Increased HIV-1 transcriptional activity and infectious burden in peripheral blood and gut-associated CD4\(^+\) T cells expressing CD30

Louise E. Hogan\(^1\)*, Joshua Vasquez\(^1\), Kristen S. Hobbs\(^1\), Emily Hanhauser\(^1\), Brandon Aguilar-Rodriguez\(^1\), Rajaa Hussien\(^1\), Cassandra Thanh\(^1\), Erica A. Gibson\(^1\), Alexander B. Carvidi\(^1\), Louis C. B. Smith\(^1\), Shahzada Khan\(^2\), Martin Trapecar\(^2\), Shomyseh Sanjabi\(^2,3\), Ma Somsouk\(^4\), Cheryl A. Stoddart\(^1\), Daniel R. Kuritzkes\(^5\), Steven G. Deeks\(^6\), Timothy J. Henrich\(^1\)*

1 Division of Experimental Medicine, University of California San Francisco, San Francisco, California, United States of America, 2 Virology and Immunology, Gladstone Institutes, San Francisco, California, United States of America, 3 Department of Microbiology and Immunology, University of California San Francisco, San Francisco, California, United States of America, 4 Division of Gastroenterology, Department of Medicine, University of California San Francisco, San Francisco, California, United States of America, 5 Division of Infectious Diseases, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts, United States of America, 6 Positive Health Program, Department of Medicine, University of California San Francisco, San Francisco, California, United States of America

* lou.hogan3@gmail.com (LEH); timothy.henrich@ucsf.edu (TJH)

Abstract

HIV-1-infected cells persist indefinitely despite the use of combination antiretroviral therapy (ART), and novel therapeutic strategies to target and purge residual infected cells in individuals on ART are urgently needed. Here, we demonstrate that CD4\(^+\) T cell-associated HIV-1 RNA is often highly enriched in cells expressing CD30, and that cells expressing this marker considerably contribute to the total pool of transcriptionally active CD4\(^+\) lymphocytes in individuals on suppressive ART. Using \textit{in situ} RNA hybridization studies, we show co-localization of CD30 with HIV-1 transcriptional activity in gut-associated lymphoid tissues. We also demonstrate that \textit{ex vivo} treatment with brentuximab vedotin, an antibody-drug conjugate (ADC) that targets CD30, significantly reduces the total amount of HIV-1 DNA in peripheral blood mononuclear cells obtained from infected, ART-suppressed individuals. Finally, we observed that an HIV-1-infected individual, who received repeated brentuximab vedotin infusions for lymphoma, had no detectable virus in peripheral blood mononuclear cells. Overall, CD30 may be a marker of residual, transcriptionally active HIV-1 infected cells in the setting of suppressive ART. Given that CD30 is only expressed on a small number of total mononuclear cells, it is a potential therapeutic target of persistent HIV-1 infection.

Author summary

Previous studies have shown that higher levels of soluble CD30 are associated with HIV-1 disease progression. Many of these studies, however, were performed prior to the
implementation of combination ART, and the relationship between surface CD30 expression, soluble CD30 and HIV-1 infection in ART suppressed individuals, or those with viremic control off ART, is not known. We demonstrate that cell-associated HIV-1 RNA is highly enriched in CD4⁺ T cells expressing CD30, a member of the tumor necrosis factor receptor superfamily. These findings were observed in several HIV-1 infected donor groups, regardless of whether or not the participants were receiving suppressive ART. Furthermore, we demonstrate that ex vivo treatment with brentuximab vedotin, an antibody-drug conjugate that targets CD30, reduces the total amount of HIV-1 DNA in PBMC obtained from infected individuals. Finally, we show through in situ RNA hybridization studies that CD30 and HIV transcriptional activity co-localize in cells from gut biopsies obtained from HIV-1 infected donors. These data suggest that CD30 may be a marker of residual, transcriptionally active HIV-1 infected cells in the setting of suppressive ART.

Introduction

CD30, a member of the TNF receptor superfamily, is expressed on tumor cells found in Hodgkin and other aggressive lymphomas [1,2] but only on a very small percentage of lymphocytes in healthy individuals [2,3,4,5,6,7]. Although its functions are largely undefined, CD30 has been implicated in the activation, proliferation and death of selected cell populations [2,4,8,9]. Stimulation of this receptor has been shown to activate NF-κB, a protein complex that regulates immune responses [2,9,10]. Infections with viral pathogens, such as human T-cell lymphotropic virus (HTLV), Epstein-Barr virus (EBV), and poxviruses markedly increase surface expression of CD30 compared with cytokine activation alone [4,11,12]. Given the rarity of CD30 expression in vivo, the dramatic increases in the setting of these infections may be secondary to virus-specific cell stress responses.

Prior studies have demonstrated that stimulation of CD30 results in increased HIV-1 expression ex vivo in cells obtained from untreated individuals, and higher levels of soluble CD30 are associated with HIV-1 disease progression [4,5,8,13,14,15,16,17,18,19,20]. However, many of these studies were performed in the setting of untreated HIV-1 infection and the relationship between surface CD30 expression, soluble CD30 (sCD30) and viral persistence in ART suppressed individuals or those with viremic control off ART is unknown. It is possible that CD30 is expressed on transcriptionally active HIV-1-infected residual cells in the setting of ART. Therefore, we investigated the relationship between CD30 and HIV-1 burden in peripheral blood and gut-associated lymphoid tissue from HIV-1-infected individuals on and off therapy. Overall, we demonstrate that HIV-1 infected CD30⁺ T cells markedly contribute to the total population of HIV-1 infected and transcriptionally active CD4⁺ T cells in several individuals regardless of ART use. In addition, we observed that CD30 and HIV-1 transcriptional activity co-localized in gut-associated lymphoid tissues in individuals on or off ART. Finally, we showed that brentuximab vedotin, significantly reduced the total amount of HIV-1 DNA in PBMC obtained from infected individuals.

Results

CD30 expression on CD4⁺ T cells is increased in HIV-1 infection

We analyzed the peripheral blood of HIV-1 infected and uninfected individuals (S1 Table, S1 Fig) for CD30 expression on PBMC, and plasma sCD30 levels. The frequency of CD30⁺CD4⁺
T cells was significantly higher among viremic and ART-suppressed HIV-1 infected groups, irrespective of ART regimen, compared to HIV-1-uninfected controls (p = 0.045 and p = 0.002 respectively). However, no significant differences in the frequency of CD30+CD4+ T cells were observed between the untreated and treated HIV-1 infected cohorts (Fig 1A). Plasma soluble CD30 levels were significantly higher in the viremic donor group as compared to the suppressed and uninfected group (p<0.001 and p<0.001 respectively) (Fig 1B), but sCD30 levels did not correlate significantly with CD30 surface expression (Fig 1C).

Fig 1. Assessment of CD30 expression on CD4+ T cells. (A) Percentage of CD4+ T cells expressing CD30 in samples from HIV-1-uninfected (n = 10) ART suppressed (n = 17) and viremic donors (n = 9) are shown. Surface expression was higher in ART suppressed and viremic HIV-1-infected individuals (p = 0.0023 and p = 0.045 respectively). (B) sCD30 (IU) in plasma was highest in viremic (n = 4) compared with HIV-1-uninfected (n = 10) and ART suppressed donors (n = 9) (p<0.001 and p<0.001 respectively). (C) No significant correlations were identified between the percentage of CD4+CD30+ T cells and sCD30 levels (r = 0.20, P = 0.35 by Spearman rank correlation analysis). The percentage of CD4+ T cells co-expressing CD69 in each cohort are shown in (D) and the percentage of CD69+CD4+ T cells expressing CD30 are shown in (E). The percentage of CD4+ T cells co-expressing HLA-DR (F) and HLA-DR+CD4+ T cells expressing CD30 (G) are shown. Despite significant increases in CD30+ cells expressing CD38/HLA-DR, there were no significant differences in the frequency of CD30 expression in CD38/HLA-DR-expressing CD4+ T cell populations. (H) The highest frequency of CD30+ T cells observed in HIV-1-infected individuals were of transitional/effector memory phenotype, compared to naïve T cells in healthy controls. (I) CD30+CD4+ and CD30-CD4+ T cells from viremic donors expressed significantly more PD1 than healthy controls (p = 0.02 and p = 0.02 respectively). CD30+CD4+ and CD30-CD4+ T cells from ART suppressed donors also expressed significantly more PD1 than healthy controls (p = 0.002 and p = 0.002 respectively). (J) Conversely, very few CD4+PD1+ T cells co-expressed CD30, but ART suppressed donors had significantly higher expression compared to healthy controls (p = 0.01). T Bars represent median ± interquartile range for all data. *p<0.05; **p < 0.01; ***p < 0.001. Significant intergroup differences were determined using rank Kurskal-Wallis tests incorporating Dunn’s tests for multiple comparisons. Wilcoxon matched-pairs signed rank tests were used to determine statistical significant between CD30 intergroup, paired samples.

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CD30 expression is preferentially expressed on activated effector memory cells in HIV-1 infected individuals

The relationship between CD30 expression and immunological phenotypes in the setting of HIV-1 infection was determined on fresh peripheral blood CD4⁺ T cells isolated from HIV-1-infected and uninfected individuals. Both the frequencies of CD69 and co-expression of HLA-DR and CD38 were significantly increased in healthy controls and ART suppressed participants within CD30⁺CD4⁺ T cells compared to the CD30⁻CD4⁺ T cells (CD69; p = <0.002 and p = 0.001, respectively; HLA-DR; p = 0.002 and p = 0.03, respectively) (Fig 1D and 1F).

The expression of CD30 is known to increase during many biological processes, including cellular activation [21]. However, the expression of CD30 is rare in individuals without concomitant viral infection or specific hematological malignancies. Therefore, to clarify whether we were simply selecting a highly activated population of CD4⁺ T cells, we determined the percentage of CD69⁺, HLA-DR⁺ or PD-1⁺ cells that expressed CD30 in addition to the percentages of CD30 that express these markers. Our data showed that few cells expressing markers associated with early or late activation (CD69 and CD38/HLA-DR, respectively) expressed CD30 (Fig 1E and 1G).

CD30 is thought to be up-regulated on activated CD4⁺ T cells, and while studies have demonstrated this following artificial stimulation in vitro [21,22,23], we are unaware of any data examining CD30 expression on unstimulated, non-malignant cells ex vivo. Therefore, we determined the expression of CD30 on unstimulated CD4⁺ T cell subsets in HIV-uninfected individuals. Interestingly, CD30 expression in HIV-uninfected donors was observed primarily on naïve CD4⁺ T cells, whereas most CD30-expressing CD4⁺ T cells in HIV-1-infected individuals, on or off ART, were found to be of effector/transitional memory phenotype (Fig 1H). A significantly higher percentage of CD30-expressing cells in healthy controls and HIV-1 infected donors also co-expressed PD-1 (p = 0.0020, p = 0.015), but again, less than 10% of all PD-1 expressing cells co-expressed CD30 (Fig 1I and 1J).

HIV-1 enrichment in CD30-expressing CD4⁺ T cells

To assess the relationship between CD30 expression, HIV-1 transcriptional activity, and cell-associated HIV-1 DNA and RNA levels, we utilized fluorescence activated cell sorting to isolate CD30⁺CD4⁺ and CD30⁻CD4⁺ T cells obtained from 29 HIV-1-infected donors on suppressive ART (n = 17), viremic donors with high viral loads (n = 9), and individuals able to control HIV-1 without ART (HIV-1 controllers, n = 3; HIV-1 plasma RNA levels <500 copies/mL). HIV-1 genomic DNA and unspliced RNA were quantified by PCR for each subset [24,25]. Cell-associated HIV-1 RNA was significantly enriched within the CD30⁺CD4⁺ T cell population (p = 0.007 and p = 0.008 for ART suppressed and viremic groups, respectively) (Fig 2A and 2B). Despite CD30⁺ cells having several orders of magnitude higher HIV-1 DNA levels in samples from several individuals, no intragroup statistical significance was identified. Samples from all participants were included in the analysis, regardless of CD30 recovery from sorting (which at times was low, e.g. <50 cells) or if HIV-1 RNA or DNA were not detected in cells from these participants to reduce selection bias.

CD30 and HIV-1 transcriptional activity

In order to determine the contribution of CD30⁺CD4⁺ T cells to the total RNA and DNA burden of HIV-1 infection in peripheral CD4⁺ T cell compartments, we compared the total HIV-1 RNA and DNA recovered from each T cell subset, taking into account the number of cells obtained, from ART suppressed (n = 17), viremic donors (n = 9) and HIV controllers (n = 3).
The average percentage of CD4+ T cells expressing CD30 was found to be 3.97%, 1.05% and 0.03% in suppressed, viremic and controller groups, respectively (Fig 2C). Despite the rarity of these cells, an average of 21%, 28.5% and 51.4% HIV-1 RNA was attributed to CD30+CD4+ T cells in suppressed, viremic and controller groups, respectively. Strikingly, >90% of detectable...
cell-associated HIV-1 RNA was found within CD30^+CD4^+ T cells in samples from five individuals either on or off ART (Fig 2D). The largest contribution of CD30^+ T cells to the peripheral HIV-1 DNA burden was observed in ART suppressed individuals, and 18.3%, 2.2% and 0.05% of HIV-1 DNA was attributed to CD30^+CD4^+ T cells in suppressed, viremic, and controller donor groups, respectively (Fig 2E). Moreover, in three ART-suppressed individuals, >50% of peripheral cell-associated HIV-1 DNA was found within CD30^+CD4^+ T cells. Overall, there was a high degree of inter-participant variability in the contribution of CD30^+CD4^+ T cells to the peripheral HIV-1 burden.

No significant relationship between the frequency of CD30^+CD4^+ T cells and CD4^+ T cell count, age, or number of years since diagnosis was identified in non-parametric analyses (all P > 0.05). However, the contribution of CD30^+CD4^+ T cells to the total pool of CD4^+ T cell-associated HIV-1 RNA was found to be significantly higher in African American individuals (mean = 42.7%) compared to white individuals (mean 12.8%), irrespective of ART regimen (P = 0.0103) (Fig 2F). There were no significant differences in HIV-1 DNA contribution between racial groups (Fig 2G).

In order to verify that the rare population of CD30^+ cells obtained from cell sorting was not from non-specific staining or other flow cytometric artefact, we performed mRNA gene expression PCR assays incorporating mRNA for CD3 complex genes, CD4, CD8α, and CD80 (a surface marker expressed on B cells and non-lymphocyte cells) on 5 additional sorted blood samples (S2 Fig). These additional extractions did not involve the use of HIV-uninfected carrier cells, in order to obtain undiluted human RNA from CD30^+ and CD30^- cells. Overall, a majority of 2^−ΔΔCt values from the CD3 and CD4 mRNA PCR arrays comparing CD30^+ to CD30^-CD4^+ T cells were equal to or greater than one, suggesting that CD30^+ cells have similar, if not higher levels of these mRNA transcripts. 2^−ΔΔCt is a measure of difference between two populations within individual samples based on the cycle threshold values obtained by quantitative PCR. A value of one indicates no difference and values greater than one indicate a greater number of RNA transcripts. In contrast, CD8 and CD80 mRNA were not detected in CD30^+ cells from 3 and 4 samples, respectively (S2 Fig) whereas CD80 and CD8 mRNA could be detected in all of the sorted CD30^- cell populations. These results indicate that the sorted CD30^+ cells have mRNA profiles consistent with CD3^+CD4^+ lymphocytes.

**Lymphocyte phenotypes and HIV-1 burden in rectal CD4^+ T cells expressing CD30 and CD32**

We obtained rectal tissue from five ART-suppressed individuals for phenotypic analysis and flow sorting of CD4^+ T cells into CD30^-, CD32^-, CD30^-CD32^-, and remaining CD30^+CD32^- subsets (S3 Fig & S3 Table) and incorporated CD13 into the staining panel of two donors to ascertain potential non-lymphocyte cell contamination (of note, CD14 is not expressed in gut-associated monocytes). Overall, we detected HIV-1 DNA and RNA in all of the CD30^-CD32^-CD4^+ lymphocytes but none in the CD30^+CD32^- population. HIV-1 RNA and DNA were intermittently detected in the CD30^-CD32^+ and CD30^-/CD32^- subsets, at times, 1–2 log_{10} higher than the CD30 CD32^-CD4^+ T cells (Fig 3A and 3B). CD4^+ T cells had significantly higher expression levels of HLA-DR within the CD32 expressing subpopulations compared to CD30^-CD32^- and CD30 CD32^- cells (Fig 3C), and up to 42% of HLA-DR^+CD30^-CD32^-CD4^+ T cells co-expressed CD13 compared to <11% of CD30^-CD32^- cells. No significant differences in HIV-1 RNA or DNA were observed between cell subsets obtained from the gut samples. It is possible that the extensive processing and collagenase treatment used to isolate cells from GALT samples may have altered surface phenotypes. Therefore, we proceeded to examine tissue samples directly using direct mRNA staining.
HIV-1 RNA and CD30 mRNA are co-localized in human gut tissues

To determine the single-cell co-localization of HIV-1 and CD30 mRNA transcriptional activity in tissue, we performed in situ hybridization (ISH) on tissue samples obtained from ileum and rectum of HIV-1-infected individuals on (n = 6) and off (n = 3) ART, and one aviremic HIV-1 controller (S1 Table). In HIV-1-uninfected control tissue samples, <0.01% of all gut-associated lymphoid tissue (GALT) cells expressed CD30 RNA (Fig 4). However, the observed CD30 expression was almost exclusively co-localized with HIV-1 RNA in gut samples from both viremic and ART suppressed individuals (Fig 4). Interestingly, we found a significantly higher percentage of co-localization of CD30 and HIV-1 RNA in ART-suppressed individuals (88% of all HIV-1 RNA expressing cells in gut tissues expressed CD30) compared with co-localization in viremic participants (32.5%; P = 0.008).

Quantitative viral outgrowth

In order to determine whether or not CD4+ T cells expressing CD30 harbour replication competent virus, we sorted PBMC from eight additional ART-suppressed individuals and
performed traditional quantitative viral outgrowth assays (qVOA) incorporating serial dilutions of CD30\(^+\) T cell subsets. We were only able to detect positive p24 in CD30\(^-\)CD32\(^-\)CD4\(^+\) T cells in two of eight participant samples (total input cells ranged from 3 to 10 million) after 21 days of co-culture (infectious units per million cells (IUPM) of 0.11 and 0.23). We were not able to detect p24 in any wells containing CD30\(^+\) cells, but experiments were limited by the very small numbers of cells that could be recovered and incorporated in the qVOA experiments (< 1,000 input cells/well).

**Brentuximab vedotin reduces total cell-associated HIV-1 DNA in PBMC**

Brentuximab vedotin, an anti-CD30 antibody-drug conjugate approved for the treatment of various haematological malignancies in which CD30 is over expressed, has shown efficacy in reducing the burden of CD30\(^+\) malignant cells in vivo [10]. In preliminary experiments, we observed a 43% to 80% decrease in the percentages of CD4\(^+\) T cells expressing CD30 obtained from three ART-suppressed donors following 48 hours of \textit{ex vivo} brentuximab vedotin exposure (10\(\mu\)g/ml) compared with parallel experimental wells with no antibody-drug conjugate (S4 Fig). Next, we exposed PBMC obtained from seven HIV-1-infected individuals on suppressive
ART to various concentrations of brentuximab vedotin. Following culture of PBMC in the presence of antiretroviral drugs (raltegravir, 3TC) and brentuximab vedotin for 5 days, we observed a significant reduction in the mean total cell-associated HIV-1 DNA within samples at the higher input concentrations (100μg/ml p = 0.047, 500μg/ml p = 0.039; Fig 5). However, no reduction in the viability of total input PBMC was observed, suggesting that brentuximab vedotin may have selectively depleted CD30+ cells enriched in HIV-1 DNA. Overall, variations in DNA levels in no-ADC control wells were observed between participant samples. However, participants were not chosen based on baseline HIV reservoir size and all experimental wells, including those with and without brentuximab vedotin were exposed to the same input cell number and culture conditions.

Undetectable cellular HIV-1 levels in an individual treated with brentuximab vedotin

Successful use of the brentuximab vedotin for refractory lymphomas has been described in two HIV-1-infected patients[26], but assessments of viral reservoir size after therapy have not been reported. We identified an individual who lacked detectable HIV-1 in cells after receiving six cycles of brentuximab vedotin for anaplastic and cutaneous T-cell lymphomas. The patient had low-level plasma viremia detectable after conventional chemotherapy (<400 RNA copies/ml), but no HIV-1 was detected in purified CD4+ T cells using sensitive real-time PCR assays for cell-associated RNA or DNA, and no plasma virus was detected by clinical viral load assays after six cycles of brentuximab vedotin. Unfortunately, the individual passed away from central nervous system tumor recurrence, and further longitudinal sampling or tissue collection was not possible. Furthermore, cells were not able to be collected prior to initiation of brentuximab vedotin. Nonetheless, this finding was unexpected as we have observed detectable HIV-1 DNA and RNA in PBMC from all other HIV-1-infected individuals in a cohort of 15 patients who completed multiple cycles of systemic chemotherapy (not including Brentuximab) for a variety of solid organ and hematological malignancies, as we have previously reported [27] (CD4+ T cell-associated HIV-1 DNA increased from a mean of 2,204 copies/10^6 cells to 43,961 copies/10^6 cells following completion of cancer chemotherapy in 10 individuals with pre- and post-chemotherapy time-points available). The HIV-1 DNA and RNA assays had a detection limit of <15 copies/10^6 CD4+ T cells given the available number of input cells for this individual.

Discussion

Previous studies have shown that higher levels of sCD30 are associated with HIV-1 disease progression [4,5,8,13,14,15,16,17,18,19,20]. However, the relationship between surface CD30 expression, sCD30 and HIV-1 burden in ART suppressed individuals and HIV controllers, is currently unknown. Here, we demonstrate that cell-associated HIV-1 RNA is significantly enriched in CD4+ T cells expressing CD30, a member of the tumor necrosis factor receptor superfamily, and this effect was observed in many, but not all, individuals. These findings were observed in several HIV-1 infected donor groups, regardless of whether or not the participants were receiving suppressive ART. HIV-1 DNA was also enriched in CD4+ lymphocytes expressing CD30 from some individuals, with HIV-1 DNA exclusively detected in CD30+ cells from one participant. When present, the magnitude of nucleic acid enrichment within CD30+CD4+ T cells was higher than previously reported for peripheral blood CD4+ T cells co-expressing PD-1 alone, or PD-1, TIGIT and LAG-3 (<1 log_{10} fold change) [28], and roughly similar to the frequency of lymph node-derived PD-1+CD4+ T cells that harbor inducible HIV-1 and total HIV-1 DNA burden in peripheral blood CD32+CD4+ T cells as previously described.
Fig 5. Anti-CD3 therapy reduces total HIV-1 DNA burden ex vivo. Following ex vivo culture of PBMC in the presence of antiretroviral drugs (raltegravir, 3TC) and brentuximab vedotin for 5 days, we observed a significant reduction in the mean total cell-associated HIV-1 DNA from seven ART-suppressed individuals, despite no change in viable cell numbers. Significant overall decreases were found within samples at the higher input concentrations (100μg/ml \( p = 0.0469 \), 500μg/ml \( p = 0.0390 \)). Significant intergroup differences were determined using paired, nonparametric Friedman tests incorporating Dunn’s tests for multiple comparisons. All experimental wells with and without brentuximab vedotin were subject to the same input cell number and culture conditions.

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Overall, our results suggest that CD30 may be a marker of residual, transcriptionally active HIV-1 infected cells in setting of suppressive ART.

CD30 was found to be primarily expressed on peripheral blood CD4\(^+\) T\(_{TM/EM}\) subsets in HIV-1 infected donors. Although central memory T cells (T\(_{CM}\)) have been shown to be a major peripheral blood reservoir of HIV [31], T\(_{EM}\) and T\(_{TM}\) subsets contribute significantly to the amount of cell-associated HIV-1 RNA and DNA in blood, and a majority of viral nucleic acid found in GALT, a major anatomic site of HIV persistence [32,33]. In addition, a recent study of six individuals on suppressive ART demonstrated that T\(_{EM}\) cells contained the largest proportion of intact HIV sequences [34]. Given the possibility that CD30 expression on CD4\(^+\) T\(_{EM}\) and T\(_{TM}\) subsets may be due to cellular activation, we sought to clarify if we were simply selecting a highly activated portion of CD4\(^+\) T cells, rather than a potential marker of viral infection. Encouragingly, the majority of activated T cells in all individual samples in our study did not co-express CD30. Furthermore, it is established that viremic individuals have higher levels of immune activation than individuals on ART [35]. In contrast, we observed that individuals on or off ART have similar frequencies of CD30\(^-\)CD4\(^+\) T cells. This observation, in addition to our finding that <1% of CD4\(^+\) T cells, on average, were CD30 positive, suggest that CD30 is not a marker commonly expressed on activated T cells in the setting of HIV-1 infection, and may be due to more specific viral cellular stress responses.

Results from the gut in-situ hybridization studies also support the conclusion that HIV-1 RNA is enriched in CD30-expressing cells in individuals on suppressive ART. Although we were unable to differentiate between CD4\(^+\) T cells and tissue-resident macrophages or dendritic cells, nearly all CD30 mRNA\(^+\) cells co-expressed HIV-1 RNA, and a larger majority of HIV-1 RNA\(^+\) cells expressed high levels of CD30 mRNA. We did not find significant differences between HIV-1 RNA and DNA levels determined by qPCR in sorted CD30\(^-\) and CD30\(^+\) CD4\(^+\) T cell subsets isolated from fresh rectal tissue in ART-suppressed individuals, but the sample size was small and the number of CD32\(^+\) and/or CD30\(^+\) cells that could be recovered was often low. It is possible that gut tissue collagen digestion and processing influenced transcriptional regulation or surface presentation of CD30 and other surface markers, a phenome-non which has been previously described for other surface proteins [36]. The highest levels of HIV-1 RNA and DNA from rectal tissue-derived cells were obtained from cells co-expressing CD30 and CD32. Interestingly, this subset had the highest HLA-DR expression and greatest frequency of CD13 cells, suggesting that there may have been some degree of macrophage/myeloid carryover during tissue sorting. It is also possible that the HIV-1 RNA was enriched in activated gut-associated CD4\(^+\) T cells, as lymphocyte activation has been shown to be higher in GALT than peripheral blood [32]. As a result, further phenotypic characterization and study of HIV persistence in these tissue-derived cell subsets is needed.

Overall, our results suggest that CD30\(^+\) expression appears to be a marker of residual HIV-1 transcriptional activity in the setting of suppressive ART, at least in some individuals. However, several of our observations indicate that CD30 may also be a marker of more stable HIV-1 infection in the setting of ART or that CD30 expression waxes and wanes over time in infected cells. For example, sCD30 was only found to be higher in viremic individuals and sCD30 levels in ART-treated individuals were similar to those in uninfected controls. These data suggest that sCD30 may be increased as a result of persistent active viral production or excessive immune activation, whereas the surface expression of CD30 may represent a more stable, general HIV-related marker in both viremic and suppressed individuals. The mechanisms for the upregulation of CD30 in the setting of HIV-1 infection are unknown and warrant further investigation.

Finally, we demonstrated that ex vivo treatment with brentuximab vedotin, an antibody-drug conjugate that targets CD30, significantly reduced the total amount of HIV-1 DNA in...
PBMC obtained from seven HIV-infected individuals. These data suggest that CD30 may have a role as a potential HIV-1 therapeutic target, as it is highly correlated with residual HIV-1 transcriptional activity, even in individuals on suppressive ART, but is not expressed on a vast majority of otherwise healthy cells. Other cell surface markers that have previously been associated with HIV-1 enrichment (e.g. PD-1 or CD32) are expressed on a variety of non-T lymphocyte cell types which may make clearance of rare HIV-infected cells without significant off-target toxicities challenging. Furthermore, there are very few cytoreductive ADCs in clinical use, and these drugs are typically directed towards disease-specific targets [10].

We observed variation in responses to ex vivo brentuximab vedotin within this study, which is expected given the described variation within HIV-1 burden and CD30 expression between individuals in blood. It is possible that factors such as cellular HIV-1 DNA burden and the level of CD30 expression within individuals may influence the reservoir responses to brentuximab vedotin, and further studies involving concomitant latency reversal are warranted. While the observed loss of CD4+ T cell-associated HIV-1 DNA and RNA from the individual who received multiple cycles of brentuximab vedotin is only anecdotal, this case finding inspired the detailed ex vivo work of CD30 as a potential marker for HIV-1 infection as described above. Further identification of HIV-infected individuals receiving brentuximab vedotin for malignancy and detailed viral reservoir investigation are needed.

This study has several limitations. We observed that CD30 expression is significantly altered following freeze thaw procedures, and fresh blood draws were required. It is also highly possible that collagenase treatment of GALT samples altered surface phenotype, as previously described with other surface markers[36]. Additionally, the number of participants that could be recruited to the study was restricted and dependent on long cell-sorting time or the need for freshly reconstituted brentuximab vedotin. As a result, pre-selection of participants was limited and different individuals were included in each of the subsequent studies.

Overall, we noted high inter-participant variability in the HIV-1 reservoir measurements. However, variation between study participants is expected given the complex interplay between infection and expression of surface markers. At times, samples with low CD30 recovery from cell sorting resulted in the inability to detect HIV-1 RNA or DNA. Variability in the lower limits of detection has been previously documented using flow cytometric sorting of rare target cells [23]. Nevertheless, rather than exclude these samples, we included results from all participants to avoid biasing our results. In some instances, very high levels of HIV-1 RNA enrichment were observed in CD30+ T cells from a minority of participants. These high measurements may have partially been due to artefact from calculations involving very small denominators (e.g. small numbers of cells surveyed). However, we identified a very close relationship between HIV-1 RNA and CD30 mRNA expression in gut using in situ hybridization studies which provides additional support using an independent method that CD30 expression is directly related to cellular HIV-1 burden and transcriptional activity. We did not measure CD30 expression following ADC treatment in the brentuximab vedotin dose response experiments, but did observe large reductions in the percentage of CD4+ T cells expressing CD30 in the presence of ADC in preliminary experiments performed on samples from three ART-suppressed individuals. While this reduction may represent ADC-targeted cell killing, CD30 staining may also have been affected by steric interference with ADC-bound receptor or receptor downregulation.

We were unable to detect positive viral outgrowth in experiments including blood CD4+ T cells expressing CD30. This is likely due to limitations in the traditional qVOA when using very low numbers of input cells (in some cases, less than 100 cells per well for CD30+ subsets), and may or may not reflect the potential for CD30 expressing cells to harbor replication.
competent virus. Further studies involving methods, such as whole genome sequencing, may prove more useful in estimating intact proviral burden within CD30+ subsets.

In conclusion, we describe the enrichment of HIV-1 RNA in CD30+ CD4+ T cells from HIV-1 infected individuals on suppressive ART. Furthermore, a large proportion of total CD4+ T cell-associated HIV-1 RNA is found within CD30 expressing cells from suppressed and viremic individuals, and bretuximab vedotin is capable of reducing HIV-1 DNA burden. Further investigation is warranted to evaluate the stability of CD30 following HIV-1 infection and the development of latency, and to determine the efficacy of CD30 as a potential therapeutic target.

Methods

Study population and peripheral blood mononuclear cell collection

In total, 43 HIV-1-infected and ten HIV-uninfected participants ≥18 years of age were enrolled. Clinical data obtained included history of ART, viral load measurements, and CD4+ T cell counts, race/ethnicity, age, and detailed medical histories. Individuals on or off ART were included in the study. Leukapheresis was performed through the UCSF SCOPE cohort for the collection of >1 billion PBMC used in cell sorting and viral outgrowth assays (VOA).

Ethics statement

The study was approved by the UCSF Committee for Human Research and the Dana-Farber/Harvard Cancer Centres Institutional Review board. All volunteers provided written informed consent.

Peripheral blood cell isolation and rectal tissue processing

Peripheral blood mononuclear cells were separated from whole blood using density centrifugation over Histopaque (Sigma). Isolation of CD4+ T cells was then performed using EasySep Human CD4+ T Cell Enrichment Kits (Stem Cell Technologies), following the manufacturer’s protocol. For rectal tissue, to remove the epithelium and epithelial cells, 30 tissue biopsy tissue pieces were incubated in pre-warmed buffer containing 10mM DTT, 5mM EDTA, 10mM HEPES and 5% FBS for 20 minutes at 37˚C under continuous rotation, vortexed and the supernatant aspirated. Tissue pieces were then incubated for a second time in the same pre-warmed buffer for a further 20 minutes at 37˚C under continuous rotation. The sample was again vortexed, and then rinsed for 20 minutes at 37˚C under continuous rotation with RPMI, 10mM HEPES and 5% FBS. To then isolate lymphocytes from the lamina propria, a second, pre-warmed buffer containing RPMI, 10mM HEPES, 7.5μg/ml DNAse and 5% FBS was applied to the tissue pieces, and these were again incubated for 20 minutes at 37˚C under continuous rotation. The samples were then vortexed, and aspirated numerous times with a blunt 20G needle until tissue was viably broken down. The cells were then rinsed twice with RPMI, 10mM HEPES and 5% FBS and passed through a 70μm cell strainer, pelleted and stained as described below for fluorescent activated sorting.

Antibody staining and fluoresce activated cell sorting

CD4+ T cells were stained in PBS with either Brilliant Violet 605-conjugated anti-CD4 (OKT4) (Biolegend), allophycocyanin (APC)-conjugated anti-CD30 (BY88) (Biolegend) and fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (SK7) (BD Biosciences) for sorting only, or phycoerythrin (PE)-Cy7-conjugated anti-CCR7 (3D12) (BD Biosciences), Brilliant Violet 711-conjugated anti-CD3 (UCHT1) (BD Biosciences), Brilliant Violet 421-conjugated
anti-PD-1 (EH12.1) (BD Biosciences), Brilliant Violet 650-conjugated anti-CD4 (SK3) (BD Biosciences), alexa Fluor 700-conjugated anti-HLA-DR (G46-6) (BD Biosciences), PE-conjugated anti-CD38 (HB7) (BD Biosciences), fluorescein isothiocyanate (FITC)-conjugated anti-CD69 (L78) (BD Biosciences), Qdot 605-conjugated anti-CD8 (3B5) (Life Technologies), PE-Texas Red-conjugated anti-CD45RA (MEM-56) (Life Technologies), allophycocyanin (APC)-conjugated anti-CD30 (BY88) (BioLegend) and LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (ThermoFisher Scientific) for CD4⁺CD30⁺ T cell phenotypical analysis. For the rectal tissue samples, cells were stained with LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (ThermoFisher Scientific), PE-CF594-conjugated anti-CD45RA (HI100) (Fisher Scientific), Brilliant Violet 711-conjugated anti-CD4 (SK3) (BD Biosciences), Brilliant Violet 650-conjugated anti-CD3 (SP34-2) (BD Biosciences), allophycocyanin (APC)-conjugated anti-CD32 (FUN-2) (BioLegend), PE-conjugated anti-CD30 (BERH8) (BD Biosciences), APC-H7-conjugated anti-HLA-DR (L243) (BD Bioscience), Brilliant Violet 586-conjugated anti-CD13 (WM15) (BD Biosciences) and Brilliant Violet 421-conjugated anti-CD38 (HIT2) (Fisher Scientific).

Cells were then analyzed and sorted on a BD FACSAria II (BD Biosciences), or analyzed on a LSR-II (BD Bioscience). Single stained beads (Life Technologies) were used for software-based compensation. During some sorts, data for phenotyping was also acquired on all events and analyzed in FlowJo V10 (TreeStar). Examples of gating strategies are shown in S1 & S3 Figs.

**Quantification of cell-associated HIV-1 RNA and DNA**

Purification of HIV-1 DNA and RNA from sorted cell populations was achieved using a Qiagen AllPrep DNA/RNA mini Kit, and following the manufacturer’s standard protocol, with an additional DNase treatment (QIAGen). Given the small number of CD30⁺ cells that could be obtained from flow sorting, these cell fractions were spiked into uninfected carrier PBMC to maximize HIV-1 DNA and RNA recovery and normalize extraction efficiency between CD30⁺ and CD30⁻CD4⁺ T cell populations. Spiking rare cells also allowed for the input of similar amounts of RNA into each PCR reaction. Quantitative PCR was performed to determine the levels of HIV-1 cell-associated RNA (caRNA), proviral DNA (pvDNA), and CCR5 in each subgroup. CCR5 was used to calculate assay cell concentration and extraction efficiency. Primer pairs and probe sequences were used as described in [24,37]. Briefly, the same primer and probe sequences were used for both total HIV-1 DNA and unspliced RNA and sit near the Gag-LTR junction, a highly conserved region among all group M HIV-1 sequences. PCR reactions were performed on an ABI OneStep qPCR machine (Applied Biosystems) using either the ABI TaqMan Universal PCR Master Mix for DNA or the ABI TaqMan Fast Virus 1-Step Master Mix for RNA for up to 45 cycles as we have previously described [24].

**Determination of soluble CD30**

Plasma from EDTA anticoagulant blood was isolated by density centrifugation followed by an additional spin and frozen until all donors had been recruited. Plasma was then thawed, and analysed using Human CD30 (soluble) Elisa Kit (Life Technologies) following the manufacturer’s standard protocol.

**Cell culture and antibody-drug conjugate treatment**

PBMC were cultured at 1 x 10⁶ cells/mL in RPMI medium (Life Technologies) supplemented with 10% heat-inactivated FBS (Gemini BioProducts), 100IU/ml penicillin and 100μg/ml streptomycin (Gemini BioProducts). Where required, Brentuximab Vedotin (Seattle Genetics) was added to cells at various concentrations. All cell cultures were performed in the presence
of antiretroviral treatment [raltegravir (40nM), and efavirenz (8nM) or T20 (20ng/mL) (Sell- eck) and IL-2 (500ng/mL) (Peprotech). Cells were cultured for five days, after which analysis by flow cytometry, DNA extraction, and quantitative PCR were performed, as described above.

In situ hybridization (ISH) of ileal and rectal tissue

Existing gut tissue was obtained through the UCSF SCOPE cohort by colonoscopy. Three millimeter sections were obtained from the gut and/or ileum (by jumbo forceps) of HIV-1-infected individuals on or off ART. Informed consent was obtained from all participants under the approval of the UCSF Committee on Human Research. Tissue was promptly preserved in 4% paraformaldehyde before paraffin embedding and sectioning for ISH. RNA-ISH was performed using RNAscope branched-DNA technology distributed by Advanced Cell Diagnostics (ACD), Newark, CA. The RNAscope assay was performed on 4-micron thick sections of FFPE human gut tissue (ileal and rectal) using the RNAscope 2.5 HD duplex kit. RNA-scope probes targeting HIV-1 RNA, and the anti-sense sequence of the Hs-TNFRSF8 gene (CD30) were developed by ACD and used according to the manufacturer’s recommendations. The HIV-1 infected ACH2 T cell line and HIV-negative rectal tissues were used as positive and negative controls, respectively. Sections were stained in multiplex for CD30 and HIV-1 RNA species and analyzed by bright-field microscopy. Whole slide digital images were obtained using the Leica Aperio slide scanner and total HIV-1-RNA+ or HIV-1-RNA+/CD30-RNA+ cells were manually counted in digital images of each section of tissues. Signal intensity was used as a qualitative measure of the level of transcription where small-punctate signals are thought to represent a single transcript and larger-darker clusters are thought to represent multiple transcripts in close proximity [38].

PCR T cell array

PBMC from an additional five individuals on suppressive ART were sorted into CD30+ and CD30- subsets followed by direct cell lysis and reverse transcription of mRNA to cDNA using TaqMan Gene Expression Cells-to-CT Kit (Life Technologies/Ambion) following manufacturer protocols, with the exception of the final preamplification step. cDNA of the CD3 receptor complex, CD4, CD80, CD8α and reference gene (18S and GUSB) mRNA were then quantified using the ABI OneStep-compatible Human T-Cell Receptor and CD3 Complex TaqMan Array (ThermoFisher) following manufacturer protocols. 2-ΔΔCt values were calculate as described [39] comparing CD30+ and CD30-CD4+ T cell subsets. B cells purified by negative magnetic bead selection (StemCell Technologies) were used as a control.

Viral outgrowth assays

Following fluorescent cell sorting of CD4+ T cell subsets based on surface marker expression as described above, cells were incorporated into a standard quantitative viral outgrowth assay exactly as previously describe [40]. In brief, this assay uses irradiated PMBC blasts, PHA and IL2 to maximally stimulate input cells, and is considered a gold-standard for quantitative co-culture. If sufficient cells were recovered, 5-fold serial dilutions starting with one to two million input cells per well were performed in duplicate. HIV-1 p24 levels were then determined by ELISA (PerkinElmer, San Jose, CA) following 21 days of co-culture. A well was considered positive if p24 levels were significantly higher than background levels from control wells containing uninfected cells. IUPMs and confidence intervals were determined using an online calculator incorporating maximum likelihood statistics as described [41].
**Statistical methods**

Significant intergroup differences were determined using rank Kurskal-Wallis of Friedman tests (depending on paired or unpaired data) incorporating Dunn’s tests for multiple comparisons. Wilcoxon matched-pairs signed rank tests were used to determine statistical significant between CD30 intergroup, paired samples. (Graphpad Software, vs. 7).

**Supporting information**

S1 Table. Patient Demographics, HIV-1 disease status and antiretroviral therapy for HIV-1-infected participants. (DOCX)

S2 Table. Experimental data for HIV-1 DNA and RNA PCR calculations. (DOCX)

S3 Table. Absolute cell numbers obtained following fluorescence-activated cell sorting of rectal tissue samples. (DOCX)

S1 Fig. Example of gating strategy used in flow cytometric and fluorescent activated cell sorting experiments. (A) Lymphocytes populations were first selected using forward and side scatter characteristics. Following this, the exclusion of doublets was performed by plotting cells on SSC-W versus SSC-H and similarly for FSC-W and FSC-H. Live and then CD3⁺ lymphocytes were selected, and further gated for CD4 expression. Following this CD4⁺ CD30⁺ T cells and CD30⁻ CD4⁺ T cells were selected in individual gates and sorted (for sorting experiments). (B) For further phenotypical analysis, the same gating was applied as described in (A) and then extended, allowing the identification of CD30 expressing cells with CD45RA and CCR7 populations (T cell subsets), CD69 (early activation), CD38 and HLA-DR (Late activation) and PD-1 expression. (C) CD30 expressing cells (red) are then compared and contrasted to CD30 negative CD4⁺ T cell populations (Blue), shown together on the same plots. A fluorescence minus one for APC-conjugated anti-CD30 was included to establish CD30 gating. (DOCX)

S2 Fig. \(2^{\Delta\Delta Ct}\) values comparing mRNA from CD30⁺ versus CD30⁻ CD4⁺ T cell subsets obtained from five individuals on suppressive ART were determined by Taqman PCR gene array for CD3 complex, CD4, CD8 and CD4 genes. Of note, no CD80 mRNA could be detected in four samples (†) and no CD8α mRNA could be detected in three samples (†). Overall, \(2^{\Delta\Delta Ct}\) values of CD3 complex and CD4 mRNA were similar or higher comparing CD30⁺ and CD30⁻ CD4⁺ T cells. No CD3 complex, CD4 or CD8 mRNA could be detected from purified B cells obtained from an uninfected donor which served as a control. \(2^{\Delta\Delta Ct}\) values represent a function comparing CD30⁺ to CD30⁻ CD4⁺ T cell mRNA levels. A value of 1 represents no difference between populations within an individual sample and values greater than 1 indicate a greater number of mRNA transcripts. (DOCX)

S3 Fig. Example of gating strategy used in GALT sorting of CD4⁺ T cells to determine CD30 and CD32 expression. Lymphocytes populations were first selected using forward and side scatter characteristics. Following this, the exclusion of doublets was performed by plotting cells on FSC-W and FSC-A and similarly for SSC-W versus SSC-A. Live and then CD45⁺ lymphocytes were selected, and further gated for CD3⁺CD4⁺ T cells. Following this CD4⁺ CD30⁺, CD4⁺ CD32⁺, and CD4⁺ CD30⁺ CD32⁺ T cells were selected in individual gates and sorted.
Further phenotypical information was collected on each T cell subset, including CD38, HLA-DR and CD13 expression.

**S4 Fig.** The use of the anti-CD30 cytotoxic antibody-drug conjugate, brentuximab vedotin (10 μg/mL), reduced the percentage of CD4+ T cells expressing CD30 after 48 hours of culture in the presence of ART (n = 3). While this reduction may represent ADC-targeted cell killing, CD30 staining may also have been affected by steric interactions with ADC-bound receptor or receptor downregulation.

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**Author Contributions**

**Conceptualization:** Louise E. Hogan, Joshua Vasquez, Timothy J. Henrich.

**Data curation:** Louise E. Hogan, Joshua Vasquez, Steven G. Deeks, Timothy J. Henrich.

**Formal analysis:** Louise E. Hogan, Joshua Vasquez, Timothy J. Henrich.

**Funding acquisition:** Timothy J. Henrich.

**Investigation:** Louise E. Hogan, Joshua Vasquez, Kristen S. Hobbs, Emily Hanhauser, Brandon Aguilar-Rodriguez, Raja Hussien, Cassandra Thanh, Erica A. Gibson, Alexander B. Carvidi, Louis C. B. Smith, Shahzada Khan, Ma Somsouk, Timothy J. Henrich.

**Methodology:** Louise E. Hogan, Kristen S. Hobbs, Alexander B. Carvidi, Shahzada Khan, Martin Trapecar, Shomyseh Sanjabi, Timothy J. Henrich.

**Project administration:** Louise E. Hogan, Timothy J. Henrich.

**Resources:** Ma Somsouk, Cheryl A. Stoddart, Steven G. Deeks.

**Supervision:** Daniel R. Kuritzkes, Steven G. Deeks, Timothy J. Henrich.

**Validation:** Louise E. Hogan, Shomyseh Sanjabi.

**Visualization:** Louise E. Hogan.

**Writing – original draft:** Louise E. Hogan, Timothy J. Henrich.

**Writing – review & editing:** Louise E. Hogan, Joshua Vasquez, Kristen S. Hobbs, Emily Hanhauser, Brandon Aguilar-Rodriguez, Cassandra Thanh, Erica A. Gibson, Louis C. B. Smith, Martin Trapecar, Shomyseh Sanjabi, Cheryl A. Stoddart, Daniel R. Kuritzkes, Steven G. Deeks, Timothy J. Henrich.

**References**

1. Stein H, Mason DY, Gerdes J, O’Connor N, Wainscoat J, et al. (1985) The expression of the Hodgkin’s disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 66: 848–858. PMID: 3876124

2. Falini B, Pileri S, Pizzolo G, Durkop H, Flenghi L, et al. (1995) CD30 (Ki-1) molecule: a new cytokine receptor of the tumor necrosis factor receptor superfamily as a tool for diagnosis and immunotherapy. Blood 85: 1–14. PMID: 7903786

3. Chiarle R, Poddia A, Prolla G, Gong J, Thorbecke GJ, et al. (1999) CD30 in normal and neoplastic cells. Clin Immunol 90: 157–164. https://doi.org/10.1006/clim.1998.4636 PMID: 10080826
4. Biswas P, Mantelli B, Delfanti F, Ferrarini M, Poli G, et al. (2003) CD30 ligation differentially affects CXCR4-dependent HIV-1 replication and soluble CD30 secretion in non-Hodgkin cell lines and in gamma delta T lymphocytes. Eur J Immunol 33: 3136–3145. https://doi.org/10.1002/eji.200324344 PMID: 14579282

5. Biswas P, Smith CA, Goletti D, Hardy EC, Jackson RW, et al. (1995) Cross-linking of CD30 induces HIV expression in chronically infected T cells. Immunity 2: 587–596. PMID: 7540942

6. Agrawal B, Reddish M, Longenecker BM (1996) CD30 expression on human CD8+ T cells isolated from peripheral blood lymphocytes of normal donors. J Immunol 157: 3229–3234. PMID: 8871616

7. Durkop H, Foss HD, Eitelbach F, Anagnostopoulos I, Latza U, et al. (2000) Expression of the CD30 antigen in non-lymphoid tissues and cells. J Pathol 190: 613–618. https://doi.org/10.1002/(SICI)1096-9896(200004)190:5<613::AID-PATH559>3.0.CO;2-0 PMID: 10729788

8. Biswas P, Cozzi-Lepri A, Delfanti F, Galli A, Colangeli V, et al. (2006) Significant link between sCD30 changes and HIV viremia in patients treated with HAART. J Med Virol 78: 1513–1519. https://doi.org/10.1002/jmv.20733 PMID: 17063513

9. Gillfillan MC, Noel PJ, Podack ER, Reiner SL, Thompson CB (1998) Expression of the costimulatory receptor CD30 is regulated by both CD28 and cytokines. J Immunol 160: 2180–2187. PMID: 9498756

10. Senter PD, Sievers EL (2012) The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Nat Biotechnol 30: 631–637. https://doi.org/10.1038/nbt.2289 PMID: 22781692

11. Higuchi M, Matsuda T, Mori N, Yamada Y, Horie R, et al. (2005) Elevated expression of CD30 in adult T-cell leukemia cell lines: possible role in constitutive NF-kappaB activation. Retrovirology 2: 29. https://doi.org/10.1186/1742-4690-2-29 PMID: 15876358

12. Makino M, Yashiki S, Fujiyoshi T, Baba M, Sonoda S (1998) An expression of anaplastic large cell lymphoma-associated antigens on HTLV-I-infected CD4+ T cells. Ann Hematol 76: 31–35. PMID: 9486922

13. Smith KJ, Barrett TL, Neafie R, Tomaszewski MM, Yeager J, et al. (1998) Is CD30 (Ki-1) immunostaining in cutaneous eruptions useful as a marker of Th1 to Th2 cytokine switching and/or as a marker of advanced HIV-1 disease? Br J Dermatol 138: 774–779. PMID: 9666821

14. Pizzolo G, Vinante F, Nadali G, Krampera M, Morosato S, et al. (1997) High serum level of soluble CD30 in acute primary HIV-1 infection. Clin Exp Immunol 108: 251–253. https://doi.org/10.1046/j.1365-2249.1997.d01-1005.x PMID: 9158093

15. Tsitsikov EN, Wright DA, Geha RS (1997) CD30 induction of human immunodeficiency virus gene transcription is mediated by TRAF2. Proc Natl Acad Sci U S A 94: 1390–1395. PMID: 9037063

16. Silvestri G, Munoz C, Butini L, Bagnarelli P, Montroni M (1997) Changes in CD8 cell subpopulations induced by antiretroviral therapy in human immunodeficiency virus infected patients. Viral Immunol 10: 207–212. https://doi.org/10.1089/vim.1997.10.207 PMID: 9473151

17. Rizzardi GP, Barcellini W, Tambussi G, Lillo F, Malnati M, et al. (1996) Plasma levels of soluble CD30, tumour necrosis factor (TNF)-alpha and TNF receptors during primary HIV-1 infection: correlation with HIV-1 RNA and the clinical outcome. Aids 10: F45–50. PMID: 8931778

18. Manetti R, Annunziato F, Biagiotti R, Giudizi MG, Piccinni MP, et al. (1994) CD30 expression by CD8+ T cells producing type 2 helper cytokines. Evidence for large numbers of CD8+CD30+ T cell clones in human immunodeficiency virus infection. J Exp Med 180: 2407–2411. PMID: 7964515

19. Pizzolo G, Vinante F, Morosato L, Nadali G, Chilosi M, et al. (1994) High serum level of the soluble form of CD30 molecule in the early phase of HIV-1 infection as an independent predictor of progression to AIDS. Aids 8: 741–745. PMID: 8086130

20. Sadeghi M, Susal C, Daniel V, Naujokat C, Zimmermann R, et al. (2007) Short communication: decreasing soluble CD30 and increasing IFN-gamma plasma levels are indicators of effective highly active antiretroviral therapy. AIDS Res Hum Retroviruses 23: 886–890. https://doi.org/10.1089/aid.2006.0228 PMID: 17678471

21. Azcona M, Jack HM, Fisher RI, Ellis TM (1994) CD30 defines a subset of activated human T cells that produce IFN-gamma and IL-5 and exhibit enhanced B cell helper activity. J Immunol 153: 2861–2867. PMID: 8089475

22. Chan KW, Hopke CD, Krams SM, Martinez OM (2002) CD30 expression identifies the predominant proliferating T lymphocyte population in human alloimmune responses. J Immunol 169: 1784–1791. PMID: 12165500

23. Ellis TM, Simms PE, Silvnick DJ, Jack HM, Fisher RI (1993) CD30 is a signal-transducing molecule that defines a subset of human activated CD45RO+ T cells. J Immunol 151: 2380–2389. PMID: 8103064
24. Henrich TJ, Gallien S, Li JZ, Pereyra F, Kuritzkes DR (2012) Low-level detection and quantitation of cellular HIV-1 DNA and 2-LTR circles using droplet digital PCR. J Virol Methods 186: 68–72. https://doi.org/10.1016/j.jviromet.2012.08.019 PMID: 22974526

25. Henrich T, Hu Z, Li J, Sciaranghella G, Busch M, et al. (2013) Long-Term Reduction in Peripheral Blood HIV-1 Reservoirs Following Reduced-Intensity Conditioning Allogeneic Stem Cell Transplantation. J Infect Dis 207:1694–702. https://doi.org/10.1093/infdis/jit886 PMID: 23460751

26. Gandhi M, Petrich A (2014) Brentuximab vedotin in patients with relapsed HIV-related lymphoma. J Natl Compr Canc Netw 12: 16–19. PMID: 24453289

27. Henrich TJ, Hobbs KS, Hanhauser E, Scully E, Hogan LE, et al. (2017) Human Immunodeficiency Virus Type 1 Persistence Following Systemic Chemotherapy for Malignancy. J Infect Dis 216: 254–262. https://doi.org/10.1093/infdis/jix265 PMID: 28239760

28. Fromentin R, Bakeman W, Lawani MB, Khoury G, Hartogensis W, et al. (2016) CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. PLoS Pathog 12: e1005761. https://doi.org/10.1371/journal.ppat.1005761 PMID: 27415008

29. Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, et al. (2016) PD-1 and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. Nat Med 22:754–61 https://doi.org/10.1038/nm.4113 PMID: 27239760

30. Descours B, Petitjean G, Lopez-Zaragoza JL, Bruel T, Raffel R, et al. (2014) CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. Nature 543: 564–567. https://doi.org/10.1038/nature21710 PMID: 28239760

31. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, et al. (2009) HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med 15: 893–900. https://doi.org/10.1038/nm.1972 PMID: 19543283

32. Yukl SA, Shergill AK, Ho T, Killian M, Girling V, et al. (2013) The distribution of HIV DNA and RNA in cell subsets differs in gut and blood of HIV-positive patients on ART: implications for viral persistence. J Infect Dis 208: 1212–1220. https://doi.org/10.1093/infdis/jit308 PMID: 23852128

33. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, et al. (2008) Relationship between T cell activation and CD4+ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis 197: 126–133. https://doi.org/10.1086/524143 PMID: 18171295

34. Rosenbloom DI, Elliott O, Hill AL, Henrich TJ, Siliciano JM, et al. (2015) Designing and Interpreting Limiting Dilution Assays: General Principles and Applications to the Latent Reservoir for Human Immunodeficiency Virus-1. Open Forum Infect Dis 2: ofv123.