Immune checkpoints and the HIV-1 reservoir: proceed with caution

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Successfully identifying and targeting immune checkpoints on latently HIV-1-infected CD4+ T cells could be a key component in HIV-1 eradication therapies [1,2]. Immune checkpoints are negative regulators of: (i) T cell activation; (ii) T cell proliferation; and (iii) effector functions including cytokine production [3]. Thus, inhibiting immune checkpoints could influence the resting status of latently infected cells [1,2], which are key obstacles to curing HIV-1 [4,5]. Candidate immune checkpoints in this regard include programmed cell death-1 (PD-1), T cell immunoreceptor with immunoglobulin and ITIM-domains (TIGIT), lymphocyte-activating protein-3 (LAG-3) and type-1 transmembrane immunoglobulin and mucin-3 (TIM3) [1,2,6–8]. Antibodies blocking immune checkpoints have been hypothesised to disrupt the resting status of T cells and hence have been utilised as latency-reversing agents [5,7,9] and may enhance CD8+ T cell effector functions in HIV eradication trials [1,2,6–8].

We propose that distinguishing between total and memory CD4+ T cell subsets is fundamental when interpreting data regarding HIV-1 DNA and immune checkpoints. To ensure clarity: 'total CD4+ T cells' refers to all CD3+CD4+ lymphocytes and encompasses naïve and memory subsets; 'memory CD4+ T cells' includes the different memory subsets but excludes naïve cells (Figure 1a).

Chomont *et al.* originally demonstrated that memory CD4+ T cells highly expressing PD-1 were enriched for HIV-1 DNA [2]. This key finding inspired others to examine immune checkpoint expression on CD4+ T cells and subsequent studies described a positive correlation between multiple immune check points (TIGIT, PD-1, LAG-3 or TIM-3) and HIV-1 DNA in total CD4+ T cells [10–12]. The rationale for examining immune checkpoints on total CD4+ T cells appears strong given that HIV-1 DNA in total CD4+ T cells is a crude but relatively reproducible approximation of the viral reservoir size. HIV-1 DNA also predicts time to viral rebound following analytical treatment interruption [13,14].

However, the original findings of Chomont *et al.* were from memory CD4+ T cells and subsequent studies have been from total CD4+ T cells. Therefore, we decided to analyse the expression of two immune checkpoints (PD-1 and TIGIT) on both total and memory CD4+ T cells in a cohort of 22 aviraemic HIV-infected individuals on long-term ART, to elucidate whether memory subset proportions could be a confounding factor when performing the analyses in total CD4+ T cells (cohort previously described [15]).

We found highly variable proportions of naïve and memory subsets between individuals (naïve CD4+ T cell range: 13–75%, Figure 1b) as previously published [16,17]. This variation exemplifies the heterogeneity in clinical cohorts encompassing HIV-infected individuals [18,19]. As also shown by others, we demonstrated that PD-1 and TIGIT are almost exclusively expressed on memory CD4+ T cells [2,8,16] (Figure 1c). To stress the importance of these findings, we ranked the 22 HIV-positive individuals according to the percentage of memory CD4+ T cells (low to high) (Figure 1d) and displayed the percentage of PD-1 or TIGIT-positive total CD4+ T cells for each individual (Figure 1e). These data demonstrated that a low proportion of memory CD4+ T cells corresponded to a low PD-1 or TIGIT expression on total CD4+ T cells, whereas a high proportion of memory CD4+ T cells corresponded to high PD-1 or TIGIT expression on total CD4+ T cells (Figures 1d, e). This linkage is substantiated by a highly significant positive correlation between the size of the memory CD4+ T cell compartment and the percentage of total CD4+ T cells expressing PD-1 or TIGIT (Figures 1f, g).

Adding our analytic approach to the current knowledge, two essential points should be stressed: (1) the majority of CD4+ T cells harbouring HIV-1 DNA are memory cells [2,20] (Figure 1h); and (2) a higher proportion of memory cells express immune checkpoints compared to naïve cells [2,8,16] (Figure 1c). The concomitant presence of HIV-1 DNA and immune checkpoints in memory CD4+ T cells means that the relative memory proportions could be a confounder when examining these parameters in total CD4+ T cells.

To explore the potential confounding effect of memory proportions, we investigated how the correlation between HIV-1 DNA and PD-1 or TIGIT change when performing the analysis in total CD4+ T cells versus memory CD4+ T cells (Figure 2). We demonstrated that HIV-1 DNA and percentage of total CD4+ T cells expressing PD-1 or TIGIT (Figure 2a) positively correlates as recently published [10–12]. However, correlating HIV-1 DNA and percentage of CD4+ T cells expressing PD-1 or TIGIT in memory CD4+ T cells results in different *r*-values compared to the analyses performed in total CD4+ T cells (Figures 2a–d). We estimated the magnitude of this change in *r*-value (Δr) using bootstrap analyses to estimate 95% confidence intervals (CI) and the permutation test to estimate *P*-values (Figure 2e). The difference in correlation for PD-1 ($\Delta r_{PD\text{-}1}$) and TIGIT (Δr_{TIGIT}) are, respectively, 0.496 (95% CI: 0.266–0.697; P=0.001) and 0.187 (95% CI: –0.059–0.542, P=0.1884; Figure 2e), demonstrating that memory subset proportion is a confounder when analysing potential immune checkpoint biomarkers for HIV-infected CD4+ T cells. These results imply that any correlation between HIV-1 DNA and immune checkpoints on total CD4+ T cells is largely driven by the proportions of memory versus naïve cells. Therefore, we argue that it cannot be inferred that CD4+ T cells expressing immune checkpoint are enriched for HIV-1 DNA based on analyses performed in total CD4+ T cells.

In conclusion, these data reveal the importance of quantifying individual memory subsets when analysing immune checkpoints on CD4+ T cells in order to evaluate their usage as biomarkers of infected cells or when defining candidate immune checkpoint(s) for targeting during HIV-1 eradication strategies.

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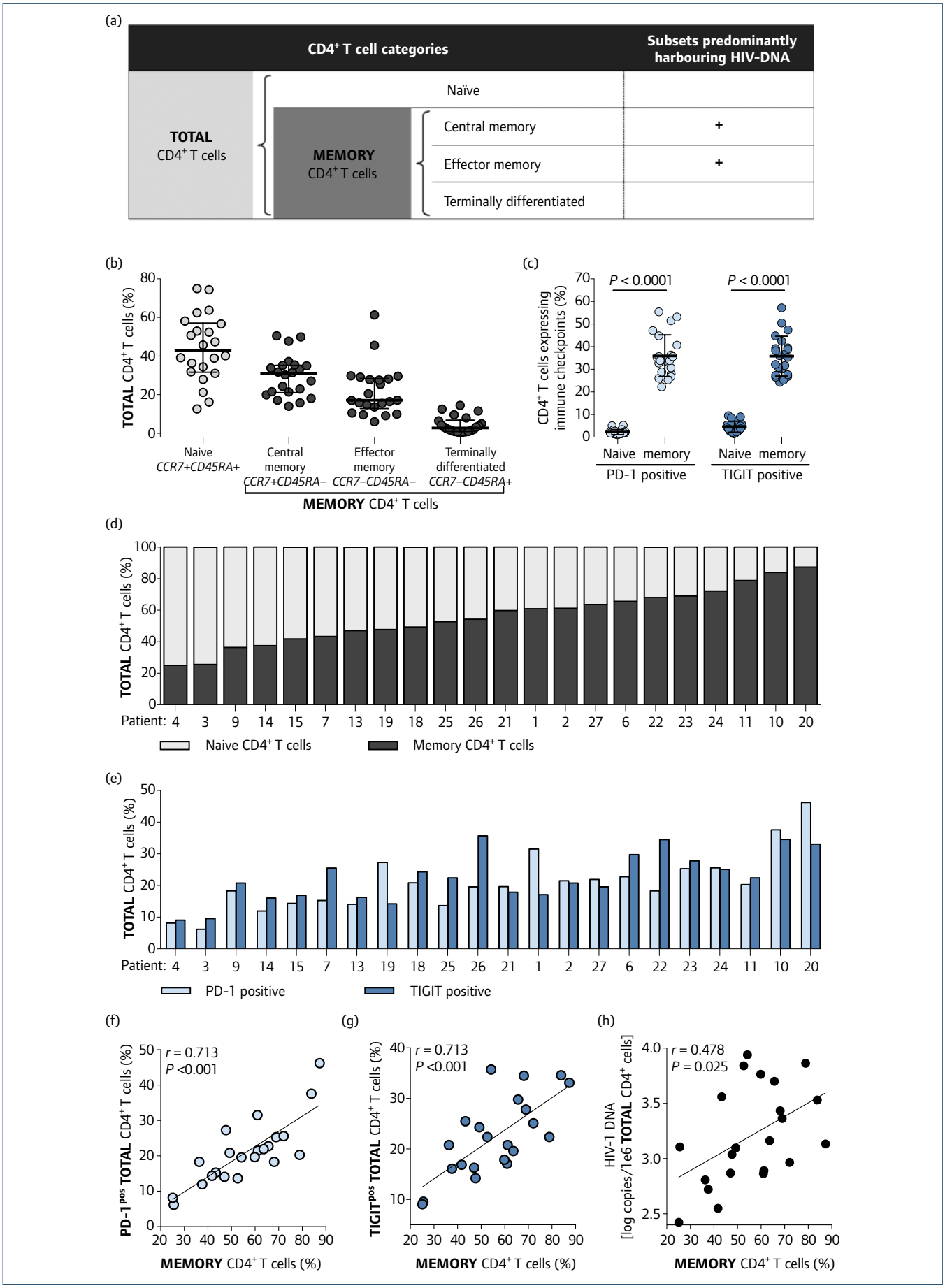

Figure 1. PD-1 and TIGIT are primarily expressed on memory CD4+ T cells. (a) Definition of total and memory CD4+ T cells. (b–e) Flow cytometric characterisation of CD4+ T cell memory subsets and PD-1 and TIGIT expression (n=22). (b) Distribution of CD4+ T cell memory subsets. (c) PD-1 and TIGIT expression on naïve and memory (i.e. central memory, effector memory and terminally differentiated) CD4+ T cells. Statistics: Student's paired t-test. (d) Bar graph illustrating proportions of naïve and memory CD4+ T cells in all individuals. (e) PD-1 and TIGIT expression on total CD4+ T cells. (d, e) Individual data ranked according to percentage memory CD4+ T cells. (g–h) Graphical illustration of the coinciding correlation of (f) PD-1, (g) TIGIT and (h) HIV-1 DNA in total CD4+ T cells with the percentage of memory CD4+ T cells

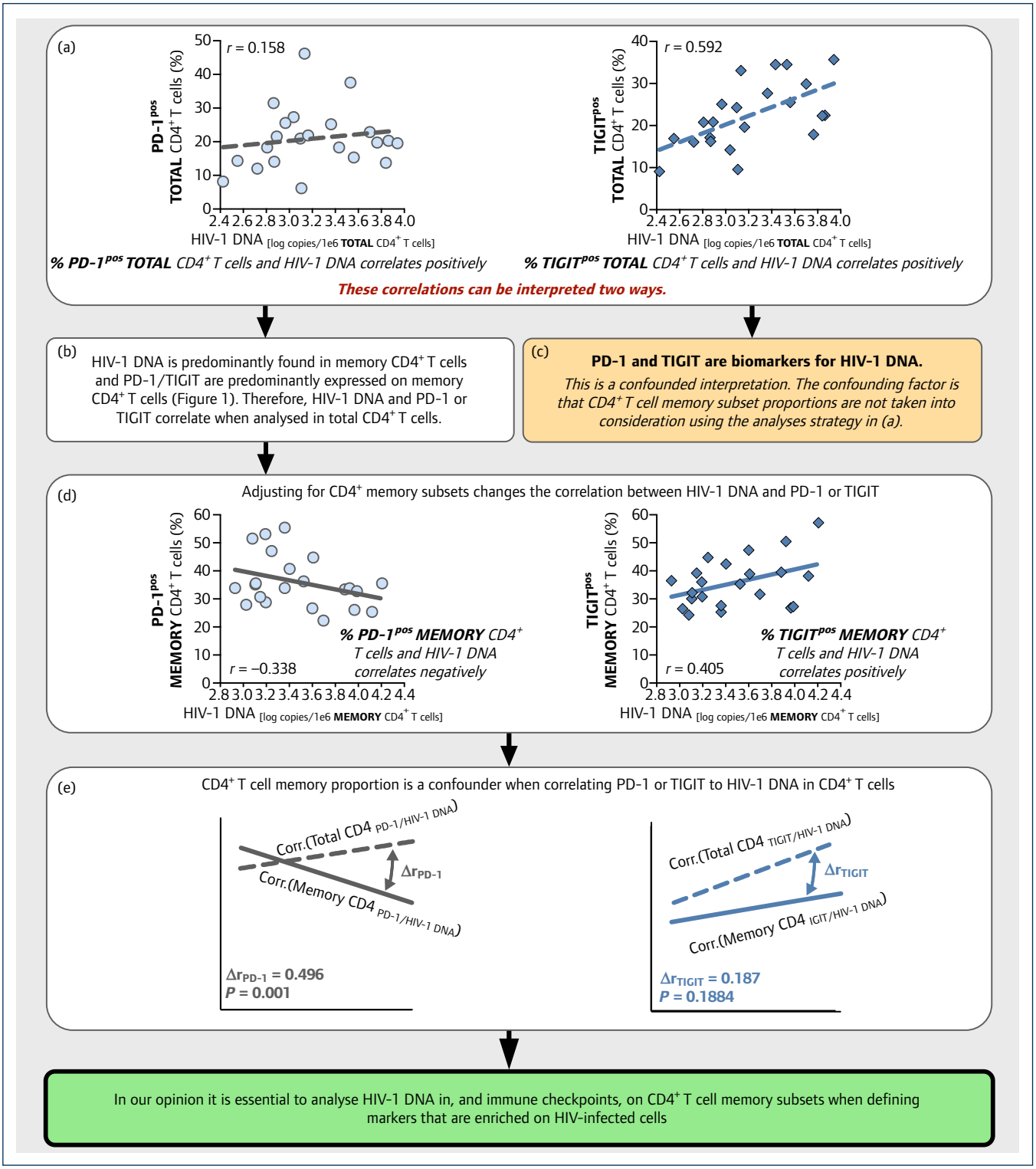

Figure 2. Decision algorithm for evaluating PD-1 and TIGIT as biomarkers for HIV-infected cells. (a) Pearson correlation of HIV-1 DNA and PD-1 (left) or TIGIT (right) on total CD4+ T cells. (b and c) Two potential interpretations of the data depicted in (a). (d) Pearson correlation of PD-1 (left) or TIGIT (right) and HIV-1 DNA in memory CD4+ T cells (estimated by adjusting for the relative contribution of naïve CD4+ T cells for each individual as previously published [15]). (e) Δr_{PD-1} and Δr_{TIGIT} estimated by bootstrap analyses for 95% confidence interval and permutation test for *P*-value

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