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The Effect of Successful Antiretroviral Therapy on Immune Activation and Reconstitution in HIV-Infected Adults: A Systematic Review and Meta-Analysis

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Abstract

We performed a systematic review and meta-analysis to investigate the impact of antiretroviral therapy (ART) on immune activation and reconstitution in people living with HIV (PLWH). The PubMed electronic database and gray literature were searched from inception until March 2020. Studies were included if they reported the levels of immune activation and reconstitution at baseline and post-treatment. The random-effect model was used to calculate effect sizes. We included a total of ten studies comprising of 1 553 PLWH with an average age of 38.02 ± 10.10 years and a male/female ratio of 3.76. Pooled estimates showed a modest increase in the level of immune activation post-treatment (SMD: 0.64 [95% CI: -1.34, 2.63]; I^2 = 98%, p^H < 0.00001). In addition, treatment with ART significantly reconstituted the immune system (SMD: 0.70 [95% CI: 0.27, 1.44]; I^2 = 68%, p^H = 0.009). Notably, the level of immune reconstitution was independent of viral load or the treatment duration but dependent on the class of ARV drugs. Consequently, protease inhibitors were associated with the highest degree of immune restoration, followed by chemokine antagonists and lastly integrase inhibitors. In conclusion, immune activation persists in PLWH despite viral suppression and the degree of immune reconstitution is dependent on the drug class. Therefore, inclusion of protease inhibitors in ART may be of great benefit in immune restoration in patients with very low CD4 count. ***(AIDS Rev. 2020;22:1-12)***

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Key words

Antiretroviral Therapy. HIV. Immune Activation and Reconstitution. T-Cells.

Introduction

The progressive loss of T-helper (CD4) cells and chronic immune activation is a hallmark of HIV infection[1,2]. In fact, in people living with HIV (PLWH), increased levels of plasma viral RNA have been associated with immune suppression and by-stander death of CD4 T-cells[2,3]. Moreover, persistent immune activa-

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tion can induce a state of immune dysfunction[3,4], which gradually leads to T-cell exhaustion[5,6] and the development of AIDS[7]. In PLWH, viral replication is associated with the development of cardiovascular, gastrointestinal, pulmonary, metabolic, hepatic, and renal complications that accelerate the mortality rate of these patients[8]. Fortunately, the discovery of antiretroviral (ARV) drugs and the initiation of combined antiretroviral therapy (cART) strategies such as highly active ART (HAART) has increased the overall life span of PLWH, which is now almost similar to that of uninfected individuals[9–11]. In addition, the incidence of opportunistic infections has been significantly reduced while the general quality of life of PLWH has been improved[11,12]. However, the effects of ART on immune activation and reconstitution remains unclear and controversial[12,13].

Chronic inflammation and immune activation in PLWH has been linked to immune dysfunction which is associated with an increased risk of cardiovascular disease[14–16]. The expression of CD38 and HLA-DR as well as their coexpression on T-cells has been widely accepted as markers of generalized immune activation[5,6,17]. In that context, the degree of T-cell activation in PLWH on cART directly influences on the levels immune reconstitution defined by CD4 T-cell gains[18,19]. Notably, immune activation is associated with viral load and the expression of HLA-DR and CD38 on CD8 T-cells[20]. However, in PLWH on ART, increased expression of CD38 and HLA-DR on T-cells is associated with the rapid decline in CD4 T-cell counts and clinical progression to AIDS independently of HIV ribonucleic acid (RNA) levels[21,22]. Increased levels of T-cell activation concomitant with poor immune reconstitution have also long been described in PLWH on cART[18,19]. Notably, the levels of immune activation[23,24] and reconstitution[25-27] following cART are highly variable, with age, CD4 nadir level, viral load, and coinfections being some of the dependent factors[1,28]. Although recently published reviews provided an overview of HIV-associated inflammation and immune exhaustion in PLWH[14,16], to the best of our knowledge, these aspects have not yet been qualitatively or quantitatively synthesized. Moreover, the impact of ARV drug classes on immune restoration has not been assessed. Therefore, this systematic review and meta-analysis aimed at assessing how successful ART influences immune activation and to further explore how the effect of different classes of antiretroviral drugs influence the degree of T-cell recovery in PLWH.

Methods

This systematic review and meta-analysis were prepared and conducted following the preferred reporting items for systematic-reviews and meta-analysis (PRISMA) guidelines[29]. Although this systematic review and meta-analysis has no registered protocol, we searched the international prospective register of systematic reviews to ensure there is no registered systematic review on a similar topic. This study was conducted to address the following questions;

Question 1: Is successful ART in PLWH associated with reduced levels of generalized immune activation?

Question 2: Are the variable outcomes regarding immune reconstitution in PLWH associated with different classes of ARV drugs?

Search strategy

A systematic search was conducted on the MEDLINE and ProQuest gray literature databases, for relevant randomized controlled trials (RCT's) from inception until on March 23, 2020. Briefly, the MEDLINE database search strategy was adapted without any language restrictions using medical subjects heading (MeSH) terms and keywords such as "CD38", "HLA-DR", "HIV", "T-cells", and their respective synonyms and associated words or phrases. The PubMed search strategy is summarized in supplementary file (Table 1S). The search was conducted by two independent reviewers (TMN and BBN) and inconsistencies were resolved through discussion and arbitration by a third reviewer (PVD).

Study selection criteria

The selection of relevant RCT's was independently conducted by two reviewers (TMN and VM) with the help of third review (PVD) in cases of disagreements. Briefly, studies involving adult PLWH on ART that reported on the primary and secondary outcomes of immune activation were included in the study. The selected studies were only included in the meta-analysis based on the availability of study-level data. Observational studies, reviews, books, editorials, and letters were excluded from this study.

Outcomes

The primary outcome of this systematic review and meta-analysis was immune activation which was

measured by the expression of CD38 or HLA-DR expression on T-cells. While the secondary outcome was immune reconstitution, which was evaluated by the levels of circulating T-cell counts. Both outcomes were continuous and were reported as standardized mean difference (SMD).

Data extraction and management

Two independent investigators (TMN and VM) extracted study-level data items using a pre-defined data extraction sheet. In cases of disagreements, a third reviewer (BBN) was consulted for arbitration. The extracted data items included the names of the authors, year of publication, sample size, age of included participants, viral load, ARV drugs administered and duration of treatment, the levels of CD4 and CD8, the expression of CD38 and HLA-DR on CD4 and CD8 T-cells, and the main findings. The study level data items were exported to Review Manager Version 5.3 software (Cochrane Collaboration, Oxford, UK) for statistical analysis.

Assessment of risk of bias and quality of evidence

The risk of bias of included studies was independently assessed by two reviewers (TMN and VM) using the modified Downs and Black checklist[30]. Discrepancies in rating were resolved through discussion or consulting third reviewer (BBN). This checklist assesses four domains, namely, reporting bias, external validity, internal validity, and selection bias. The overall scores were rated as; poor if the score was (< 12 points), fair if (13-18 points), good if (19-23 points), and excellent if the score was 24-27. The quality of evidence was assessed using the Grading of Recommendations Assessment Development and Evaluation (GRADE) approach[31].

Statistical analysis

Cohen's kappa scores were used to measure inter-rater reliability and a score of 0.00 was considered poor, (0.01-0.20) slight, (0.21-0.40) fair, (0.41-0.60) moderate, (0.61-0.80) substantial, and (0.81-1.00) perfect[32]. The mean and standard deviation for each continuous effect measure was extracted or calculated from median range using *Hozo* et al. method[33]. In cases where the mean and 95% confidence interval (CI) were reported, the standard deviation was estimated using the Cochrane guidelines[34]. The Higgin's I^2 index[35] was used to quantify the levels of heterogeneity and a fixed or random-effects model was used depending on the degree of statistical heterogeneity[36]. Statistical significance of heterogeneity was reported as P^H and $P < 0.05$ was considered statistically significant. The effect estimates were reported as SMD and 95% CI and were interpreted using the Cohen's *d* method[37], whereby an SMD of 0.2, 0.5, and 0.8 was equated to small, medium, and large, respectively.

Sensitivity analysis and publication bias

We performed a sensitivity analysis to test the robustness of the reported effect estimates, by following a step-wise omission of studies. This was accomplished by performing a repeated the meta-analysis based on characteristics of participants and study design. Briefly, the sensitivity analysis was performed based on viral load and treatment duration. Publication bias was assessed by visual inspection of funnel plots, whereby a perfect symmetry is indicative of no publication bias.

Results

Study selection

We identified 24 citations through PubMed electronic database. A total of ten studies were excluded at the abstract screening stage, as these were deemed irrelevant and not describing findings related to the outcomes of the present study. The full-texts of the remaining 14 studies were assessed for eligibility and four studies were excluded due to study designs (n = 4). As a result, a total of ten studies met the inclusion criteria (overall agreement 92%, kappa = 0.84) and were included in this systematic review and meta-analysis whereas only six studies included in the quantitative synthesis (Fig. 1).

Characteristics of included studies

The characteristics of included studies are shown in Table 1. Briefly, the included studies were randomized controlled trials published between 1996 and 2014 from Europe (n = 4)[23,27,38,39], North America (n = 3)[24,25,40], Australia (n = 2)[26,41], and Africa (n = 1)[42]. In total, the included studies comprised 1 553 participants with an average age of 38.02 ± 10.10 years and a male/female ratio of 3.76. A total of 1414 participants

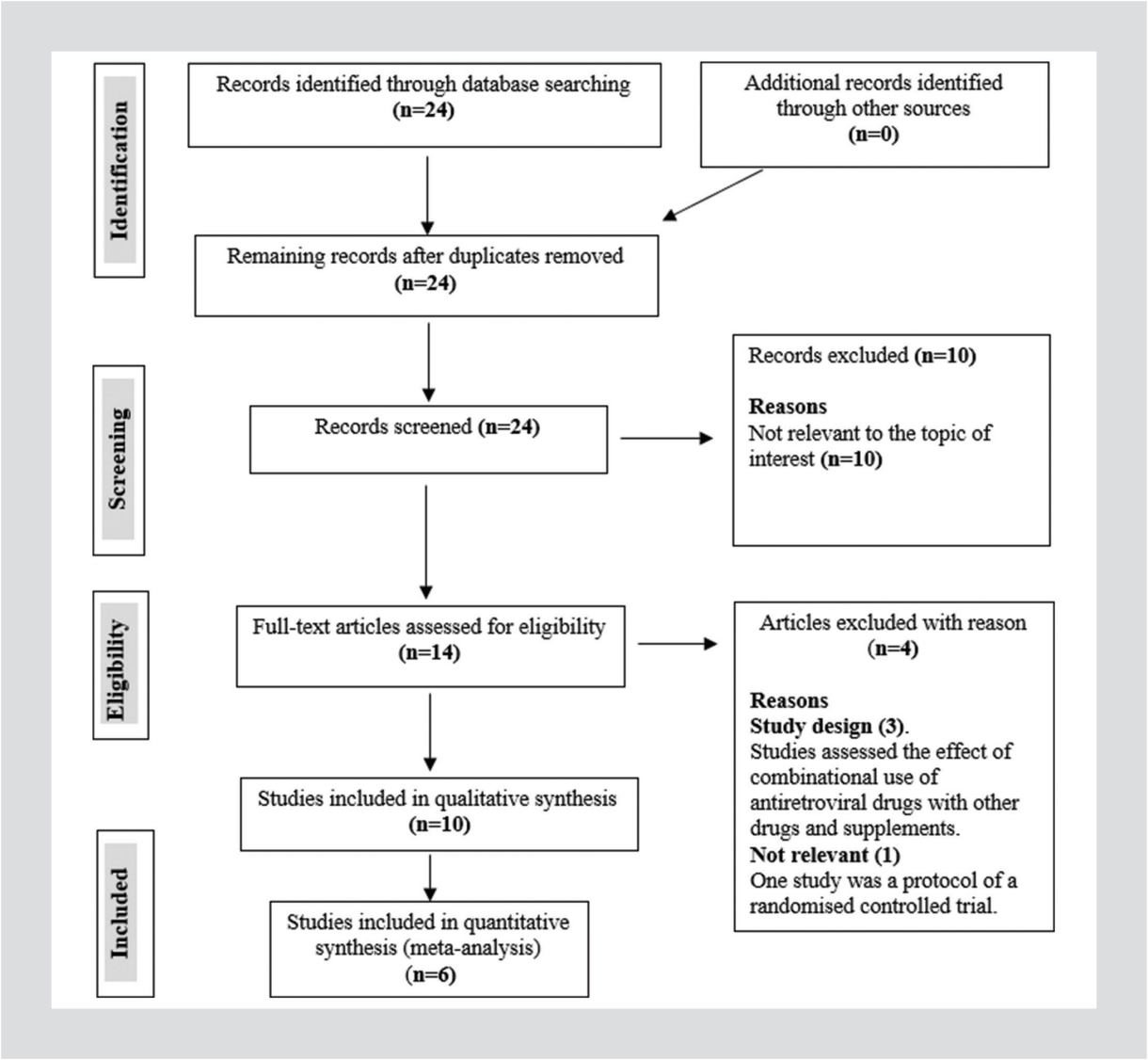

Figure 1. *PRISMA flow diagram illustrating the study selection process.*

were on HAART and 139 on intensified HAART with a median treatment duration of 12 (3-36) months.

Quality assessment and risk of bias

The quality of evidence presented herein was rated as high due to the nature of study designs and low risk of bias of included studies. The median range of the overall risk of bias in all included studies was 20 (15-24) with the majority of studies scored as good[23,25–27,38,39,41,42] and a few as excellent[24] or fair[40] (Table 2S). The median range in the reporting bias was 9 (8-10) (overall agreement 96%, kappa = 0.92) and the median external validity score was 2 (1-2) (overall agreement 80%, kappa = 0.60). In addition, the median range in selection bias was 4 (2-5) and internal validity was 5 (3-7) in domain.

Data synthesis

The meta-analysis involved a total of six studies comprising 224 participants, of which 59 were on integrase inhibitors, 68 on the chemokine antagonist (C-C chemokine receptor type 5 [CCR5]), 29 on protease inhibitors and 78 unspecified ARV classes. Raltegravir, an integrase inhibitor was reported in two of the included studies[25,27], and so was maraviroc[23,24], CCR5 antagonist and ritonavir[38,41], protease inhibitor. Cohorts

Table 1. Characteristics of included studies reporting on the effect of antiretroviral treatment on immune activation and reconstitution (n = 10)

Author, year	Country	Study design	Male, n (%)	Age (years)	Reported effect measures	Main findings	Risk of Bias
Kelleher et al., 1996[41]	Australia	21 participants with CD4 cell counts > 50 cells/μL off treatment for 2 weeks prior to initiating ritonavir therapy for 5 months	NR	NR	CD38 and HLA-DR expression on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Treatment with ritonavir decreased viral load and the expression of CD38 on both CD4 and CD8 T-cells. However, the expression of HLA-DR on CD8 cells significantly increased while it remained constant on CD4 cells. Ritonavir treatment increased both CD4 and CD8 T-cells counts. Nonetheless, there was no correlation between viral load reduction and increased T-cell counts.	Low
Plana et al., 2000[38]	Spain	26 ART naïve participants with plasma HIV-1 RNA > 10 000 copies/ml HAART (n = 18) HAART plus protease inhibitor (n = 8) for 12 months	NR	NR	CD38 expression on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Both treatment regimens significantly reduced viral load with triple treatment lowering HIV-RNA to undetectable levels. In addition, treatment reduced the expression of CD38 on both CD4 and CD8 T-cells. Notably, the reduction in viral load directly correlated with the levels of $CD38^+CD8^+$ T-cells. Treatment regimens increased CD4 T-cell counts and reduced CD8 counts. The latter count correlated with viral load.	Low
Scott-Algara 2001[39]	France	355 ART naïve participants Group 1 Plasma HIV-1 RNA < 500 copies/ml (n = 240) Group 2 Plasma HIV-1 RNA > 500 copies/ml (n = 115) for 18 months	264 (74.4%)	35.7 ± 9.1	CD38 and HLA-DR expression on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Treatment significantly suppressed viral load in both groups. The levels of $CD4^+CD38^+$, $CD4^+HLA\text{-}DR^+$ and $CD4^+CD38^+HLA\text{-}DR^+$ T-cells significantly increased post-treatment in both groups. However, with respect to CD8 T-cells, the frequency of $CD8^+CD38^+$ and $CD8^+CD38^+HLA\text{-}DR^+$ we decreased in both groups with only $CD8^+HLA\text{-}DR^+$ increased in groups 2. ART increased CD4 T-cell count and reduced CD8 cells. The changes in T-cell counts were independent of the baseline viral load.	Medium
Dahl et al., 2011[25]	USA	23 participants on HAART for an average of 7 years with plasma HIV-1 RNA < 50 copies/ml 9 on HAART 14 on HAART plus raltegravir for 3 months	21 (91%)	53.93 ± 6.13	$CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Both treatment regimens did not alter viral load or the expression of $CD38^+HLA\text{-}DR^+$ on T-cells. HAART and intensified HAART increased and decreased CD4 T-cell count, respectively. However, both treatments reduced CD8 T-cell counts.	Low
Byakwaga et al., 2011[26]	Australia	35 participants on HAART for an average of 4.5 years with plasma HIV-1 RNA < 50 copies/mL 17 on HAART 18 on HAART plus raltegravir for 6 months	34 (97%)	52.35 ± 10.17	Frequency of $CD4^+$ T-cells	Treatment regiments did not alter CD4 cell count.	Low

(*Continues*)

Table 1. Characteristics of included studies reporting on the effect of antiretroviral treatment on immune activation and reconstitution (n = 10) (*Continued*)

Author, year	Country	Study design	Male, n (%)	Age (years)	Reported effect measures	Main findings	Risk of Bias
Llibre et al., 2012[27]	Spain	67 participants on HAART for an average of 5 years with plasma HIV-1 RNA < 50 copies/ml 22 on HAART 45 on HAART plus raltegravir for 12 months	NR	Over 18 years of age	$CD38^+HLA\text{-}DR^+$ expression on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Treatments did not alter viral load and the levels of $CD4^+CD38^+HLA\text{-}DR^+$ T-cells. However, it lowered the frequency of $CD8^+CD38^+HLA\text{-}DR^+$ T-cells. Both treatments increased the levels of CD4 and CD8 T-cell counts with the increase more pronounced in the intensified HAART group.	Low
Rusconi et al., 2013[23]	Italy	90 immunological non-responders on HAART for an average 9.03 years with plasma HIV-1 RNA < 50 copies/mL 45 on HAART 45 on HAART plus maraviroc for 12 months	74 (82%)	NR	$CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Viral load was significantly decreased in the HAART group only, whereas it remained unchanged in the intensified HAART. Both treatment regimens did not alter the expression of $CD38^+HLA\text{-}DR^+$ on T-cells except for intensified HAART which increased the expression of $CD38^+HLA\text{-}DR^+$ on $CD4^+$ T-cells. Intensified HAART increased CD4 T-cell count while HAART had no effect on the count. Moreover, both treatments regimens did not change CD8 counts.	Low
Roxby et al., 2013[42]	Kenya	58 pregnant women with HIV-1 and HSV-2 co-infection with CD4 count > 250 cells/L on HAART for 12 months	0 (0%)	25.25 ± 2.04	$CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells	Treatment lowered vial load but did not have an effect on the expression of $CD38^+HLA\text{-}DR^+$ on T-cells.	Low
Hunt et al., 2013[24]	USA	45 participants on HAART for an average of an average of 2.68 years with plasma HIV RNA < 500 copies/mL 22 on HAART 23 on HAART plus maraviroc for 6 months	43 (96%)	50.26 ± 3.01	$CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells Frequency of $CD4^+$ and $CD8^+$ T-cells	Treatment regimens lowered viral load. In addition, HAART reduced $CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells while intensified HAART increased the levels of $CD38^+HLA\text{-}DR^+$ on $CD8^+$ T-cells with no effect on CD4 T-cells. Both treatments increased CD4 counts. Moreover, intensified HAART significantly increased CD8 T-cell count with no change in the HAART only group.	Low
Zheng et al., 2014[40]	USA	833 participants on HAART with plasma HIV-1 RNA ≤ 200 copies/mL for 36 months	701 (84%)	38.2 ± 9.3	$CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells	Treatment lowered viral load and the expression of $CD38^+HLA\text{-}DR^+$ on $CD4^+$ and $CD8^+$ T-cells. Moreover, the degree of immune activation was directly and inversely proportional to viral load and $CD4^+$ T-cells, respectively.	Medium

ART: antiretroviral therapy; HAART, highly active antiretroviral therapy; NR, not reported; RNA, ribonucleic acid; USA, United States of America.

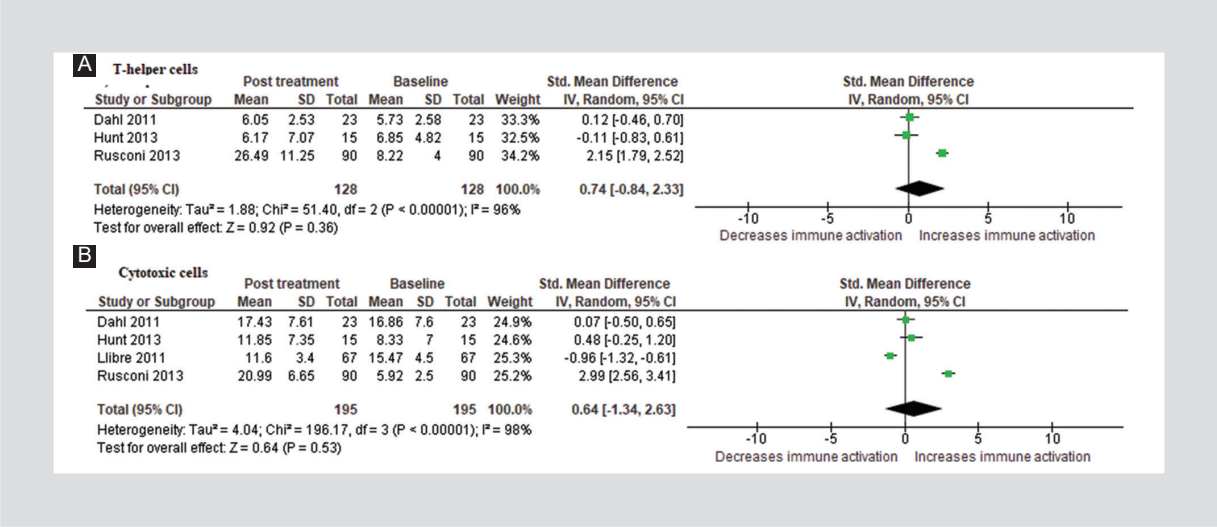

Figure 2. *The effect of ARV treatment on generalized immune activation measured by the expression of CD38⁺HLA-DR⁺ on CD4* **(A)** *and CD8* **(B)** *T-cells.*

of patients from the included three studies[23,25,27] were virologically suppressed with plasma HIV-1 RNA < 50 copies/ml.

Antiretroviral treatment in HIV-infected patients promotes immune activation

In all, 80% (n = 8) of included studies reported on the expression of CD38 and HLA-DR-markers on T-cells. A total of three studies[23,24,39] reported increased expression of CD38⁺HLA-DR⁺ on CD4 T-cells, and 1 study[40] demonstrated decreased expression on both CD4 and CD8 T-cells, while others[25,27,42] described comparable levels of these immune activation markers post-treatment. Other studies have reported on the expression of CD38 on T-cells whereby two of the studies[38,41] demonstrated decreased expression of CD38 on both CD4 and CD8 T-cells while the remaining study[39] showed elevated expression of CD38 on CD4 T-cell subset. Dysregulated expression of HLA-DR on CD4 and CD8 T-cells was reported in two studies[39,41].

Despite these inconsistencies identified with individual study analysis, overall pooled estimates showed a medium effect size between baseline and post-treatment levels of T-helper cell (SMD: 0.74 [95% CI: –0.84, 2.33]; I^2 = 96%, p^H < 0.00001) (Fig. 2a), as well as the levels of cytotoxic T-cell activation (SMD: 0.64 [95% CI: -1.34, 2.63]; I^2 = 98%, p^H < 0.00001) (Fig. 2b). We performed a subgroup analysis to explore the potential sources of unexplained statistical heterogeneity based on the different geographic regions. The analysis showed that studies from North America had substantially lower levels of statistical heterogeneity in the reported levels of immune activation (SMD: 0.23 [-0.22, 0.68]; I^2 = 0%, p^H = 0.39) compared to studies from Europe (SMD: 1.01 [-2.86, 4.88]; I^2 = 99%, p^H<0.00001) (Table 3S).

The effect of different antiretroviral treatment classes on the levels of immune reconstitution

Changes in the levels of T-cell counts post-ART have been reported as a measure of immune reconstitution[18,19]. A total of eight out of the ten included studies reported on the effect of different ARV classes on immune reconstitution. Whereby, CD4 T-cell gains were reported post-treatment with protease inhibitors (n = 2)[38,41] and reverse transcriptase inhibitors (n = 1)[39] while both CD4 gains and loss were demonstrated in CCR5 antagonists (n = 2)[23,24]. The use of integrase inhibitors was associated with CD4 gains (n = 2)[25,27] although others found no change in the levels post-treatment (n = 1)[26]. On the other hand, both CD8 gains and losses were described in PLWH on protease inhibitors[38,41], reverse transcriptase inhibitors[39], integrase inhibitors[25,27], and CCR5 antagonist[23,24]. The pooled effect estimates showed that successful ART is associated with immune reconstitution (SMD: 0.70 [95% CI: 0.27, 1.44]; I^2 = 68%, p^H = 0.009) (Fig. 3a). However, due to high statistical heterogeneity amongst included studies, we performed a subgroup analysis

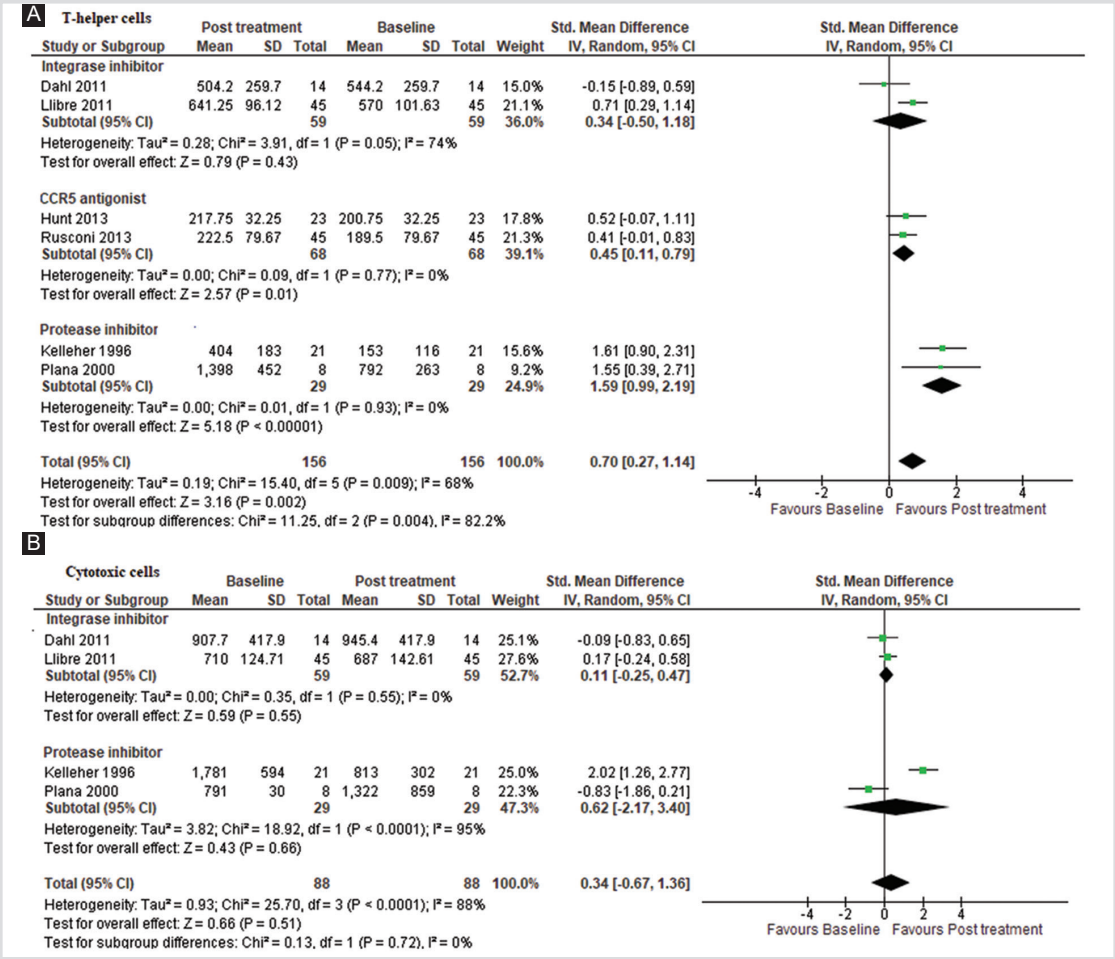

Figure 3. *The impact of different classes of ARV drugs on immune reconstitution measured by CD4* **(A)** *and CD8* **(B).**

to assess whether the different classes of ART modified the effect size.

The test for subgroup differences showed a significant subgroup effect (p = 0.004). Thus, the different classes of ARV drugs modified the overall effect of immune reconstitution in PLWH although there were substantial levels of unexplained heterogeneity between included studies (I^2 = 73.4%). The use of protease inhibitors significantly reconstituted the immune system (SMD: 1.59 [95% CI: 0.99, 2.19]; I^2 = 0%, p^H = 0.009) compared to CCR5 antagonist (SMD: 0.45 [95% CI: 0.11, 0.79]; I^2 = 0%, p^H = 0.77) and integrase inhibitors (SMD: 0.34 [95% CI: –0.50, 1.18]; I^2 = 74%, p^H = 0.05) (Fig. 3a).

With regard to cytotoxic T-cell reconstitution, ART showed a small increase in cell counts post treatment (SMD: 0.34 [95% CI: –0.67, 1.36]; I^2 = 88%, p^H < 0.0001) (Fig. 3b). Despite substantial levels of heterogeneity, the test for subgroup differences showed no significant subgroup effect (p = 0.72). Therefore, different classes of ARV drugs had no influence on the levels of immune reconstitution in HIV infected patients. Subgroup analysis based on geographic locations showed that studies from North America had lower levels of heterogeneity in immune reconstitution (SMD: 0.26 [–0.20, 0.72]; I^2 = 48%, p^H = 0.17) when compared to studies from Europe (SMD: 0.95 [0.40, 1.49]; I^2 = 70%, p^H = 0.02, respectively (Table 3S).

Sensitivity analysis and publication bias

We assessed the robustness of our results and further explored sources of heterogeneity in the re-

Table 2. Summary of findings

Post-treatment levels compared to baseline levels
Patient or population: Adults (> 18 years of age) living with HIV
Intervention: Antiretroviral drugs
Comparison: Baseline levels

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Baseline	Post-antiretroviral treatment				
Immune activation Measured by the coexpression of CD38 and HLA-DR on $CD8^+$ T-cells	-	The SMD post-treatment was 0.64 higher (−1.34 to 2.63)	NE	195 (4 randomized control trials)	⊕⊕⊕⊕ HIGH	
Immune reconstitution Measured by $CD4^+$ T-cell count	-	The SMD post-treatment was 0.70 higher (0.27 to 1.14)	NE	156 (6 randomized control trials)	⊕⊕⊕⊕ HIGH	Test for subgroup differences based on antiretroviral drug classes was significant (p = 0.004). Protease inhibitors had the highest CD4 cell gain with an effect size of 1.59. Integrase inhibitors had the least CD4 cell gain with an effect size of 0.34.

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: Confidence interval; SMD: Standardized Mean difference; OR: Odds ratio; NE: Not estimable; GRADE: Working Group grades of evidence. High certainty: We are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect. Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

ported outcomes by performing a sensitivity analysis. The meta-analyses were repeated by following a stepwise omission of studies based on viral load and treatment duration on each reported outcome. Notably, this did not affect the overall effect size nor change the direction of the reported pooled estimates (Table 4S). The levels of heterogeneity remained high in all parameters except for treatment duration whereby they were low in > 12 months (I^2 = 45%). Thus, suggesting treatment duration to be a potential source of statistical heterogeneity in the included studies. The assessment of funnel plots indicated no publication bias (Fig. 1S).

Discussion

The primary aim of this systematic review and meta-analysis was to determine whether ART reduces the level of generalized immune activation in PLWH. Furthermore, to assess whether the degree of immune reconstitution was dependent on the classes of ARV drugs used. Notably, the qualitative synthesis of evidence was inconsistent in both outcomes due to variations in characteristics of cohorts and ARV drugs used. However, pooled estimates showed increased level of generalized immune activation in these patients despite successful viral load suppression. The degree of immune reconstitution in PLWH on ART is dependent on the antiretroviral drug class. In that context, protease inhibitors were associated with the highest CD4 count gain while integrase inhibitors had the least. The overall main findings of this systematic review and meta-analysis are summarized in Table 2.

Various mechanisms including loss of mucosal integrity, immune response to viral replication, homeostatic proliferation, and altered pro- and anti-inflamma-

tory CD4 T-cell subsets have been postulated to induce systemic chronic immune activation in PLWH[13]. As such, ART suppresses the viral load and reduce HIV-related opportunistic infections and AIDS-related mortality[1,43]. We, therefore, explored whether viral suppression in successful ART had any effect on the level of immune activation. Interestingly, despite contradictory findings reported in the included studies, pooled estimates showed that elevated levels of immune activation persisted despite viral suppression. In agreement with our findings, various mechanisms contributing to HIV-associated chronic immune activation in PLWH on ART have been reported[6,13,44]. In this study population, persistent T-cell activation is the most likely applicable mechanism as reported elsewhere[18,44,45]. Thus, it is extremely important to monitor immune activation in successful ART in efforts to prevent T-cell exhaustion and loss of effector function[5,6,44].

There are currently five overall classes of ARV drugs, namely, fusion inhibitors, reverse transcriptase inhibitors, integrase inhibitors, chemokine antagonists, and protease inhibitors[1,43]. The mechanism of action of these drugs is centered on the inhibition of key HIV enzymes such as reverse transcriptase, protease, and integrase as well as the viral replication process at various stages of its cell cycle. Briefly, fusion inhibitors and chemokine antagonists act extracellularly by blocking the fusion of HIV to the host target cells while reverse transcriptase inhibitors prevent the transcription of viral RNA genome[1]. Integrases inhibitors block the incorporation of viral genome into host, whereas protease inhibitors hinder the synthesis and assembling of infectious viral particle[43,46]. Interestingly, different classes of ARV drugs may be combined in ART such as HAART to improve their efficacy[25,27,38,39]. This is especially important since it has been postulated that ART may not always be effective in restoring immune system despite a decrease in viral load[12]. For instance, HAART was only associated with immune reconstitution in 39% of PLWH whose CD4 T-cell counts increased to > 500 cells/μL[47] and it did not alter the levels of CD4 cell count in virally suppressed PLWH[23,26]. Nonetheless, pooled estimates from included studies showed that ART is concomitant with restoration of both CD4 and CD8 T-cell counts, with more profound magnitude in the former. Our findings showed that the level of immune reconstitution is dependent on the class of ARV drugs used rather than viral load and treatment duration as previously reported[1,28]. In that context, our study revealed that protease inhibitors had the largest effect size in CD4 increase, followed by CCR5 antagonists and lastly integrase inhibitors. In agreement with our findings, others reported a significant increase in CD4 counts following therapy with protease inhibitor[48]. Increased efficacy of protease inhibitors could be attributed to the ability of ritonavir in particular, to block cytochrome P450-mediated drug metabolism[49]. Cytochrome P450 is a hepatic enzymes involved in metabolism of vitamins, steroids. Thus its inhibition leads to enhanced pharmacokinetics of other protease inhibitor drugs in HAART. We could not statistically assess the impact of fusion and reverse transcriptase inhibitors on immune reconstitution due to lack of eligible studies reporting on these classes of ARV in PLWH. Among the three classes of ARV drugs reported in this study, the inclusion of protease inhibitors in HAART may be considered in efforts of restoring CD4 counts, particularly in PLWH with very low CD4 nadir. This is in- line with a recently published protease inhibitors milestone[50]. However, it must also be noted that in addition to its high toxicity, protease inhibitors are closely associated with the immune reconstitution inflammatory syndrome[48], an inflammatory reaction that occurs in response to a pre-existing opportunistic infection due to immune recovery following a successful ART[43].

A few limitations should be considered when interpreting the findings of this systematic review and meta-analysis. Firstly, due to the study designs of the pooled studies, our conclusions on immune activation and reconstitution were based on within group analyses which are prone to difference in nominal significance error[51]. Comparisons between the HAART and intensified HAART groups could not be assessed due to the participants in the former group who were already on the same therapy before being recruited in the study. Secondly, the reported classes of ARV drugs comprised one drug in that particular class; thus it is unclear whether these findings are applicable to the general ARV class or are only limited to the specific drugs reported. That is maraviroc, raltegravir, and ritonavir for CCR5 antagonist, integrase, and protease inhibitor classes, respectively. Finally, there was significant amount of statistical heterogeneity in the included studies. Therefore, the findings of this study need to be applied in clinical setting with caution.

Despite these limitations and, to the best of our knowledge, this is the first meta-analysis to assess the impact of ART on immune activation and reconstruc-

tion in PLWH. In addition, all included studies had a low risk of bias and the evidence synthesized was of high quality which is representative of PLWH and applicable to the population outside the geographic setting of this systematic review and meta-analysis study populations. The sensitivity analysis performed in this study revealed the robustness of the findings reported herein since they were not influenced by a single study. Finally, our findings show the need for treatment strategies that modulate immune activation in PLWH on successful ART.

In conclusion, the level of generalized immune activation in PLWH on ART is elevated despite viral load suppression and the degree of immune reconstitution in these patients is depended on the antiretroviral drug class. Therefore, it is important to monitor immune activation in PLWH with successful ART to delay the deleterious consequences of immune exhaustion.

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Authors' contribution

TMN, PVD, and BBN conceptualized, designed, and drafted this manuscript. TMN, and VM – data extraction and study appraisal; TMN, and BBN – statistical analysis, TMN, PVD, VM, and BBN – editing and final approval of manuscript. TMN is the guarantor of this systematic review and meta-analysis.

Supplementary data

Supplementary data are available at AIDS Reviews online (http://www.aidsreviews.com/). These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

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