁₀ copies/mL. Frequency of cells with total HIV DNA and 2-LTR circles in PBMCs tended to be higher in the megaHAART arm but these did not reach statistical significance. Most were infected with CRF01_AE and R5 tropic virus. Genotypic drug resistant mutations were observed in three patients. One had mutations to NRTI (T215F), NNRTI (Y181C) and protease inhibitor (M46I), one had NNRTI mutations (G190A and Y181C) and one had NRTI mutation (M41L).

Safety profile and discontinuations

The adverse events from antiretrovirals and their discontinuation rates did not differ between arms. Of 62 patients treated, eight (five in megaHAART and three in HAART arms) discontinued efavirenz because of adverse events ($n=6$) or primary NNRTI resistance ($n=2$). The adverse events were dizziness ($n=5$) and alanine aminotransferase elevation ($n=1$). The median time from baseline to switch was 12 [interquartile range (IQR) 9–14] days. There were 38 grade 3 and 4 events (23 in megaHAART and 15 in HAART, $P>0.05$). Most events (30/38) were related to treatment, and all of these were assessed to be due to efavirenz with the most common events being central nervous system-related symptoms ($n=15$) and elevated liver function test results ($n=14$). The five patients in the megaHAART arm who discontinued efavirenz were treated with the remaining four drugs (tenofovir/emtricitabine/raltegravir/maraviroc) until they discontinued maraviroc as per protocol at week 24, whereas three in the HAART arm took tenofovir/emtricitabine/raltegravir for the remainder of the study.

Adherence by history was similar between arms. From weeks 0–24, eight megaHAART patients reported missing a median of one dose (range 1–14) whereas four HAART patients reported missing a median of two doses (range 1–2; $P=0.34$). Over the course of 96 weeks, nine megaHAART and six HAART patients reported missing a median of 1 (range 1–14) and 2 (range 1–7) doses, respectively ($P=0.55$).

Clinical parameters

The median HIV RNA in plasma declined more rapidly during the first 4 weeks in the megaHAART arm. The rate of viraemic decline during the first 4 weeks was 3.3 [95% confidence interval (CI) 3.0–3.5] log₁₀ copies/mL in the megaHAART and 2.6 (95%CI 2.4–2.8) log₁₀ copies/mL in the HAART arm. This resulted in a statistically significant faster time to achieve plasma HIV RNA <50 copies/mL of 55 (IQR 35–102) days with megaHAART vs 83 (IQR 54–131) days with HAART ($P=0.04$). By week 12, plasma HIV RNA was comparable between arms and remained so until week 96 (Figure 2a). The proportion of subjects displaying plasma HIV RNA levels below 50 copies/mL was not statistically different between the megaHAART and HAART arms: 77% vs 58% at week 12, 90% vs 87% at week 24, 93% vs 100% at week 48 and 93% vs 100% at week 96 ($P>0.05$). The AUC of plasma HIV RNA between weeks 0 and 24 was 51.8 in the megaHAART and 56.3 in the HAART arm ($P=0.06$). After achieving HIV RNA suppression, three patients in the megaHAART arm had viral load blips (range 57–937 copies/mL) as did four in the HAART arm (range 52–102 copies/mL). One patient in the megaHAART arm experienced treatment failure at week 48 and was lost to follow-up at week 156. One patient in the HAART arm self-interrupted treatment at weeks 36 and 72 with corresponding

Table 1. Baseline characteristics of acutely HIV-infected subjects

Characteristics	All	MegaHAART [†]	HAART [†]
Median (IQR) age, years	28 (24–34)	25 (23–30)	30 (24–34)
Gender male:female, <i>n</i>	58:4	29:2	29:2
Risk behaviour, <i>n</i> (%)			
MSM	58 (94)	29 (94)	29 (94)
Heterosexual female	4 (4)	2 (4)	2 (4)
Median (IQR) duration since history of HIV exposures, days	17 (12–22)	17 (12–22)	16 (12–23)
Fiebig stage, <i>n</i> (%)			
I (RNA+, p24 antigen-, HIV IgM-)	22 (35)	10 (32)	12 (39)
II (RNA+, p24 antigen+, HIV IgM-)	5 (8)	2 (6.5)	3 (10)
III (HIV IgM+/WB-)	29 (47)	16 (52)	13 (42)
IV (HIV IgM+/WB indeterminate)	3 (5)	1 (3)	2 (6)
V (HIV WB+ without p31) [‡]	3 (5)	2 (6.5)	1 (3)
Acute retroviral syndrome, <i>n</i> (%)	49 (79)	25 (81)	24 (77)
Median (IQR) CD4 cells/mm ³	388 (293–538)	392 (339–559)	352 (257–534)
Median (IQR) plasma HIV RNA, log ₁₀ copies/mL	5.6 (5.1–6.1)	5.6 (5.1–6.1)	5.5 (4.9–6.2)
Median (IQR) colonic HIV RNA, copies/mg tissue	3.1 (2.6–4.0)	3.0 (2.8–4.0)	3.1 (2.0–4.3)
Median (IQR) HIV DNA in PBMCs, copies/10 ⁶ cells			
Total	94 (7–550)	135 (7–814)	63.4 (3–366)
Integrated	0 (0–33)	0 (0–33)	0 (0–14)
2-LTR circles	16 (0–84)	20 (0–66)	9 (0–201)
Major drug mutations, <i>n</i> (%)			
NRTI	2 (3)	1 (3)	1 (3)
NNRTI	2 (3)	1 (3)	1 (3)
PI	1 (2)	0 (0)	1 (3)
HIV subtype by MHAbsce*, <i>n</i> (%) <i>n</i> =59			
CRF01_AE	52 (88)	24 (80)	28 (97)
B	1 (2)	1 (3)	0 (0)
CRF01_AE/B recombinant	4 (7)	3 (10)	1 (3)
Non-typable	2 (3)	2 (7)	0 (0)
Tropism by Trofile, <i>n</i> (%)			
R5	45 (73)	22 (71)	23 (74)
Dual R5/X4	3 (5)	2 (6)	1 (3)
Unable to be amplified	14 (22)	7 (23)	7 (23)

[†]*P* value >0.05 for all parameters; [‡]Three patients at Fiebig IV at screening progressed to Fiebig V at enrolment and were included in the analysis;

*Multiregional hybridisation assay

Ig: Immunoglobulin; WB: Western blot; PBMC: peripheral blood mononuclear cells; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor

viral load rebound at both time points. He subsequently achieved viral suppression on the same regimen.

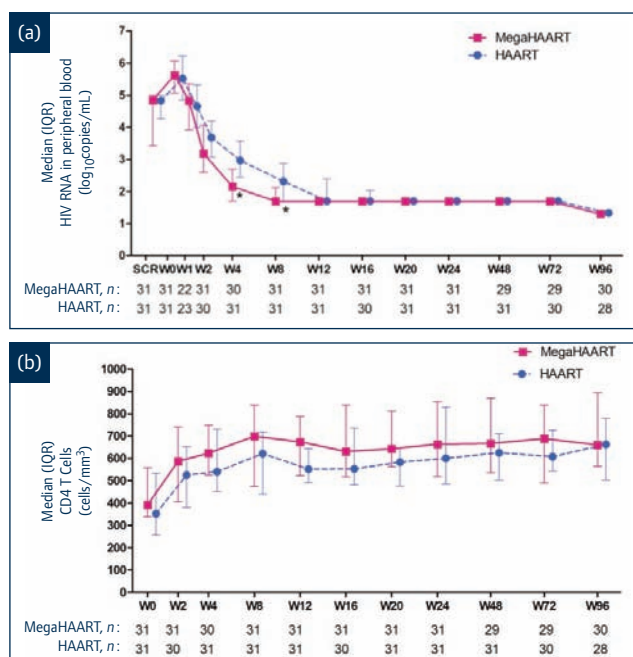


Figure 2. Median (a) plasma HIV RNA and (b) CD4 T cell counts after megaHAART or HAART initiated in acute HIV infection (*P* value <0.05).

The total CD4 cell counts were not different between arms (Figure 2b). Overall, there was a rapid rise in CD4 cell count of 216 cells/mm³ within the first 8 weeks after ART (211 cells/mm³ and 218 cells/mm³ in the megaHAART and HAART arms, respectively (*P*=0.45). By week 24, the total CD4 cell counts were 663 (IQR 520–853) cells/mm³ in the megaHAART arm and 600 (IQR 486–830) cells/mm³ in the HAART arm.

Proviral and viral burden in the peripheral blood and sigmoid colon

The median frequency of PBMCs harbouring total HIV DNA was comparable between arms, except for the transiently elevated value at week 24 in the megaHAART arm that was likely to have been driven by an increase in total HIV DNA levels in two patients who had a temporary rise in their plasma HIV RNA (Figure 3a). The median fall in total HIV DNA from baseline to week 12 was 109 (IQR 0–659) copies/10⁶ PBMCs in the megaHAART arm vs 42 copies/10⁶ (IQR 7–267) copies/10⁶ PBMCs in the HAART arm (*P*=0.82). Median total HIV DNA after treatment was low, and not different between the megaHAART and HAART arms: 13 copies/10⁶ (IQR 2–61, min 0–max 344) vs 3 (IQR 0–35, min 0–max 284) copies/10⁶ PBMCs at week 48 and 7 (IQR 0–23, min 0–max 127) vs 4 (IQR 0–29, min 0–max 53) copies/10⁶ PBMCs at week 96 (*P*=0.41). The median value for integrated HIV DNA in PBMCs reached 0 at all time points after ART in both groups (data not shown). The number of 2-LTR circles per million PBMCs was higher in the megaHAART arm at weeks 2 and 12. At week

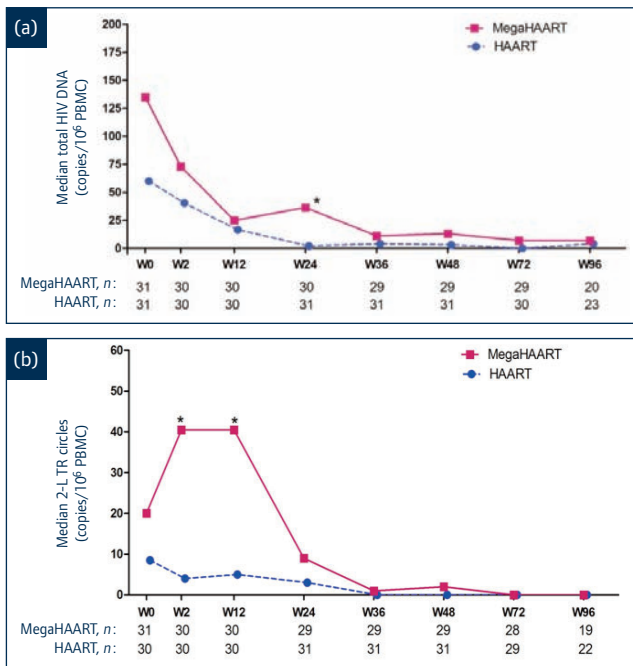


Figure 3. Total (a) HIV DNA and (b) 2-LTR circles in peripheral blood mononuclear cells after megaHAART or HAART in acute HIV infection (P value <0.05)

12, the median number of 2-LTR circles was 41 (IQR 1–139) for megaHAART vs 3 (IQR 0–16) for HAART ($P=0.03$) (Figure 3b).

In 25 subjects who underwent sigmoid colon biopsy at baseline (15 in the megaHAART and 10 in the HAART arm), the median gut HIV RNA was 3.1 (IQR 2.6–4.0) copies/mg tissue [3.0 (IQR 2.8–4.0) and 3.1 (IQR 2.0–4.3) copies/mg tissue in the megaHAART and HAART arms, respectively; $P=1.00$]. In participants who agreed to repeated biopsy, 11/12 (92%) in the megaHAART and 7/7 (100%) in the HAART arm had gut HIV RNA below 50 copies/mg tissue at week 24. At week 96, this was achieved in 3/5 megaHAART and 4/4 HAART patients.

Total HIV DNA values in cells from the sigmoid colon were similar between arms for all time points (Figure 4). After 24 weeks of treatment, the median values for total HIV DNA were 87 (IQR 18–109, min 0–max 570) and 0 (IQR 0–120, min 0–max 298) copies/10⁶ cells in the megaHAART and HAART arms, respectively; this difference was not statistically significant ($P=0.16$). At week 96, the median total HIV DNA was 10 (IQR 0–62, min 0–max 342) and 3 (IQR 0–6, min 0–max 6) copies/10⁶ cells in the megaHAART and HAART arms, respectively. A similar total HIV DNA decline from baseline to week 24 was observed between arms: megaHAART arm had a median fall of 888 (IQR 213–10,318) vs 610 (IQR 0 to 1,587) copies/mg tissue in the HAART arm ($P=0.3$). The integrated HIV DNA tended to be higher in the megaHAART arm at baseline (median 58, IQR 0–232 copies/mg tissue) vs the HAART arm (median 0, IQR 0–122 copies/mg tissue; $P=0.48$). After treatment, the median values of integrated HIV DNA reached 0 at weeks 24 and 96 in both groups. The numbers of 2-LTR circles were significantly higher in the megaHAART arm only at week 24: median values were 4 (IQR 0–19) in the megaHAART and 0 (IQR 0–0) in the HAART arm ($P=0.03$).

Markers of inflammation and immune activation

The levels of CRP, the clinical marker for inflammation, were not different between arms at all time points (Figure 5). The median values for CRP were 1.25 (IQR 0.68–0.45) and 1.45 (IQR 0.66–3.40) mg/L in the megaHAART and HAART arms at baseline, respectively. They declined to 0.51 (0.16–1.38) and 0.47

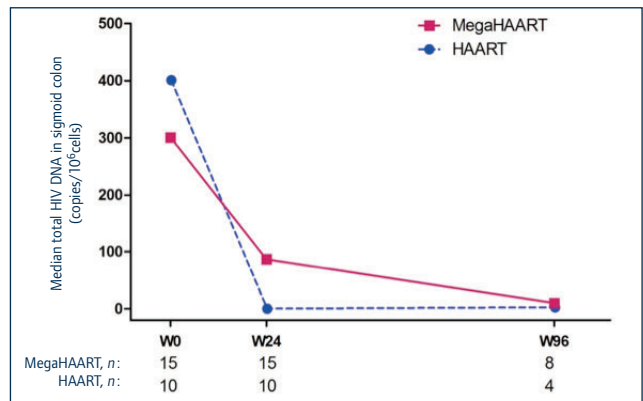


Figure 4. Total HIV DNA in sigmoid colon after megaHAART or HAART in acute HIV infection

(0.15–0.73) mg/L at week 96, respectively ($P=0.02$, compared to baseline).

The frequencies of activated CD8+ T cells (CD38+ and HLA-DR+; Figure 6) and cycling CD8+ T cells (Ki67+, data not shown) were not different between arms at any time point. They declined significantly after initiation of treatment in both arms at week 96 ($P<0.05$). At baseline, the frequencies of activated CD8+ T cells were 14% (IQR 9–17) and 14% (IQR 8–20) in the megaHAART and HAART arms, respectively ($P=0.77$). These reduced to 5% (IQR 3–8) and 5% (IQR 3–6), respectively, at week 96 ($P<0.05$, compared to baseline). The frequencies of cycling CD8+ T cells at baseline were 9% (3–11) and 3% (1–6) for the megaHAART and HAART arms, respectively, and 0.82 (0.67–1.61) and 0.82 (0.37–1.1), respectively, at week 96 ($P<0.05$, compared to baseline). There was also a significant reduction in the frequencies of activated and cycling CD4+ T cells between baseline and 96 weeks post-ART initiation in both arms. However, there were no differences between the megaHAART and HAART group (data not shown).

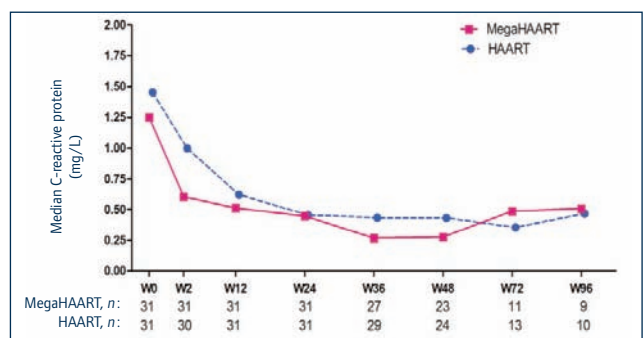


Figure 5. Median levels of plasma CRP after megaHAART or HAART in acute HIV infection

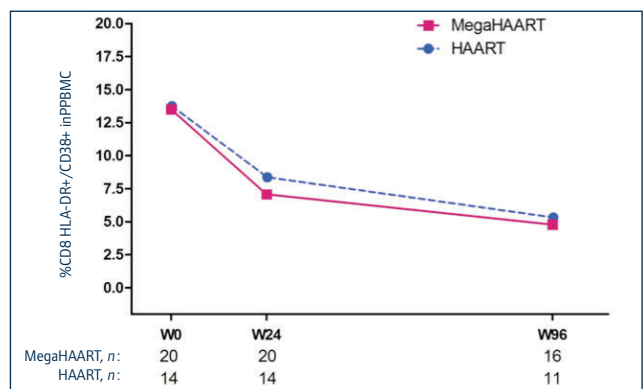


Figure 6. Frequencies of activated CD8+ T cells (CD38+ and HLA-DR+) after megaHAART or HAART in acute HIV infection

Discussion

In individuals treated during AHI in our study, intensification of standard NNRTI-based HAART with raltegravir and maraviroc during the first 24 weeks resulted in comparable levels of HIV reservoir size and immune activation compared with standard HAART alone. Although plasma HIV RNA did decline more rapidly during the first 4 weeks in the megaHAART arm, the frequencies of peripheral blood and sigmoid cells harbouring HIV DNA after treatment did not differ between arms. Levels of CRP and frequencies of activated T cells were also similar between treatments. Regardless of the regimens used, markers of the HIV reservoir size and immune activation declined with treatment.

Acute HIV infection is highly dynamic in the first month of infection when the virus multiplies rapidly before the body is able to mount an effective immune response against it [27]. During this period, infection of long-lived cells establishes HIV persistence and gut CD4 T cells are massively depleted, igniting the vicious cycle of inflammation and immune activation [28]. Emerging data support the role of early ART in containing the HIV reservoir and CD4 depletion [4–6,9]. In chronic HIV infection, despite successful ART, the HIV reservoir size remains large and stable [29,30]. Consistent with our previous data [3], we have demonstrated that when treatment is initiated within the first month of infection, a marked decline in proviral HIV DNA can be achieved. In the ANRS PRIMO cohort, proviral DNA levels were significantly lower when ART was initiated by 15 days vs 3 months after infection [31].

Our decision to intensify an NNRTI-based standard regimen with raltegravir and maraviroc during the first 24 weeks of acute HIV treatment was based upon the knowledge of the viral dynamics during AHI [32] and the mode of action of these drugs [16,17]. It was hypothesised that adding drugs that can block cellular entry (maraviroc) and integration (raltegravir) to a regimen with NRTIs (tenofovir and emtricitabine) and NNRTI (efavirenz) could be beneficial in reducing the viral burden and HIV reservoir seeding. This did not manifest in this study possibly because the NRTI/NNRTI-based standard regimen was already effective in reducing the HIV viral burden. The difference in plasma HIV RNA between arms occurred early at weeks 4 and 8, and by week 12, the majority had achieved viral undetectability. Two other randomised studies observed similar outcomes with PI-based regimens. Markowitz *et al.* reported a more rapid initial plasma HIV RNA decline in the five-drug arm (tenofovir/emtricitabine/ritonavir-boosted darunavir/raltegravir/maraviroc, $n=26$) compared to the three-drug arm without raltegravir/maraviroc ($n=14$). The cell-associated HIV DNA and HIV RNA, replication-competent virus, activated T cells and sCD14 after 96 weeks did not differ between arms [16]. Ninety patients in the OPTIPRIM study were randomly allocated to the same regimens as in the Markowitz study, and proviral DNA in PBMCs was similar between arms at week 96. More patients in the five-drug arm had low-level viraemia attributed to poor adherence [17]. A non-randomised study also showed no benefit to HIV RNA and CD4 levels with either raltegravir or maraviroc intensification [14]. The addition of maraviroc to a raltegravir-based regimen during AHI in another study facilitated a slightly faster but modest decline in proviral DNA compared to a raltegravir-based regimen [33]. We observed higher levels of 2 LTR circles in PBMCs and gut in the megaHAART arm during the first 24 weeks, which were likely to be due to raltegravir blocking proviral integration, as previously described [20,34].

Given the absence of detectable integrated DNA in PBMCs and gut, analytical treatment interruption (ATI) would be the other

measure of the difference between the two regimens. The absence of detectable reservoir has not necessarily predicted the outcome of ATI [7,35,36]. Carefully executed ATI could potentially permit the identification of correlates for control of viraemia, and establish a reservoir assay reasonably predictive of remission outcome.

An important observation from this study was the high rates of efavirenz-related toxicities leading to grades 3 and 4 adverse events in 10 (16%) and efavirenz discontinuation in eight (13%) patients. These are markedly higher than those reported in Thai patients who initiated efavirenz-based treatment in chronic HIV infection [37]. It is possible that concomitant acute retroviral syndrome during AHI could potentiate symptoms and signs related to efavirenz.

Several published studies corroborated our findings that early treatment reduces the frequencies of activated T cells and levels of soluble inflammatory biomarkers [11,16] but some did not [38,39]. We did not observe differences in markers of immune activation between arms. Published studies have reported favourable inflammatory profiles with maraviroc [18,40,41], but some offered an opposing view [19,42]. Whether or not using maraviroc for longer than 24 weeks could have a greater benefit on markers of immune activation is unclear. The implication of the more rapid decline in HIV RNA with megaHAART on infectiousness and risk of onward HIV transmission is a subject of ongoing investigation in our study.

The strength of this study is in its randomisation design, the inclusion of participants who initiate ART as early as 2–3 weeks following onset of infection and the gut tissue sampling in a subset of subjects. However, we recognise the inherent limitation of the small sample size that could have resulted in our inability to observe statistically significant differences between arms. Because raltegravir was substituted for efavirenz in cases of toxicity and resistance, a small number of patients in the HAART group were on raltegravir. Analysis that excluded these patients showed similar results (data not shown). There were also a few subjects with dual R5/X4 virus that would render maraviroc ineffective. Excluding these patients did not change the results (data not shown). We are also aware of the limitations of tests, including ours, in detecting small HIV reservoir size. We demonstrated HIV DNA values that were lower than those previously reported [10,43,44] with some patients achieving levels below the detection threshold of one copy per million PBMCs, which could be due to the earlier onset of ART in our study.

In summary, in AHI patients who were randomised to NRTI/NNRTI-standard regimen vs standard regimen intensified with raltegravir and maraviroc, we did not observe differences between arms for markers of HIV reservoir size and immune activation. Both types of treatment resulted in the decline of all markers. A larger sample size in each randomised arm, and further characterisation of the replicative ability of the HIV reservoir and of other inflammatory biomarkers in blood and tissues will contribute to the knowledge gaps on timing and selection of ART for the treatment of AHI.

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