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ORIGINAL RESEARCH

Rapid Initiation of Antiretroviral Therapy Suppresses T Cell Pathological Proliferation and Improves Immune Recovery in People Living with HIV

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Purpose: Initiating antiretroviral therapy promptly (rapid ART) is linked to better immune recovery in people with HIV (PWH), although its specific effects on immune dysregulation remain partially understood. We have discovered a "pathological proliferation" phenomenon, marked by T cell over-proliferation and exhaustion in PWH, potentially hindering full immune recovery. The objective of this research is to examine how rapid ART affects T-cell pathological proliferation, immune recovery, and systemic inflammation in PWH.

Patients and Methods: In this cross-sectional study (conducted at Beijing Youan Hospital, Capital Medical University, China, from April 1 to September 18, 2022), we recruited 39 PWH, including 23 in the rapid ART group (within 30 days) and 16 in the non-rapid ART group (after 180 days). Fasting venous blood samples were collected in the morning. Immune phenotypes of T cells were analyzed using mass cytometry and Luminex.

Results: The rapid ART group demonstrated a significant decline in $Ki67^+$ $CD4^+$ and $CD8^+$ T cells. Within this group, a higher percentage of naive T (T_N) cells was observed in $CD4^+$ T cells, along with a remarkable reduction in Ki67 expression. Additionally, $CD8^+$ T cells in the rapid ART group exhibited an increased presence of T_N cells while showing a decreased proportion of PD-1/HLA-DR/CD38 high-expressing cells. In addition, the rapid ART group exhibited significantly lower IL-18 levels. T_N cells ($CD31^+$ $HLA\text{-}DR^-$ $CD38^-$ $CD57^-$ $PD\text{-}1^-$) and central memory T (T_{CM}) cells ($HLA\text{-}DR^+$ $CD38^-$ $PD\text{-}1^-$ $CD57^-$) that were not suppressed by rapid ART showed significant correlations with baseline CD4 counts, HIV loads, and recent CD4/CD8 ratio.

Conclusion: These findings suggest rapid ART may curb pathological T cell proliferation and improved immune recovery in PWH. Despite these benefits, persistent immune activation in some individuals highlights the need for targeted immune monitoring and potential adjunctive interventions to optimize long-term immune health in PWH.

Keywords: rapid ART, pathological proliferation, immunological non-responders, INRs, human immunodeficiency virus, HIV

Introduction

Despite the substantial advancements in treatment for people with HIV (PWH) due to antiretroviral therapy (ART), immune dysregulation still poses a significant challenge.[1] The rapid initiation of ART (Rapid ART) following an HIV diagnosis, is currently recommended by international guidelines and authoritative bodies[2,3] due to its numerous clinical

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benefits. Although existing data clearly indicate that rapid ART can provide numerous clinical advantages for PWH, there is still limited understanding of the specific effects of rapid ART on immune dysregulation.

T cells are crucial for immune recovery in PWH,[4] but HIV-induced activation and exhaustion impair their function.[5–8] Prior studies from our group have indicated that individuals with acute HIV-1 infection experience heightened T cell hyperactivation and excessive proliferation before the initiation of ART.[9] Persistent PD-1^{+} T cell expansion following ART initiation exhibits an inverse correlation with CD4^{+} T cell levels. Although early ART helps to restore T cell activity, it does not entirely normalize proliferation rates.[10–14] Sustained T cell proliferation may negatively impact CD4^{+} T cell restoration in individuals undergoing early ART. Pathological proliferation, identified in immune non-responders among PWH, involves excessive CD4^{+} T cell proliferation alongside heightened activation and senescence.[15] We suggest that throughout the progression of HIV infection, a persistent state of "pathological proliferation" remains, marked by sustained T cell overgrowth, immune activation, senescence, and exhaustion.

Naive T (T_N) cells and central memory T (T_{CM}) cells represent critical components of the adaptive immune system, playing key roles in immune surveillance, long-term memory, and immune reconstitution.[16,17] Given their functional importance, detailed immunophenotyping of T-cell subsets—including naive, central memory, and effector memory cells, is essential for understanding immune dysregulation and recovery in PWH. Rapid ART may improve the immune status by suppressing these aberrant immune phenotypes, potentially facilitating immune reconstitution in PWH.

Previous research from our group has demonstrated the other aspects of rapid ART in PWH, as reported by He et al,[18] which focused on brain structure and function outcome. Here, we investigate whether rapid ART more effectively suppresses T cell over-proliferation, immune activation, and senescence, which were not analyzed in the previous work. To achieve this objective, we employed advanced methodologies, including mass cytometry and Luminex technology. This research is anticipated to offer important insights into how rapid ART aids immune reconstitution in PWH, thus aiding clinical decisions with evidence-based outcomes.

Materials and Methods

Participants

This cross-sectional study was conducted at Beijing Youan Hospital, Capital Medical University (Beijing, China) from April 1 to September 18, 2022. Potential participants were identified and screened using the hospital' s outpatient electronic medical records. Participants provided written informed consent following a comprehensive explanation of the study's procedures and associated risks. Details regarding the inclusion and exclusion criteria were outlined in Supplementary Methods 1. Based on a thorough screening process, participants were classified into two distinct groups: those who commenced ART within thirty days of an HIV diagnosis (rapid ART group) and those who initiated ART more than six months post-diagnosis (non-rapid ART group). Age was calculated at the time of study enrollment based on the participant's date of birth recorded in their medical records. All participants were male, consistent with the men who have sex with men demographic most commonly represented in our clinical population. Fasting venous blood samples were collected from all participants in the morning on the day of enrollment. These samples were used for mass cytometry analysis of T cell phenotypes and for cytokine/chemokine assays.

Analysis of Data from Mass Cytometry

The details of monoclonal antibodies labeled with metal isotopes can be found in Supplementary Table 1. To differentiate immune cell populations, 23 distinct pre-conjugated antibodies were employed. Cell labeling was performed based on previously established protocols.[19] The experimental procedures were performed following our previously published protocol.[15] The methodologies used for mass cytometry data analysis are provided in Supplementary Methods 2. The thresholds for Ki67 and PD-1 positivity were determined following the gating principles established in our previously published work.[15] Consistent gating strategies were applied across T cell subsets, with adaptations based on their respective expression patterns to ensure accurate and reproducible classification.[15]

Cytokine and Chemokine Assay

Luminex Technology Was Performed to Evaluate Cytokine/Chemokine Levels (Supplementary Methods 3).

Statistical Analysis

Statistical analysis was performed using R (v3.6.0) and SPSS (v18.0). The normality of continuous variables was assessed using the Shapiro-Wilk test. Categorical variables were compared using the χ^2-test or Fisher's exact test, while continuous variables were analyzed using Student's *t*-test or the Mann-Whitney *U*-test as appropriate. Spearman's rank correlation analysis was performed to investigate the relationships among inflammatory cytokine levels, T cell subpopulations, and clinical parameters. Data visualization and analysis were conducted using R (v3.6.0), GraphPad Prism (v9.5.1), and Origin (v2021). A P-value below 0.05 was considered statistically significant.

Results

Features of Participants

Thirty-nine participants met the eligibility requirements for the study after a thorough screening process. These individuals were allocated into two groups: the rapid ART group (n = 23) and the non-rapid ART group (n = 16). The median duration between HIV diagnosis and ART initiation was significantly shorter in rapid ART group at 13 days (IQR 8–18 days) compared to 645 days (IQR 390.75–1419 days) in non-rapid ART group (Table 1). The two groups had comparable demographic and clinical characteristics (Table 1).

Rapid ART Suppress the Over-Proliferation of T Cells in PWH

Ki67 overexpression marks incomplete reconstitution.[15] We categorized $CD3^+$ T cells into $CD4^+$ and $CD8^+$ subsets (Figure 1A). We identified $Ki67^+$ $CD4^+$ /$CD8^+$ T cells within these populations (Figure 1B–C). The findings demonstrated that the rapid ART group exhibited a notable decline in Ki67 positive $CD4^+$ and $CD8^+$ T cells.

A prior study has indicated that PD-1 positive T cells maintain proliferation,[9] prompting us to analyze the level of $Ki67^+$ PD-1^+ CD4/$CD8^+$ T cells. The rapid ART group exhibited notably higher frequencies in $Ki67^+$ PD-1^+ $CD4^+$ T cells, while lower frequencies in $Ki67^+$ PD-1^+ $CD4^+$ T cells (Figure 1D and E).

Rapid ART Normalized the Proliferation and Differentiation of CD4+ T Cells

Definitions of cell subpopulations and clusters are shown in Figure 2A–C and the Supplementary Results 1, and Supplementary Table 2. Among the 21 clusters, C18, characterized as $CD38^+$ HLA-DR^- PD-1^- $CD57^-$ T_N cells, was notably more common in the rapid ART group (Figure 2D). Furthermore, Ki67 expression in C18 was notably higher in the rapid ART group (Figure 2E). The proportions of T_N cell clusters C21 (HLA-DR^- $CD38^-$ PD-1^- $CD57^+$) and C14 (HLA-DR^- $CD38^-$ PD-1^- $CD57^-$) did not differ significantly between the two groups (Figure 2F and G). Additionally, clusters C8, C13, and C15 also displayed significant intergroup differences (Figures 2H–J). According to these results, rapid ART may increase the frequency of functionally active naive T cells (eg C18: $CD38^+$HLA-$DR^-$$CD57^-$PD-$1^-$ T_N cells) while not increasing senescent naive T cells (eg C21: $CD57^+$ T_N cells). We noted a higher frequency of C13 (HLA-DR^- $CD38^-$ PD-1^- $CD57^-$ $CCR2^-$ T_{CM}) and C15 (HLA-DR^- $CD38^-$ PD-1^- $CD57^-$ $CCR2^-$ T_{CM}) in the rapid ART group (Figure 2H and I). The frequency of C8 (HLA-DR^+ $CD38^+$ PD-1^- $CD57^-$ $CCR2^+$ T_{CM}), a highly activated and inflammation-associated population, was notably reduced (Figure 2J).

The rapid ART group demonstrated a decrease in Ki67 expression in both C4 (HLA-DR^- $CD38^-$ PD-1^- $CD57^-$ $CCR2^+$ T_{CM} cells) and C6 (HLA-DR^- $CD38^-$ PD-1^+ $CD57^-$ $CCR2^+$ T_{CM} cells) (Figure 2K and L). Our observations revealed that cluster C11 ($Ki67^{high}$ $CD38^-$ HLA-DR^- PD-1^- $CD57^-$ $CCR2^-$ T_{CM}) exhibited no significant differences in activation, senescence, programmed death, or non-specific cytotoxic function markers between the two groups.

Rapid ART Inhibits Hyperactivation and Exhaustion of CD8+T Cells

Figures 3A–C, Supplementary Results 2 and Supplementary Table 2 illustrate the definitions of cell subpopulations and clusters. Notable differences were identified in the proportions of cell clusters C2, C4, C9, C12, and C15 between the two

Table 1 Basic Information for Participants

Characteristic	Rapid ART Group (n = 23)	Non-Rapid ART Group (n = 16)	Statistic	*P* value
Gender (male/female)	23/0	23/0	NA	NA
Age (years), mean ± SD	33.74 ± 6.57	38.19 ± 7.28	$t = -1.991$	0.054[1]
Education (years), median (IQR)	16.00 (15.00 ~ 16.00)	14.00 (12.00 ~ 16.00)	$Z = -1.129$	0.268[2]
Weight (kg), mean ± SD	67.87 ± 9.25	68.69 ± 9.35	$t = -0.270$	0.788[1]
Height (meters), mean ± SD	1.75 ± 0.06	1.74 ± 0.05	$t = 0.591$	0.558[1]
BMI (kg/m²), median (IQR)	22.15 (20.28 ~ 23.67)	22.11 (20.82 ~ 23.67)	$Z = -0.386$	0.708[2]
HIV baseline indicators				
CD4 counts (cells/μL), mean ± SD	345.82 ± 195.26	353.68 ± 164.15	$t = -0.132$	0.896[1]
CD8 counts (cells/μL), mean ± SD	983.23 ± 385.51	970.68 ± 247.71	$t = 0.115$	0.909[1]
CD4/CD8 ratio, median (IQR)	0.36 (0.25 ~ 0.43)	0.37 (0.28 ~ 0.47)	$Z = -0.414$	0.687[2]
HIV load (log10 copies/mL), mean ± SD	4.24 ± 0.73	4.11 ± 0.65	$t = 0.569$	0.573[1]
ART initiation indicators				
CD4 counts (cells/μL), mean ± SD	359.92 ± 210.76	362.13 ± 180.53	$t = -0.034$	0.973[1]
CD8 counts (cells/μL), median (IQR)	1041.00 (751.00 ~ 1,209.82)	904.95 (696.24 ~ 1,250.91)	$Z = -0.228$	0.832[2]
CD4/CD8 ratio, median (IQR)	0.36 (0.25 ~ 0.46)	0.36 (0.22 ~ 0.55)	$Z = 0.000$	1.000[2]
HIV load (log10 copies/mL), mean ± SD	4.18 ± 0.71	4.44 ± 0.71	$t = -1.096$	0.280[1]
ART regimen (INSTI/Non-INSTI)	1/22	3/13	NA	0.286[3]
Current HIV-associated indicators				
CD4 counts (cells/μL), mean ± SD	613.04 ± 267.74	686.10 ± 324.05	$t = -0.769$	0.447[1]
CD8 counts (cells/μL), median (IQR)	894.18 (673.00–1,062.00)	783.50 (529.75–1,225.47)	$Z = -0.157$	0.882[2]
CD4/CD8 ratio, median (IQR)	0.71 ± 0.30	0.88 ± 0.52	$t = -1.111$	0.278[1]
HIV load not detectable (yes/no)	23/0	16/0	NA	NA
ART regimen (INSTI/Non-INSTI)	13/10	9/7	NA	1.000[3]
Duration from diagnosis to ART initiation (days), median (IQR)	13.00 (8.00–18.00)	645.00 (390.75–1419.00)	$Z = -5.258$	<0.001[2]
ART duration (months), median (IQR)	87.90 (77.60–97.60)	82.20 (22.18–107.90)	$Z = -0.799$	0.437[20]
HIV diagnosis duration (months), median (IQR)	88.40 (78.00–97.60)	109.00 (87.83–122.65)	$Z = -1.942$	0.052[2]

Notes: Demographic and clinical characteristics of the study participants at the time of enrollment. Age was calculated based on each participant's age at enrollment. All participants were male. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), while categorical variables were presented as percentages. For normally distributed continuous data, two-sample *t*-tests were conducted, whereas the Mann-Whitney *U*-test was used for non-normally distributed data. Categorical variables were analyzed using Chi-square and Fisher's exact tests.

Abbreviations: ART, anti-retroviral therapy; SD, standard deviation; IQR, interquartile range; NA, not available; BMI, body mass index; INSTI, integrase strand transfer inhibitor.

groups among the 20 clusters (Figure 3D–H). The rapid ART group exhibited a significantly higher proportion of C15 ($CD31^{+}$ $HLA\text{-}DR^{-}$ $CD38^{-}$ $PD\text{-}1^{-}$ $CD57^{-}$ T_N), while proportions of C1 ($CD31^{-}$ $HLA\text{-}DR^{+}$ $CD38^{+}$ $PD\text{-}1^{-}$ $CD57^{+}$ T_N), C6 ($CD31^{-}$ $HLA\text{-}DR^{+}$ $CD38^{-}$ $PD\text{-}1^{-}$ $CD57^{+}$ T_N), C14 ($CD31^{+}$ $HLA\text{-}DR^{-}$ $CD38^{+}$ $PD\text{-}1^{-}$ $CD57^{-}$ T_N), and ($CD31^{+}$ $HLA\text{-}DR^{+}$ $CD38^{-}$ $PD\text{-}1^{-}$ $CD57^{+}$ T_N) did not differ significantly (Figure 3I–L). $CD31^{+}$ TN cells are associated with inducing regulatory T cells, which may contribute to reduced expression of PD-1, CD57, CD38, and HLA-DR. The rapid ART group exhibited reduced levels of C4 ($CD38^{-}$ $HLA\text{-}DR^{-}$ $CD57^{+}$ $PD\text{-}1^{+}$ $CD45RA^{+}$ effector memory T(T_{EMRA})), C9 ($CD38^{-}$ $HLA\text{-}DR^{+}$ $CD57^{-}$ $PD\text{-}1^{-}$ T_{EMRA}), C2 ($CD38^{+}$ $HLA\text{-}DR^{+}$ $CD57^{-}$ $PD\text{-}1^{+}$ T_{CM}), and C12 ($CD38^{-}$ $HLA\text{-}DR^{+}$ $CD57^{-}$ $PD\text{-}1^{-}$ T_{CM}).

Remarkably, we found that certain subpopulations of activated, senescent, or exhausted cells were not suppressed in the rapid ART group. These include C1 ($HLA\text{-}DR^{+}$ $CD38^{+}$ $CD57^{+}$ T_N), C6 ($HLA\text{-}DR^{+}$ $CD57^{+}$ T_N), C17 ($CD57^{+}$ T_{EMRA}), C3 ($CD57^{+}$ effector memory T (T_{EM})), C10 ($PD\text{-}1^{+}$ $CD57^{+}$ T_{CM}), C5 ($PD\text{-}1^{+}$), C16 ($CD57^{+}$ T_{EM}), C11 ($CD57^{+}$ $HLA\text{-}DR^{+}$ T_{EM}), C20 ($PD\text{-}1^{+}$ T_{CM}), and C14 ($CD38^{+}$ T_N).

Associations of Clinical Characteristics with Inflammatory Response, and Immune Dysregulation in PWH Receiving Rapid ART

Luminex technology was utilized to quantify 21 inflammatory cytokines. IL-18 levels were notably lower in rapid ART group (Figure 4A), pointing to a significantly diminished systemic inflammatory response. A correlation analysis was performed to examine the relationship between inflammatory cytokine and chemokine levels and clinical characteristics in rapid ART group (Figure 4B). The findings revealed a significant positive correlation between interferon gamma-induced protein 10 (IP-10) levels and CD8 counts both at the initial HIV diagnosis (baseline) and at the beginning of

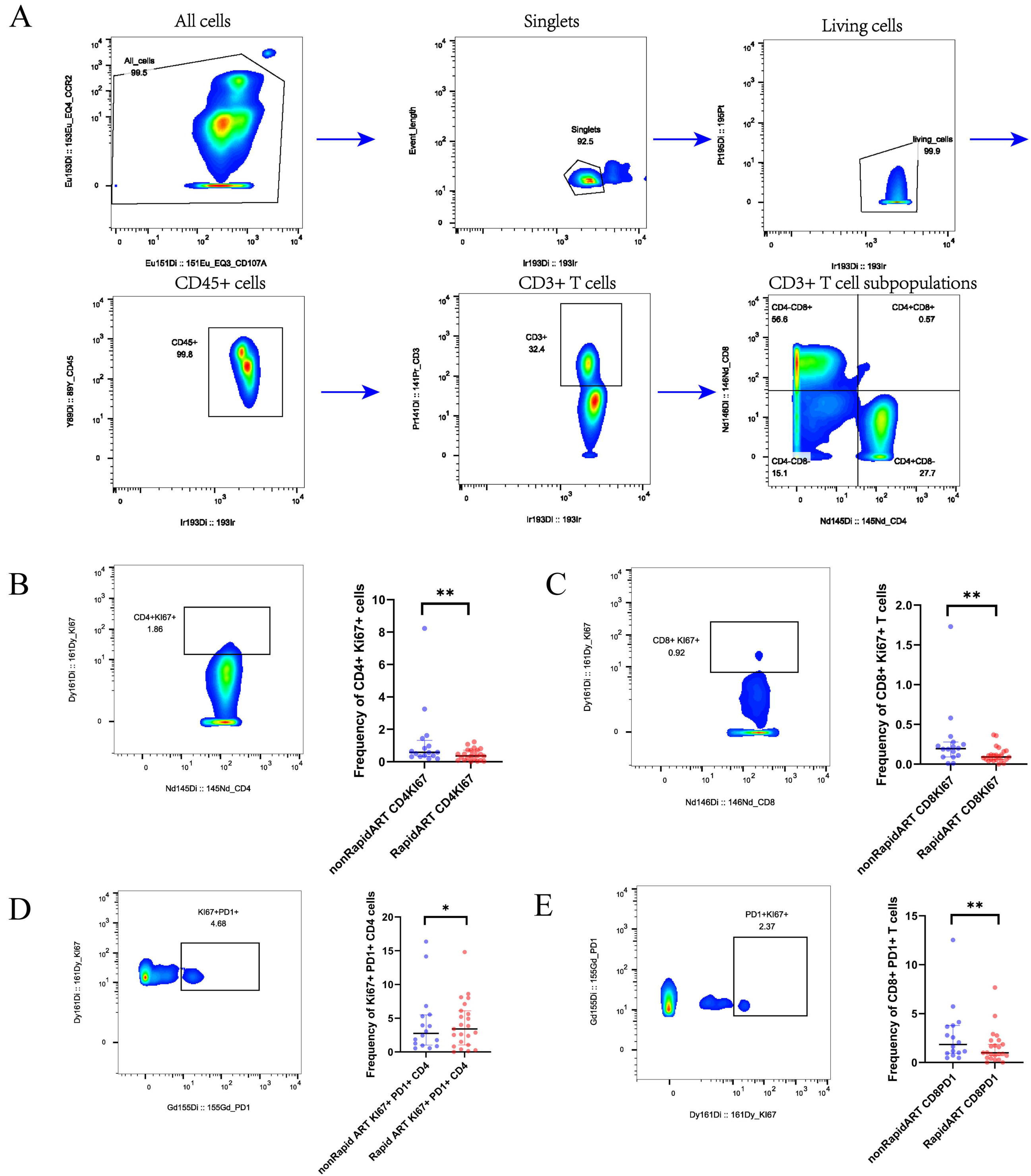

Figure 1 Rapid ART suppressed the over-proliferation of T cells in PLWH. (**A**) Gating strategy for CD45^{+} CD3^{+} T cells, distinguishing CD4^{+} CD8^{-} and CD4^{-} CD8^{+} T cell populations; (**B**) Frequencies of Ki67^{+} CD4^{+} T cells in the rapid ART group versus the non-rapid ART group; (**C**) Frequencies of Ki67^{+} CD8^{+} T cells in the rapid ART group versus the non-rapid ART group; (**D**) Frequencies of Ki67^{+} PD-1^{+} CD4^{+} T cells in both groups; (**E**) Frequencies of Ki67^{+} PD-1^{+} CD8^{+} T cells in both groups. Thresholds for Ki67^{+} and PD-1^{+} gating were determined according to our previously published mass cytometry approach, with subset-specific adjustments to reflect marker expression patterns. In all group comparisons, horizontal lines represent the median and interquartile range (IQR). Statistical comparisons for all panels (**B**–**E**) were performed using the Mann–Whitney *U*-test. *: $P < 0.05$; **: $P < 0.01$.

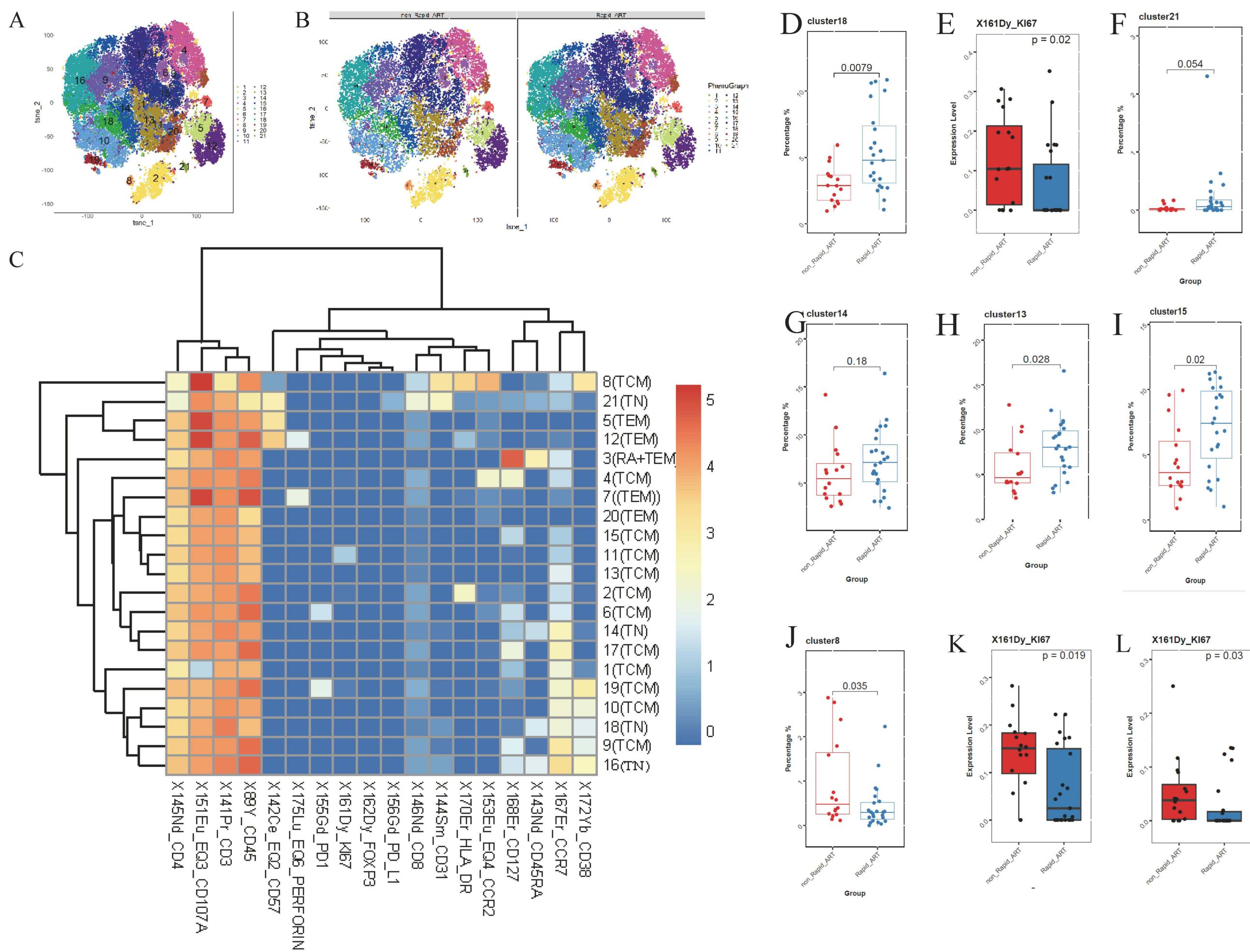

Figure 2 Rapid ART may normalize proliferation and differentiation of CD4 T cells. (**A**) Distribution of CD4^{+} T cell subpopulations categorized into T_{N}, T_{EM}, T_{EMRA}, and T_{CM} cells, analyzed using R software; (**B**) Expression of associated markers in the rapid ART and non-rapid ART groups; (**C**) Specific clusters representing T_{N}, T_{EM}, and T_{CM} cells among the 21 CD4^{+} T cell clusters; (**D**) Frequency of T_{N} cell cluster C18 in the rapid ART group versus the non-rapid ART group; (**E**) Ki67 expression levels in T_{N} cell cluster C18 between the two groups; (**F–J**) Frequencies of C21 (**F**) C14 (**G**) C13 (**H**) C15 (**I**) and C8 (**J**) cell clusters in the rapid ART group compared to the non-rapid ART group; (**K–L**) Ki67 expression levels in C4 (**K**) and C6 (**L**) cell clusters. T_{N}, naïve T cells; T_{EM}, effector memory T cells; T_{EMRA}, CD45RA^{+} TEM; T_{CM}, central memory T cells. Statistical comparisons in panels (**D–L**) were performed using the Mann–Whitney *U*-test. A *P*-value of less than 0.05 was considered indicative of statistical significance.

ART treatment (ART initiation). At both baseline and ART initiation, Monocyte chemotactic protein-3 (MCP-3) and macrophage inflammatory protein-1 alpha (MIP-1α) levels showed a positive correlation with HIV load. Additionally, vascular endothelial growth factor a (VEGF-A) levels were significantly positively correlated with age.

A correlation analysis was conducted to assess the relationship between CD4 and CD8 T cell subpopulations and clinical indicators in rapid ART group (Figure 4C and D). Within the CD4^{+} T cell subgroups (Figure 4C), C5 (CCR2^{+} HLA-DR^{-} CD38^{-} CD57^{+} PD-1^{-}T_{EM}) exhibited a positive correlation with CD4 counts and an inverse correlation with HIV load at both baseline and ART initiation. Similarly, C6 (CCR2^{+} HLA-DR^{-} CD38^{-} CD57^{-} PD-1^{+} T_{CM}) was negatively associated with CD4 counts at ART initiation and in follow-up assessments. Additionally, C12 (CCR2^{+} HLA-DR^{+} CD38^{-} CD57^{+} PD-1^{-} T_{EM}) displayed a significant correlation with baseline CD4 counts and maintained an inverse association with HIV load over time.

In CD8^{+} T cell subpopulations (Figure 4D), C3 (HLA-DR^{-} CD38^{-} CD57^{-} PD-1^{-} T_{EM}) was positively correlated with baseline CD4 counts and negatively associated with both baseline and ART initiation HIV loads. C6 (CD31^{-} HLA-DR^{+} CD38^{+} CD57^{-} PD-1^{-} T_{N}) exhibited a positive correlation with CD4 counts at both baseline and ART initiation. C8 (CD31^{+}HLA-DR^{-} CD38^{-} PD-1^{-} CD57^{-} T_{N}) was reported negatively correlated with CD4^{+} and CD8^{+} T cell counts at both baseline and upon ART initiation. C12 (HLA-DR^{+} CD38^{-} PD-1^{-} CD57^{-} T_{CM}) was inversely associated with CD4

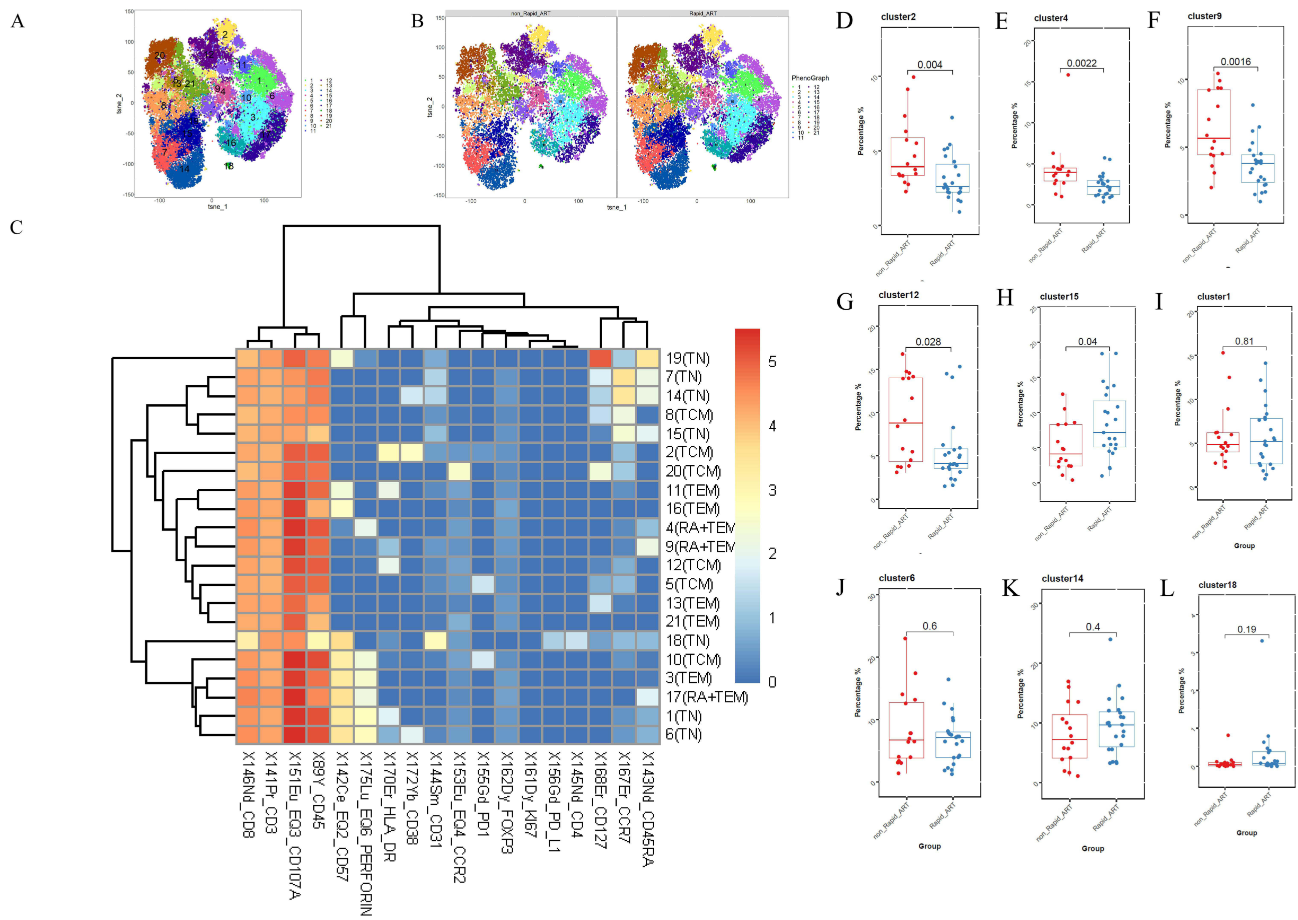

Figure 3 Rapid ART may inhibit hyperactivation and exhaustion of CD8$^+$T cells. (**A**) Distribution of CD8$^+$ T cell subpopulations categorized into T_N, T_{EM}, T_{EMRA}, and T_{CM} cells, analyzed using R software; (**B**) Expression of associated markers in the rapid ART and non-rapid ART groups; (**C**) Specific clusters representing T_N, T_{EM}, and T_{CM} cells among the 20 CD4$^+$ T cell clusters; (**D–L**) Frequencies of T_N cell clusters C2 (**D**) C4 (**E**) C9 (**F**) C12 (**G**) C15 (**H**) C1 (**I**) C6 (**J**) C14 (**K**) and C18 (**L**) in the rapid ART versus non-rapid ART groups. T_N, naïve T cells; T_{EM}, effector memory T cells; T_{EMRA}, CD45RA$^+$$T_{EM}$; T_{CM}, central memory T cells. Statistical comparisons in panels (**D–L**) were performed using the Mann–Whitney *U*-test. A *P*-value of less than 0.05 was considered indicative of statistical significance.

counts and CD4/CD8 ratio across all time points, but exhibited a positive correlation with HIV load at ART initiation. C17 (HLA-DR$^-$ CD38$^-$ PD-1$^-$ CD57$^+$ T_{EMRA}) showed a positive correlation with CD4 counts and the CD4/CD8 ratio consistently over time, while exhibiting a negative correlation with HIV load at both baseline and ART initiation.

Discussion

Rapid ART plays a critical role in alleviating immune dysregulation in PWH.[21] Our analysis initially demonstrated that rapid ART effectively suppressed T cell over-proliferation and enhanced immune recovery. However, persistent immune activation in some T cell subpopulations suggests that rapid ART alone may not fully normalize immune function. This underscores the need for ongoing immune monitoring and individualized treatment strategies to optimize immune reconstitution. Future research should examine the extended impact of rapid ART on sustained immune recovery and explore potential additional therapeutic strategies to further optimize immune function.

The significant decrease in Ki67$^+$ T cells highlights the potent inhibitory effect of rapid ART on T cell over-proliferation. Our research notably found a significant decrease in the frequencies of Ki67$^+$ CD4$^+$ and CD8$^+$ T cells within the rapid ART group. High levels of Ki67 expression suggest excessive T cell proliferation, which is frequently linked to increased immune activation and cellular senescence.[15] Such over-proliferation serves as a crucial indicator of immune dysfunction. Although CD4$^+$ T cell counts and functions might progressively improve with ART, they typically do not reach the normal levels observed in HIV-negative individuals and may even progress to immune non-responder status. By effectively inhibiting this over-proliferation, rapid ART appears to facilitate a more balanced immune response,

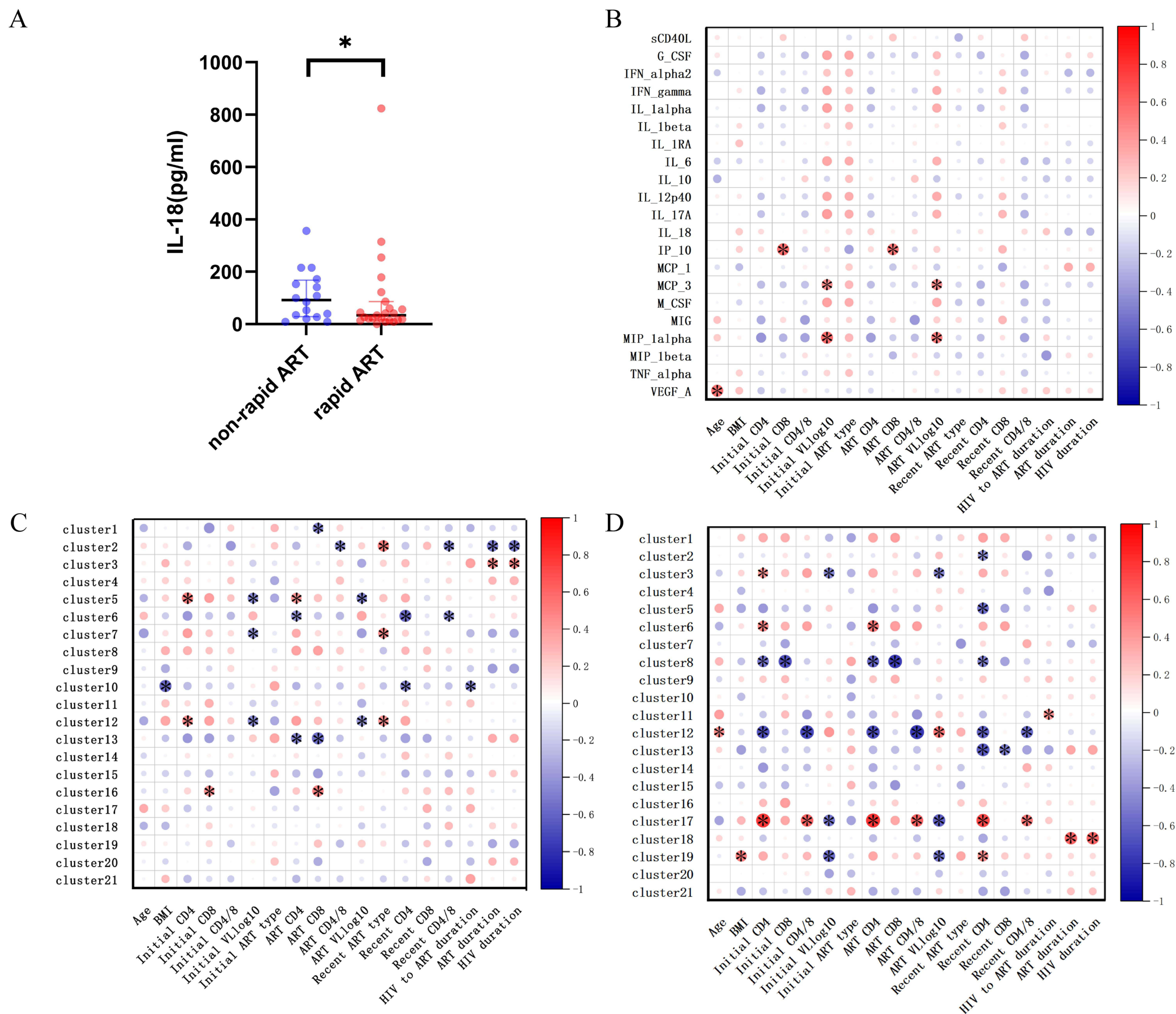

Figure 4 Immunomodulatory benefits of rapid ART are more pronounced in over-proliferated or exhausted T cells. (**A**) Differences in IL-18 levels between the rapid ART and non-rapid ART groups. Horizontal lines represent median and interquartile range (IQR). Statistical comparisons were performed using the Mann–Whitney *U*-test. (**B**–**D**) Spearman's rank correlation analysis in the rapid ART group: (**B**) Inflammatory factors and clinical parameters; (**C**) $CD4^+$ T cell subpopulations and clinical parameters; (**D**) $CD8^+$ T cell subpopulations and clinical parameters. Circle size represents the absolute value of the Spearman correlation coefficient. Red circles indicate positive correlations, while blue circles indicate negative correlations. T_N, naïve T cells; T_{EM}, effector memory T cells; T_{EMRA}, CD45RA$^+$ T_{EM}; T_{CM}, central memory T cells; ART, antiretroviral therapy. *: $P < 0.05$.

potentially obstructing the progression of HIV-related immune impairment. Recent studies support these results, highlighting the crucial role of rapid ART in lessening the adverse effects of T cell hyperactivation and exhaustion in those with chronic HIV.[20,22] The reduction in Ki67 expression within these T_N and T_{CM} cells indicates a suppression of pathological proliferation, potentially leading to a more balanced immune response. This suppression may be crucial in preventing the harmful effects of excessive T cell activation while still preserving immune function.

Rapid ART might enhance immune status by encouraging normal CD4$^+$ T_N cells differentiation and decreasing the count of abnormally proliferating T_{CM} cells.[23] Our data illuminate the specific effects of rapid ART on various T cells subpopulations. The rapid ART group showed a higher frequency of T_N cells and lower abnormal proliferation, as evidenced by decreased Ki67 expression, which suggests that rapid ART not only replenishes naive T cells but also diminishes the pathogenic hyperproliferation characteristic of immune dysregulation. Also, these results underscore the beneficial role of rapid ART in mitigating immune activation and inflammation. Importantly, this supports the idea that rapid ART could be pivotal in restoring the naive T cell pool, which is essential for effective immune responses following

HIV infection.[24] Moreover, rapid ART is more effective at inhibiting excessive proliferation in T_{CM} cells associated with exhaustion or inflammation, the findings regarding T_{CM} cells further clarify the complex interplay between ART and immune reconstitution. The significant reduction of PD-1 high-expressing T_{CM} cells in the rapid ART group implies that early intervention could be crucial in preventing programmed cell death.[25] The established association between PD-1 and T cell exhaustion underscores how rapid ART can reduce PD-1 levels in T_{CM} cells, offering a hopeful therapeutic path to boost immune function in PWH.[26] Rapid ART could improve the immune system's long-term ability to combating HIV by reducing T cell exhaustion.[27]

Rapid of ART might suppress the hyperactivation and exhaustion of $CD8^+$ T cells. Findings indicate that rapid ART significantly reduces immune-activated $CD8^+$ T cell subsets, including PD-1 high-expressing T_N cells and HLA-DR^+ T_{EMRA} cells. Meanwhile, rapid ART could significantly increase the number of functional regulatory T_N cells without elevating excessively activated or senescent cell levels. This indicates that rapid ART is not only effective in inhibiting excessive activation but also in fine-tuning the immune response, potentially leading to more effective antiviral responses from CD8 T cells.[28] Rapid ART significantly inhibits terminally differentiated T cells that are activated, exhausted, or senescent Importantly, by suppressing immune activation, rapid ART may limit the progression of T cell dysfunction and maintain a robust immune profile. Notably, the suppressive effect of rapid ART on pathological proliferation in $CD8^+$ T cells appeared to be subset-specific—while certain subsets exhibited reduced proliferation, others remained unaffected. This suggests that rapid ART may only partially limit pathological $CD8^+$ T cell responses, warranting further investigation into their role in immune recovery in PWH.

The immunomodulatory effects of rapid ART are also evident in its potential to suppress inflammation. We found that rapid ART is associated with lower systemic inflammation, evidenced by reduced IL-18 levels. This decrease in IL-18 suggests that rapid ART may mitigate chronic inflammation in PWH, which could lead to better clinical outcomes. Previous studies have similarly shown that early ART initiation reduces inflammatory markers and improves immune recovery.[29,30] We found notable correlations between inflammatory cytokines, including MCP-3, MIP-α, and IP-10, and clinical features like $CD4^+$/$CD8^+$ T cell counts and HIV load. These results emphasize the continuous impact of inflammation on the immune recovery, suggesting that one advantage of rapid ART could be its capacity to reduce chronic inflammation. Even with rapid ART, chronic systemic inflammation levels in PWH are still linked to T cell counts and viral replication levels when ART begins.

We discovered connections between specific T cell subpopulations and clinical factors, highlighting the complexity of immune restoration under rapid ART. Certain T cell clusters, such as $CD8^+$ T_{CM} and T_N cells with phenotypes such as $CD31^+$ HLA-DR^- $CD38^-$ $CD57^-$ PD-1^-T_N and HLA-DR^+ $CD38^-$ $CD57^-$ PD-1^- T_{CM}, were not completely suppressed by rapid ART. These clusters remained associated with immune status and HIV load at ART initiation and during follow-up. Persistent markers of activation and exhaustion suggest rapid ART may not be sufficient to fully normalize immune system. For instance, the HLA-DR^+ $CD38^-$ T_{CM} cells, were not adequately suppressed, indicating that these cells could continue to drive immune dysregulation despite rapid ART. These findings align with that activated and T cell profiles are linked to poorer immune reconstitution in PWH.[8,31,32] The persistence of these T cell subpopulations underlines the need for long-term monitoring and potentially the development of targeted therapies to manage these cells' impact on immune reconstitution. Further exploration is needed to clarify if these cells are a residual set of dysfunctional T cells or if they indicate ongoing pathological processes that might undermine long-term treatment effectiveness.

This study possesses several strengths that contribute to its significance in the field of HIV research. First, it emphasizes the timing of ART initiation, demonstrating that rapid ART offers advantages over non-rapid ART in improving immune dysregulation in PWH. Additionally, by employing advanced mass cytometry techniques, this research allows for a comprehensive analysis of a broader range of T cell subpopulations, as well as their immune phenotypes and functions, compared to conventional flow cytometry. This methodology offers detailed insights into rapid ART's effects on T cell phenotypes, enabling precise conclusions about its immunomodulatory effects. Moreover, the clearly defined participant groups, consisting of individuals who start ART at various stages of HIV infection, enable robust comparisons of immune responses, thereby enhancing the validity of our findings.

While this study presents notable strengths, it also has certain limitations that should be acknowledged. One key constraint is the limited sample size, which may affect the broader applicability of the findings. Although our results were statistically significant, larger and more varied populations are needed to validate these findings across different demographics and clinical contexts. The cross-sectional design of the study limits the ability to establish causality between rapid ART and the observed immune outcomes. Long-term studies are required to evaluate how rapid ART impacts immune recovery over time, especially given the changing nature of immune responses in HIV infection. Furthermore, while we focused on T cell subpopulations, other immune components and their interactions, such as B cells and innate immune cells, were not investigated, which could provide further insights into the multifaceted immune landscape in HIV-infected individuals.

Conclusion

In conclusion, we highlight that rapid ART effectively reduces T cell over-proliferation and systemic inflammation, which are critical for preventing long-term immune dysfunction. Rapid ART also influences various T cell subpopulations, contributing to a more balanced immune profile. However, the persistence of certain T cell subpopulations despite treatment indicates that additional strategies may be necessary to fully address immune dysregulation. These results highlight the critical role of rapid ART to enhance immune recovery, while emphasizing the necessity for continuous monitoring and specific interventions to maintain long-term health in PWH.

Ethics Committee Approval

The Ethics Committee of Beijing Youan Hospital, Capital Medical University, approved this study (2023057). All participants provided written informed consent following a briefing on the study's objectives. The study with human participants complied with the principles outlined in the 2013 revision of the Declaration of Helsinki.

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Disclosure

The authors declare no conflicts of interest related to this work.

References

1. Walsh D, Naghavi MH. Exploitation of cytoskeletal networks during early viral infection. *Trend Microbiol*. 2019;27(1):39–50. doi:10.1016/j.tim.2018.06.008
2. Ambrosioni J, Levi L, Alagaratnam J, et al. Major revision version 12.0 of the European AIDS clinical society guidelines 2023. *HIV Med*. 2023;24(11):1126–1136. doi:10.1111/hiv.13542
3. Gandhi RT, Bedimo R, Hoy JF, et al. Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2022 recommendations of the international antiviral society-USA panel. *JAMA*. 2023;329(1):63–84. doi:10.1001/jama.2022.22246
4. Migueles SA, Laborico AC, Shupert WL, et al. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol*. 2002;3(11):1061–1068. doi:10.1038/ni845
5. Wykes MN, Lewin SR. Immune checkpoint blockade in infectious diseases. *Nat Rev Immunol*. 2018;18(2):91–104. doi:10.1038/nri.2017.112
6. Piconi S, Trabattoni D, Gori A, et al. Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy. *AIDS*. 2010;24(13):1991–2000. doi:10.1097/QAD.0b013e32833c93ce
7. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 2006;443(7109):350–354. doi:10.1038/nature05115
8. Yang X, Su B, Zhang X, Liu Y, Wu H, Zhang T. Incomplete immune reconstitution in HIV/AIDS patients on antiretroviral therapy: challenges of immunological non-responders. *J Leukocyte Biol*. 2020;107(4):597–612.

9. Li Z, Yan P, Wang R, et al. Persistent T cell proliferation and MDSCs expansion precede incomplete CD4(+) T cell recovery in people with acute HIV-1 infection with early ART. *Heliyon*. 2023;9(5):e15590. doi:10.1016/j.heliyon.2023.e15590
10. Hellmuth J, Slike BM, Sacdalan C, et al. Very early initiation of antiretroviral therapy during acute HIV infection is associated with normalized levels of immune activation markers in cerebrospinal fluid but not in plasma. *J Infect Dis*. 2019;220(12):1885–1891. doi:10.1093/infdis/jiz030
11. Bekele Y, Lakshmikanth T, Chen Y, et al. Mass cytometry identifies distinct CD4+ T cell clusters distinguishing HIV-1-infected patients according to antiretroviral therapy initiation. *JCI Insight*. 2019;4(3). doi:10.1172/jci.insight.125442
12. Macatangay BJ, Yang M, Sun X, et al. Brief report: changes in levels of inflammation after antiretroviral treatment during early HIV infection in AIDS clinical trials group study A5217. *J Acquired Immune Deficiency Syndromes*. 2017;75(1):137–141. doi:10.1097/QAI.0000000000001320
13. Krebs SJ, Ananworanich J. Immune activation during acute HIV infection and the impact of early antiretroviral therapy. *Curr Opin HIV AIDS*. 2016;11(2):163–172. doi:10.1097/COH.0000000000000228
14. Crowell TA, Fletcher JL, Sereti I, et al. Initiation of antiretroviral therapy before detection of colonic infiltration by HIV reduces viral reservoirs, inflammation and immune activation. *J Intl AIDS Society*. 2016;19(1):21163. doi:10.7448/IAS.19.1.21163
15. Zhang Y, Ji J, Xie K, et al. Pathological proliferation: a potential mechanism for poor CD4(+) T cell recovery in people living with HIV. *Front Cell Infect Microbiol*. 2024;14:1344778. doi:10.3389/fcimb.2024.1344778
16. Song CB, Zhang LL, Wu X, et al. CD4(+)CD38(+) central memory T cells contribute to HIV persistence in HIV-infected individuals on long-term ART. *J Transl Med*. 2020;18(1):95. doi:10.1186/s12967-020-02245-8
17. Venanzi Rullo E, Pinzone MR, Cannon L, et al. Persistence of an intact HIV reservoir in phenotypically naive T cells. *JCI Insight*. 2020;5(20). doi:10.1172/jci.insight.133157
18. He Y, Zhang Y, Jiang T, et al. The association between rapid antiretroviral therapy initiation and brain structure and function based on multimodal magnetic resonance imaging in HIV-positive men who have sex with men. *BMC Infect Dis*. 2025;25(1):41. doi:10.1186/s12879-024-10397-x
19. Hallisey M, Dennis J, Gabriel EP, et al. Profiling of natural killer interactions with cancer cells using mass cytometry. *Lab Investigat*. 2023;103(8):100174. doi:10.1016/j.labinv.2023.100174
20. Hey-Cunningham WJ, Murray JM, Natarajan V, et al. Early antiretroviral therapy with raltegravir generates sustained reductions in HIV reservoirs but not lower T-cell activation levels. *AIDS*. 2015;29(8):911–919. doi:10.1097/QAD.0000000000000625
21. Naidoo KK, Highton AJ, Baiyegunhi OO, et al. Early initiation of antiretroviral therapy preserves the metabolic function of CD4+ T cells in subtype C human immunodeficiency virus 1 infection. *J Infect Dis*. 2024;229(3):753–762. doi:10.1093/infdis/jiad432
22. Casetti R, Sacchi A, Bordoni V, et al. In human immunodeficiency virus primary infection, early combined antiretroviral therapy reduced γδ T-cell activation but failed to restore their polyfunctionality. *Immunology*. 2019;157(4):322–330. doi:10.1111/imm.13089
23. Sponaugle A, Abad-Ferenandez M, Clutton G, et al. CD4 T cell memory restoration upon antiretroviral initiation in people living with HIV. *J Immunol*. 2020;204(1_Supplement):14–94. doi:10.4049/jimmunol.204.Supp.94.14
24. Petkov S, Chiodi F. Impaired CD4+ T cell differentiation in HIV-1 infected patients receiving early anti-retroviral therapy. *Genomics*. 2022;114(3):110367. doi:10.1016/j.ygeno.2022.110367
25. Rutishauser RL, Hartogensis W, Deguit CD, et al. Early and delayed antiretroviral therapy results in comparable reductions in CD8(+) T cell exhaustion marker expression. *AIDS Res Hum Retroviruses*. 2017;33(7):658–667. doi:10.1089/aid.2016.0324
26. Benito JM, Restrepo C, García-Foncillas J, Rallón N. Immune checkpoint inhibitors as potential therapy for reverting T-cell exhaustion and reverting HIV latency in people living with HIV. *Front Immunol*. 2023;14:1270881. doi:10.3389/fimmu.2023.1270881
27. Li JZ, Segal FP, Bosch RJ, et al. Antiretroviral therapy reduces T-cell activation and immune exhaustion markers in human immunodeficiency virus controllers. *Clin Infect Dis*. 2020;70(8):1636–1642. doi:10.1093/cid/ciz442
28. Fenwick C, Joo V, Jacquier P, et al. T-cell exhaustion in HIV infection. *Immunol Rev*. 2019;292(1):149–163. doi:10.1111/imr.12823
29. Rabezanahary H, Clain J, Racine G, et al. Early antiretroviral therapy prevents viral infection of monocytes and inflammation in simian immunodeficiency virus-infected rhesus macaques. *J Virol*. 2020;94(22). doi:10.1128/JVI.01478-20
30. Oliveira MF, Chaillon A, Nakazawa M, et al. Early antiretroviral therapy is associated with lower HIV DNA molecular diversity and lower inflammation in cerebrospinal fluid but does not prevent the establishment of compartmentalized HIV DNA populations. *PLoS Pathogens*. 2017;13(1):e1006112. doi:10.1371/journal.ppat.1006112
31. Zhang Y, Jiang T, Li A, et al. Adjunct therapy for CD4(+) T-cell recovery, inflammation and immune activation in people living with HIV: a systematic review and meta-analysis. *Front Immunol*. 2021;12:632119. doi:10.3389/fimmu.2021.632119
32. Chen J, Titanji K, Sheth AN, et al. The effect of age on CD4+ T-cell recovery in HIV-suppressed adult participants: a sub-study from AIDS Clinical Trial Group (ACTG) A5321 and the bone loss and immune reconstitution (BLIR) study. *Immun Age*. 2022;19(1):4. doi:10.1186/s12979-021-00260-x

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