Published in final edited form as:

Curr Opin HIV AIDS. 2019 March ; 14(2): 121–128. doi:10.1097/COH.0000000000000528.

Role of cytokine agonists and immune checkpoint inhibitors towards HIV remission

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Abstract

Purpose of the review: This article describes the current status of the use of cytokines and immune-checkpoint inhibitors as therapeutic strategies towards HIV remission.

Recent Findings: Clinical trials using IL-2 and IL-7 showed increased levels of circulating T cells, although no reduction to the viral reservoir was observed. Studies in non-human primates demonstrated that experimental IL-15 administration increased proliferation and cytotoxicity of SIV-specific CD8⁺ T cells, and promoted their localization to the lymph node B cell follicles. Immune checkpoint modulators targeting PD-1 and CTLA-4, successfully used in oncologic diseases, have shown potential to restore HIV-specific function in early stage clinical trials, while also transiently increasing plasma and cell-associated viral RNA. Due to the complexity of the mechanisms regulating HIV persistence, it is very likely that combinatorial approaches, including cytokines with immune checkpoint blockades, will be needed to achieve HIV remission.

Summary: The present review covers approaches based on cytokine agonists and immune checkpoint inhibitors that have shown promise towards therapeutic pathways for HIV remission. These strategies have been tested pre-clinically in animal models of HIV infection to determine their safety, activity, and mechanisms of action, with the goal to inform the design of the most synergistic combinatorial strategies. Several of these interventions are included in ongoing or planned clinical trials in HIV infection; these trials will elucidate the clinical efficacy of these innovative immunotherapy approaches toward HIV remission.

Keywords

Cytokine agonists; IL-15; IL-21; PD-1; CTLA-4; HIV remission

Introduction:

If left untreated, HIV infection is associated with an uncontrollable loss of memory CD4⁺ T cells, that ultimately results in immunodeficiency at which AIDS and death may occur. It has

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Conflicts of interest:

There are no conflicts of interest

been shown that during antiretroviral therapy (ART), there is persistence of residual immune activation (1, 2) and of a pool of latently infected cells that has the ability to recrudescence upon ART-treatment interruption (3–6). Therefore, in order to fully achieve HIV remission, immune activation and mechanisms of viral persistence must be targeted. During chronic infections, such as HIV, cytokine production is dysregulated with reductions in IL-2, IL-15, and IL-21 secretion, and increases in IL-7, tumor necrosis factor- α (TNF- α) and IFN- α levels within the lymphoid compartments (7–9). This imbalance in cytokine production may play a role in dysfunction of T cell homeostasis, but also impairs the HIV-specific response due to lowered CD8⁺ T cell and NK cell expansion.

The common γ -chain cytokines, interleukin-2 (IL-2), IL-7, IL-15, and IL-21 are critical to the normal proliferation, regulation, function, and homeostasis of T cells (10). IL-2 was originally discovered as a T cell growth factor (11); other findings have shown its capacity to expand and induce differentiation of B cells (12), and promote NK cell expansion and enhance effector functions (13). IL-2 also plays an essential role in the development and maintenance of regulatory T (T_{Reg}) cells, which are critical for suppression of inflammation during infection (14). IL-7 signaling supports sustained expression of anti-apoptotic proteins *Bcl-2* and *Mcl-1*, and contributes to the survival of T cells and to maintain the longevity of memory CD8⁺ T cells (15–18). IL-15 is essential in the development, maintenance, and expansion of memory CD8⁺ T cells and enhances maintenance and survival of NK cells (19, 20). IL-21 promotes the maintenance and functionality of T_H17 cells, a subset of CD4⁺ T cells that have been shown to be critical in the maintenance of mucosal integrity and an overall reduction in systemic inflammation (21–27).

Over the years, the link between type I Interferon (IFN-I) and HIV has been closely investigated. IFN-I activates NK cells and upregulates anti-viral restriction factors (APOBEC3G, TRIM5 α , and MX2) that target all facets of the HIV life-cycle. In HIV-infected patients and SIV-infected rhesus macaques (RMs), it has been shown that levels of IFN-I and interferon stimulated genes (ISGs) are elevated (28, 29). On the other side of the spectrum, IFN-I also has been implicated in increasing systemic immune activation (predicts poor CD4⁺ T cell reconstitution and progression to AIDS) and facilitates production and recruitment of target CD4⁺ T cells (30). Moreover, IFN-I can induce CD4⁺ T cell apoptosis, and dampen the effect of Ag-specific CD4⁺ and CD8⁺ T cell responses (30). Remarkably, IFN-I responses are rapidly downregulated in the natural host of SIV infection after the acute stage of the disease, whereas in RMs, IFN-I signaling remains elevated throughout acute infection into the early chronic phase (31, 32).

Co-inhibitory receptors (Co-IRs), such as PD-1 (programmed cell death-1) and CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), down-modulate immune responses to prevent hyper-activation, minimize damage to surrounding tissues, and maintain self-tolerance (33). Co-IRs are upregulated after T cell activation and function to dampen effector responses through inhibition of signaling pathways. Overexpression of Co-IRs has been associated with T cell exhaustion and overall dysfunction in cancer and chronic infections, including HIV (34–37). It has been postulated that expression of Co-IRs leads to HIV latency during ART treatment, and that CD4⁺ T cells expressing Co-IRs could be the main contributors to viral persistence (38–41). Recently, it has been shown that PD-1⁺ cells were enriched for

HIV latency, and that other markers of exhaustion were correlates of latency enrichment (42). PD-1 expression on CD8⁺ T cells was associated with HIV persistence during ART, indicating that the quality of early CD8⁺ responses could serve as a predictor of HIV persistence (43).

Current approaches to HIV remission largely involve single-agent immunotherapy, with very few current studies focusing on combinatory approaches. Several of the most recently published immune-based approaches are detailed below. This review focuses on therapies centered around cytokines and immune checkpoint blockade, however, there has been exciting data published recently detailing the use of broadly neutralizing antibodies (bnAbs) and latency reversing agents (LRA) towards HIV remission (44–47). Future immune-based therapies towards a functional or sterilizing cure will need to use combined interventions that account for the complexity of the mechanisms favoring viral persistence, including targeting HIV-associated immune activation, reverting HIV latency, and restoring HIV-specific responses. These approaches will likely include combinations of cytokine agonists and immune checkpoint blockades to achieve HIV remission.

Therapeutic strategies based on cytokine agonists

γ-chain Cytokines—Recombinant cytokines have been shown to improve immunologic function and increase expansion of CD4⁺ and CD8⁺ T cells (10). For this reason, administration of IL-2, IL-7, IL-15, and IL-21 have been tested clinically or pre-clinically in the context of HIV or SIV infection (Figure 1). There were several phase III clinical studies involving IL-2 in ART-suppressed, HIV-positive participants that showed an increase in peripheral CD4⁺ T cell counts, however there seemed to be no effect in reducing the risk of HIV-associated opportunistic infections or overall reduction in the viral reservoir (48–50). Several other studies involving IL-7 showed increased reconstitution in circulating CD4⁺ and CD8⁺ T cells, and transient increases in plasma HIV-RNA (51–53). However, IL-7 treatment resulted in a 70% increase in the absolute number of circulating CD4⁺ T cells harboring integrated HIV-DNA in virally suppressed subjects (54).

Administration of monomeric IL-15 at the initiation of ART treatment in SIV-infected RMs enhanced activation, function, and proliferation of NK cells and CD8⁺ T cells, but, failed to reconstitute SIV-specific CD4⁺ or total CD4⁺ T cells (55). A recent study in simian-HIV (SHIV) infected RMs showed that the treatment with a human heterodimeric IL-15 (hetIL-15), which circumvents the need for IL-15 to bind to the IL-15Rα for subsequent presentation to target cells, largely increased effector CD8⁺ T cells with high granzyme B (GzmB) levels in blood and LNs, as well as increased levels of Ag-specific CD8⁺ T cells within LNs (56). Quantitative image analysis of these LNs also revealed increases in numbers of GzmB⁺ CD8⁺ T cells within the B cell follicles, a potential HIV sanctuary. However, the mechanism regulating IL-15 dependent trafficking of cytotoxic CD8⁺ T cells to B cell follicles is not yet clear, and does not seem to depend on increased expression of the chemokine receptor CXCR5, thus more experiments exploring this phenomenon need to be done. These data are of great interest due to the notion that having limited homing of cytotoxic CD8⁺ T cells to follicles is a contributing factor favoring HIV persistence in follicular helper T (T_{FH}) cells (57–59). Another group, using human IL-15 superagonist

(ALT-803), also showed increased trafficking and cytolytic potential of SIV-specific CD8⁺ T cells to B cell follicles, with the LNs of elite controlling RMs containing lower frequencies of cells expressing SIV-RNA or harboring SIV-DNA post ALT-803 treatment (60). A separate study looking at the effects of ALT-803 in SIV infection of ART-naïve RMs demonstrated that ALT-803 can suppress SIV replication in RMs in the absence of ART, although all of the RMs in the study had received SIV vaccine vectors that potentially could have elicited a CD8⁺ T cell response, and three of the RMs possessed major histocompatibility complex alleles associated with control of SIV (61). These results highlight hetIL-15 as a potential component of future combinatorial approaches to target HIV reservoir sanctuaries.

Studies involving the administration of IL-21 during the acute phase of untreated SIV infection (26) or early chronic phase of ART-treated SIV-infection (27) of RMs resulted in a significant reduction of T cell activation (determined by CD38⁺ and HLA-DR⁺ expression) and cell-cycling (Ki-67⁺) in peripheral blood and intestinal mucosa. Mechanistically, the IL-21 mediated reduction in immune activation was largely related to an enhanced preservation of intestinal T_H17 cells and improved mucosal barrier integrity. Remarkably, IL-21 treatment during ART significantly reduced SIV-RNA in the plasma, SIV-DNA content within the gut, and the frequency of CD4⁺ T cells harboring replication competent virus in the LN (25, 27). A follow up study combining IL-21 and probiotic therapy showed that this therapy limited microbial translocation and systemic inflammation in ART-treated RMs (62). A schematic summary of the main effects of cytokine-based interventions that have been tested during HIV infection in humans or SIV-infection in NHPs is shown in Figure 1.

Type-I Interferon—Type I interferon receptor blockade (IFN-1ant) during acute SIV infection (1 mg IFN-ant daily for 4 weeks post challenge) caused a reduction in antiviral gene expression, increased PBMC-associated SIV-gag DNA at 28 days post infection (d.p.i.), and accelerated CD4⁺ T cell depletion with rapid progression to AIDS despite an overall decrease in systemic inflammation (63). In the same study, daily administration of IFN-α2a a week prior to infection and during acute SIV infection to a different group of RMs upregulated the expression of antiviral genes and prevented systemic infection. However, continued administration of IFN-α2a induced an IFN-I desensitization and decreased antiviral gene expression, followed by increases in PBMC-associated SIV-gag DNA and acceleration in CD4⁺ T cell loss (63). This indicates that the overall timing of IFN induced innate responses during acute infection plays a major role in the course of disease progression and dwarfs the detrimental effects of a hyperactive inflammatory response. The administration of an IFN-1ant during chronic SIV infection of ART-naïve and ART-suppressed RMs resulted in the reduction of IFN-I mediated inflammation, a reduction in expression of ISGs, but no significant effect on plasma levels of IL-1β, IL-1Ra, IL-6, and IL-8 (64). Contrary to what was seen during acute SIV infection, there was no significant increase in plasma viremia up to 25 weeks post IFN-1ant administration and no difference was seen in levels of cell-associated SIV-DNA (64). Another recent study using pegylated IFN-α2a (pIFN-α2a) in conjunction with suppressive ART showed upregulation of ISGs, but the treatment failed to significantly reduce the size of the reservoir (65).

Development of HIV therapies using cytokines requires us to have a greater understanding of the roles that each cytokine plays in the setting of a complex and still inflamed environment such as SIV/HIV during ART. Ideally, the administration of cytokines needs to be able not only to restore T cell numbers, but also improve T cell function and increase their ability to migrate to lymphoid tissues where it has been shown that the reservoirs for HIV can persist. As a result, the efficacy of cytokine agonists and therapies in conjunction with other immune-based interventions should be further tested in pre-clinical and clinical settings as a potential effective means for impacting the viral reservoirs and promoting HIV remission for infected individuals.

Therapeutic strategies based on immune-checkpoint blockade

The expression of co-inhibitory receptors (Co-IRs) on Ag-specific T cells defines a T cell population marked by diminished effector functions and decreased production of effector cytokines (34, 36). Previously, it has been shown that central memory (T_{CM}) and transitional memory (T_{TM}) $CD4^+$ T cells from ART suppressed individuals that express PD-1^{hi} had higher levels of integrated HIV-DNA as compared to the PD-1^{lo} population (38). In addition, PD-1⁺ and follicular helper T cells (T_{FH}) located within the B cell follicle were found to be the major $CD4^+$ T cell compartment for the production of replication competent and infectious HIV-1, and the persistent virus transcription in individuals on long-term ART (39). Furthermore, PD-1, TIGIT (T cell immunoreceptor with Ig and ITIM domains), and LAG-3 (Lymphocyte-activation gene 3) have been identified as Co-IRs that were positively associated with the frequency of $CD4^+$ T cells containing integrated HIV-DNA (41). It was also noted that most of the cells containing inducible HIV genomes expressed at least one of these markers. In a separate study using ART-treated SIV-infected RMs, CTLA-4⁺PD-1⁺ memory $CD4^+$ T cells with a T_{Reg} phenotype were enriched in SIV-DNA in the blood, LN, spleen, and gut (40). Interestingly, this population of SIV-enriched cells was found outside the B cell follicle of the LN, and increased in their overall contribution to the reservoir with prolonged ART-mediated suppression. Collectively, these studies propose Co-IR signaling as primary pathways favoring viral persistence, and identified $CD4^+$ T cells expressing Co-IRs as the major cellular reservoir to be targeted during ART. Importantly, several of these Co-IRs have been successfully targeted via monoclonal antibodies (mAbs) in cancer immunotherapy (immune checkpoint blockade, ICB) to augment anti-cancer $CD8^+$ T cell response, encouraging their use in HIV infection to restore antiviral T cell function and potentially target the viral reservoir. Since Co-IR signaling seems to favor HIV latency in $CD4^+$ T cells, blockade of these pathways is expected to revert latency (shock) and favor the elimination of the reservoir through the restored $CD8^+$ T cell response (kill), in the so-called “shock and kill” approach (Figure 2).

Consistent with this rationale, CTLA-4 blockade using ipilimumab in a melanoma and HIV-infected individual who was on suppressive ART therapy showed increased levels of $CD4^+$ T cell activation and also induced cell-associated unspliced HIV-RNA that correlated with a subsequent decline in plasma HIV-RNA (66). This was one of the first studies showing that using immune checkpoint inhibitors could result in activating latently infected cells. Similarly, PD-1 blockade (nivolumab) in an ART-suppressed, HIV-infected participant who was diagnosed with non-small-cell lung cancer induced a transient increase in plasma

viremia and a decrease in “exhausted” CD4⁺ and CD8⁺ T cells (67). Treatment with nivolumab was associated with transient increases in plasma viremia and decreases in “exhausted” CD4⁺ and CD8⁺ T cells (67). A clinical trial targeting the ligand for PD-1 (PD-L1) also showed an increase in frequency of HIV-1 Ag-specific CD8⁺ T cells expressing IFN- γ , indicating an increase in HIV-1 specific immunity (68). However, there were some grade 1 and 2 adverse events (AE), with clinical symptoms presenting as chest tightness, shortness of breath, headache, fatigue, and leg pain. One participant, at thirty-six weeks post-infusion had low cortisol levels and was subsequently diagnosed as hypophysitis (68). All together, these results suggest that treatment with ipilimumab or nivolumab could induce synergistic “shock and kill” mechanisms. The results of these studies are promising, but very preliminary, and need to be seen in additional cohorts of HIV infected persons. There are currently several ongoing clinical trials in HIV infected cancer patients to determine the efficacy of immune checkpoint blockade alone or with combinations of immune checkpoint inhibitors (NCT02408861, NCT03354936). In the NHP model of SIV infection, PD-1 blockade was associated with variable degrees of success. One of the first studies to use PD-1 blockade in the context of SIV infection in ART-naïve RMs demonstrated an expansion of SIV-specific CD8⁺ T cells that had an overall enhanced effector profile (69). The authors also noted significant reductions in plasma viral load and prolonged survival of SIV-infected RMs. In a follow-up study, PD-1 blockade in conjunction with ART (with anti-PD-1 started one week before ART) showed enhanced anti-viral CD8⁺ response as well as a reduction in ISGs (70). Following ART-interruption, there was an expansion of CXCR5⁺ effector CD8⁺ T cells within LNs that may favor viral clearance within LNs (57, 58, 70). A schematic summary of the main effects of ICB that have been performed in HIV-infected cancer subjects or SIV-infected NHPs is shown in Figure 2. The same figure also lists the potential advantages in terms of HIV remission for a combined PD-1 and CTLA-4 blockade as compared to the single interventions.

The use of Co-IR blockades has not been approved by the Federal Drug Administration (FDA) for HIV infection alone, thus all ongoing studies are occurring in HIV-positive individuals who are receiving immune checkpoint blockades for advance stage cancers. These therapies utilizing Co-IR blockades have inherent risks, as blocking these molecules in cancer patients can result in autoimmune or inflammatory side-effects, reactivation of underlying autoimmune conditions, or the development of new autoimmune diseases. The use of Co-IR blockades has not been tested in the context of HIV infection alone, thus it is possible these side-effects will be attenuated in that context. Due to the possibility of severe side-effects and very limited data on the anti-reservoir activity of ICB, additional studies using ART-treated SIV-infected NHPs must be performed to evaluate the clinical efficacy and side effect profile of ICB in the context of HIV cure research, in particular for strategies involving combined ICB.

Conclusion

The ability to rid the body of the persistent HIV reservoir appears to be the main hurdle in achieving a HIV cure. Treatment using recombinant cytokines or agonists have shown promising results, however, further studies still remain. Recent work and clinical trials involving Co-IR blockades have shown great promise in “shocking” the virus out of latency

and inducing a better “kill” with improved effector CD8⁺ T cell expansion and killing capacity. Thus, there is hope that recombinant cytokines or agonists combined with Co-IR blockade could synergize together as a potential means to achieving a true sterilizing cure or remission for HIV. Follow-up studies in NHPs could prove to be very valuable in determining whether combinatorial therapy may be effective, well tolerated, and scalable. The past 30 years of HIV research has provided many insights, however, there are still lingering questions that remain to be answered that pose a challenge to curing HIV.

Acknowledgments

Financial support and sponsorship:

This work was supported by: NIH/NIAID (R33 AI-104278 and R01 AI-116379)

References:

1. Sauce D, Larsen M, Fastenackels S, Pauchard M, Ait-Mohand H, Schneider L, et al. HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. *Blood*. 2011;117(19):5142–51. [PubMed: 21436070]
2. Hunt PW, Brenchley J, Sinclair E, McCune JM, Roland M, Page-Shafer K, et al. 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*. 2008;197(1):126–33. [PubMed: 18171295]
3. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997;387(6629):183–8. [PubMed: 9144289]
4. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A*. 1997;94(24):13193–7. [PubMed: 9371822]
5. Chun TW, Davey RT Jr., Engel D, Lane HC, Fauci AS. Re-emergence of HIV after stopping therapy. *Nature*. 1999;401(6756):874–5. [PubMed: 10553903]
6. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, et al. Latent infection of CD4⁺ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med*. 1999;5(5):512–7. [PubMed: 10229227]
7. Olsson J, Poles M, Spetz AL, Elliott J, Hultin L, Giorgi J, et al. Human immunodeficiency virus type 1 infection is associated with significant mucosal inflammation characterized by increased expression of CCR5, CXCR4, and beta-chemokines. *J Infect Dis*. 2000;182(6):1625–35. [PubMed: 11069233]
8. McGowan I, Elliott J, Fuerst M, Taing P, Boscardin J, Poles M, et al. Increased HIV-1 mucosal replication is associated with generalized mucosal cytokine activation. *J Acquir Immune Defic Syndr*. 2004;37(2):1228–36. [PubMed: 15385729]
9. Kotler DP, Reka S, Clayton F. Intestinal mucosal inflammation associated with human immunodeficiency virus infection. *Dig Dis Sci*. 1993;38(6):1119–27. [PubMed: 8508707]
10. Lin JX, Leonard WJ. The Common Cytokine Receptor gamma Chain Family of Cytokines. *Cold Spring Harb Perspect Biol*. 2018;10(9).
11. Morgan DA, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science*. 1976;193(4257):1007–8. [PubMed: 181845]
12. Armitage RJ, Macduff BM, Eisenman J, Paxton R, Grabstein KH. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *J Immunol*. 1995;154(2):483–90. [PubMed: 7814861]
13. Siegel JP, Sharon M, Smith PL, Leonard WJ. The IL-2 receptor beta chain (p70): role in mediating signals for LAK, NK, and proliferative activities. *Science*. 1987;238(4823):75–8. [PubMed: 3116668]

14. Malek TR, Yu A, Vincek V, Scibelli P, Kong L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rbeta-deficient mice. Implications for the nonredundant function of IL-2. *Immunity*. 2002;17(2):167–78. [PubMed: 12196288]
15. Rathmell JC, Farkash EA, Gao W, Thompson CB. IL-7 enhances the survival and maintains the size of naive T cells. *J Immunol*. 2001;167(12):6869–76. [PubMed: 11739504]
16. Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature*. 2003;426(6967):671–6. [PubMed: 14668867]
17. Sprent J, Surh CD. Normal T cell homeostasis: the conversion of naive cells into memory-phenotype cells. *Nat Immunol*. 2011;12(6):478–84. [PubMed: 21739670]
18. Cui G, Staron MM, Gray SM, Ho PC, Amezcua RA, Wu J, et al. IL-7-Induced Glycerol Transport and TAG Synthesis Promotes Memory CD8+ T Cell Longevity. *Cell*. 2015;161(4):750–61. [PubMed: 25957683]
19. Lodolce JP, Boone DL, Chai S, Swain RE, Dassopoulos T, Trettin S, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity*. 1998;9(5):669–76. [PubMed: 9846488]
20. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med*. 2000;191(5):771–80. [PubMed: 10704459]
21. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*. 2007;448(7152):480–3. [PubMed: 17581589]
22. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature*. 2007;448(7152):484–7. [PubMed: 17581588]
23. Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M, et al. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature*. 2008;454(7202):350–2. [PubMed: 18469800]
24. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. *Blood*. 2008;112(7):2826–35. [PubMed: 18664624]
25. Micci L, Cervasi B, Ende ZS, Irielle RI, Reyes-Aviles E, Vinton C, et al. Paucity of IL-21-producing CD4(+) T cells is associated with Th17 cell depletion in SIV infection of rhesus macaques. *Blood*. 2012;120(19):3925–35. [PubMed: 22990011]
26. Pallikkuth S, Micci L, Ende ZS, Irielle RI, Cervasi B, Lawson B, et al. Maintenance of intestinal Th17 cells and reduced microbial translocation in SIV-infected rhesus macaques treated with interleukin (IL)-21. *PLoS Pathog*. 2013;9(7):e1003471. [PubMed: 23853592]
27. Micci L, Ryan ES, Fromentin R, Bosinger SE, Harper JL, He T, et al. Interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques. *J Clin Invest*. 2015;125(12):4497–513. [PubMed: 26551680]
28. Gringeri A, Santagostino E, Cusini M, Muca-Perja M, Marinoni A, Mannucci PM, et al. Absence of clinical, virological, and immunological signs of progression in HIV-1-infected patients receiving active anti-interferon-alpha immunization: a 30-month follow-up report. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1996;13(1):55–67. [PubMed: 8797687]
29. Bosinger SE, Li Q, Gordon SN, Klatt NR, Duan L, Xu L, et al. Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. *J Clin Invest*. 2009;119(12):3556–72. [PubMed: 19959874]
30. Utay NS, Douek DC. Interferons and HIV Infection: The Good, the Bad, and the Ugly. *Pathog Immun*. 2016;1(1):107–16. [PubMed: 27500281]
31. Sandler NG, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat Rev Microbiol*. 2012;10(9):655–66. [PubMed: 22886237]
32. Handley SA, Thackray LB, Zhao G, Presti R, Miller AD, Droit L, et al. Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. *Cell*. 2012;151(2):253–66. [PubMed: 23063120]

33. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol*. 2005;23:515–48. [PubMed: 15771580]
34. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006;439(7077):682–7. [PubMed: 16382236]
35. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, et al. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med*. 2006;12(10):1198–202. [PubMed: 16917489]
36. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 2006;443(7109):350–4. [PubMed: 16921384]
37. Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, et al. PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med*. 2006;203(10):2281–92. [PubMed: 16954372]
38. Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009;15(8):893–900. [PubMed: 19543283]
- 39 **. Banga R, Procopio FA, Noto A, Pollakis G, Cavassini M, Ohmiti K, et al. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat Med*. 2016;22(7):754–61. [PubMed: 27239760] Here the authors showed that PD-1+ LN CD4+ T cells, which are primarily TFH CD4+ T cells, are the major source of replication-competent HIV-1 in ART-suppressed individuals
- 40 **. McGary CS, Deleage C, Harper J, Micci L, Ribeiro SP, Paganini S, et al. CTLA-4(+)PD-1(–) Memory CD4(+) T Cells Critically Contribute to Viral Persistence in Antiretroviral Therapy-Suppressed, SIV-Infected Rhesus Macaques. *Immunity*. 2017;47(4):776–88 e5. [PubMed: 29045906] In this article, the authors show that CTLA-4+ PD-1- memory CD4+ T cells with a TReg like phenotype were enriched for SIV-DNA in the blood, LN, spleen, and gut and contained replication-competent virus.
- 41 *. Fromentin R, Bakeman W, Lawani MB, Khoury G, Hartogensis W, DaFonseca S, et al. CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. *PLoS Pathog*. 2016;12(7):e1005761. [PubMed: 27415008] In this study, it was shown that most cells with an inducible HIV genome contained at least one of the following markers: PD-1, TIGIT, or LAG-3. CD4+ T Cells co-expressing the three markers were further enriched for integrated HIV genomes.
42. Evans VA, van der Sluis RM, Solomon A, Dantanarayana A, McNeil C, Garsia R, et al. Programmed cell death-1 contributes to the establishment and maintenance of HIV-1 latency. *AIDS*. 2018;32(11):1491–7. [PubMed: 29746296]
43. Ghiglione Y, Trifone C, Salido J, Rhodes A, Ruiz MJ, Polo ML, et al. PD-1 expression in HIV-specific CD8+ T-cells prior to antiretroviral therapy is associated with HIV persistence. *J Acquir Immune Defic Syndr*. 2018.
44. Bar-On Y, Gruell H, Schoofs T, Pai JA, Nogueira L, Butler AL, et al. Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. *Nat Med*. 2018;24(11):1701–7. [PubMed: 30258217]
45. Borducchi EN, Liu J, Nkolola JP, Cadena AM, Yu WH, Fischinger S, et al. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature*. 2018;563(7731):360–4. [PubMed: 30283138]
46. Lim SY, Osuna CE, Hraber PT, Hesselgesser J, Gerold JM, Barnes TL, et al. TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci Transl Med*. 2018;10(439).
47. Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, Millard K, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature*. 2018;561(7724):479–84. [PubMed: 30258136]
48. Molina JM, Levy Y, Fournier I, Hamonic S, Bentata M, Beck-Wirth G, et al. Interleukin-2 before antiretroviral therapy in patients with HIV infection: a randomized trial (ANRS 119). *J Infect Dis*. 2009;200(2):206–15. [PubMed: 19508157]

49. Levy Y, Thiebaut R, Gougeon ML, Molina JM, Weiss L, Girard PM, et al. Effect of intermittent interleukin-2 therapy on CD4+ T-cell counts following antiretroviral cessation in patients with HIV. *AIDS*. 2012;26(6):711–20. [PubMed: 22301410]
50. Group I-ES, Committee SS, Abrams D, Levy Y, Losso MH, Babiker A, et al. Interleukin-2 therapy in patients with HIV infection. *N Engl J Med*. 2009;361(16):1548–59. [PubMed: 19828532]
51. Sereti I, Dunham RM, Spritzler J, Aga E, Proschan MA, Medvik K, et al. IL-7 administration drives T cell-cycle entry and expansion in HIV-1 infection. *Blood*. 2009;113(25):6304–14. [PubMed: 19380868]
52. Imamichi H, Degray G, Asmuth DM, Fischl MA, Landay AL, Lederman MM, et al. HIV-1 viruses detected during episodic blips following interleukin-7 administration are similar to the viruses present before and after interleukin-7 therapy. *AIDS*. 2011;25(2):159–64. [PubMed: 21124203]
53. Parker R, Dutrieux J, Beq S, Lemerrier B, Rozlan S, Fabre-Mersseman V, et al. Interleukin-7 treatment counteracts IFN-alpha therapy-induced lymphopenia and stimulates SIV-specific cytotoxic T lymphocyte responses in SIV-infected rhesus macaques. *Blood*. 2010;116(25):5589–99. [PubMed: 20841508]
54. Vandergeeten C, Fromentin R, DaFonseca S, Lawani MB, Sereti I, Lederman MM, et al. Interleukin-7 promotes HIV persistence during antiretroviral therapy. *Blood*. 2013;121(21):4321–9. [PubMed: 23589672]
55. Lugli E, Mueller YM, Lewis MG, Villinger F, Katsikis PD, Roederer M. IL-15 delays suppression and fails to promote immune reconstitution in virally suppressed chronically SIV-infected macaques. *Blood*. 2011;118(9):2520–9. [PubMed: 21757617]
- 56 **. Watson DC, Moysi E, Valentin A, Bergamaschi C, Devasundaram S, Fortis SP, et al. Treatment with native heterodimeric IL-15 increases cytotoxic lymphocytes and reduces SHIV RNA in lymph nodes. *PLoS Pathog*. 2018;14(2):e1006902. [PubMed: 29474450] In this study, administration of hetIL-15 increased effector CD8+ T cell function (higher levels of Granzyme B) and their trafficking to B cell follicles of the LN, resulting in reduction of SHIV-RNA in plasma and LN.
57. Petrovas C, Ferrando-Martinez S, Gerner MY, Casazza JP, Pegu A, Deleage C, et al. Follicular CD8 T cells accumulate in HIV infection and can kill infected cells in vitro via bispecific antibodies. *Sci Transl Med*. 2017;9(373).
- 58 *. Ferrando-Martinez S, Moysi E, Pegu A, Andrews S, Nganou Makamdop K, Ambrozak D, et al. Accumulation of follicular CD8+ T cells in pathogenic SIV infection. *J Clin Invest*. 2018;128(5):2089–103. [PubMed: 29664020] It was shown that follicular CD8+ T cells (fCD8+) accumulated only during pathogenic SIV-infection, and these fCD8+ had a high cytolytic potential that could be used to target and kill HIV-infected cells through the use of bispecific antibodies.
- 59 **. Buggert M, Nguyen S, Salgado-Montes de Oca G, Bengsch B, Darko S, Ransier A, et al. Identification and characterization of HIV-specific resident memory CD8(+) T cells in human lymphoid tissue. *Sci Immunol*. 2018;3(24). In this study, it was shown that TRM CD8+ T cells accumulated in LTs and dominated the LT CD8+ response against HIV. It was also noted that within LTs of elite controllers, there was a significantly higher number of HIV-specific TRM CD8+ T cells.
- 60 **. Webb GM, Li S, Mwakalundwa G, Folkvord JM, Greene JM, Reed JS, et al. The human IL-15 superagonist ALT-803 directs SIV-specific CD8(+) T cells into B-cell follicles. *Blood Adv*. 2018;2(2):76–84. [PubMed: 29365313] The authors reported that after administration of ALT-803, there was increased trafficking of SIV-specific cytotoxic T cells to the B cell follicles of the LN, resulting in reductions of cells harboring SIV-RNA and SIV-DNA.
61. Ellis-Connell AL, Balgeman AJ, Zarbock KR, Barry G, Weiler A, Egan JO, et al. ALT-803 Transiently Reduces Simian Immunodeficiency Virus Replication in the Absence of Antiretroviral Treatment. *J Virol*. 2018;92(3).
- 62 *. Ortiz AM, Klase ZA, DiNapoli SR, Vujkovic-Cvijin I, Carmack K, Perkins MR, et al. IL-21 and probiotic therapy improve Th17 frequencies, microbial translocation, and microbiome in ARV-treated, SIV-infected macaques. *Mucosal Immunol*. 2016;9(2):458–67. [PubMed: 26286233] Supplementation of ART with probiotics and IL-21 resulted with enhanced TH17 expansion and reduced microbial translocation and associated gut dysbiosis.

- 63 **. Sandler NG, Bosinger SE, Estes JD, Zhu RT, Tharp GK, Boritz E, et al. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature*. 2014;511(7511):601–5. [PubMed: 25043006] The authors show the dichotomy of Type-I interferon (IFN-I) responses, as blockade of IFN-I reduced T cell activation but increased the size of the reservoir, and administration of IFN- α 2a increased antiviral genes while at the same time accelerating the loss of CD4+ T cells.
- 64 *. Nganou-Makamdop K, Billingsley JM, Yaffe Z, O'Connor G, Tharp GK, Ransier A, et al. Type I IFN signaling blockade by a PASylated antagonist during chronic SIV infection suppresses specific inflammatory pathways but does not alter T cell activation or virus replication. *PLoS Pathog*. 2018;14(8):e1007246. [PubMed: 30142226] Administration of PASylated IFN-Iant to ART-suppressed and ART-naïve chronically SIV-infected RMs resulted in reductions to systemic inflammation without increasing viral replication.
65. Palesch D, Bosinger SE, Mavigner M, Billingsley JM, Mattingly C, Carnathan DG, et al. Short-Term Pegylated Interferon alpha2a Treatment Does Not Significantly Reduce the Viral Reservoir of Simian Immunodeficiency Virus-Infected, Antiretroviral Therapy-Treated Rhesus Macaques. *J Virol*. 2018;92(14).
66. Wightman F, Solomon A, Kumar SS, Urriola N, Gallagher K, Hiener B, et al. Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma. *AIDS*. 2015;29(4):504–6. [PubMed: 25628259]
- 67 *. Guihot A, Marcelin AG, Massiani MA, Samri A, Soulie C, Autran B, et al. Drastic decrease of the HIV reservoir in a patient treated with nivolumab for lung cancer. *Ann Oncol*. 2018;29(2):517–8. [PubMed: 29206889] In this study, treatment with nivolumab induced a transient reactivation of HIV replication and restored function to “exhausted” HIV-specific CD8+ T cells, with reduction of HIV-DNA in a HIV+ cancer participant.
- 68 *. Gay CL, Bosch RJ, Ritz J, Hataye JM, Aga E, Tressler RL, et al. Clinical Trial of the Anti-PD-L1 Antibody BMS-936559 in HIV-1 Infected Participants on Suppressive Antiretroviral Therapy. *J Infect Dis*. 2017;215(11):1725–33. [PubMed: 28431010] This is the first time anti-PD-L1 blockade was used in the treatment of HIV-1+ participants and resulted in enhancement of HIV-1 specific immunity in a subset of participants (increased expression of IFN γ
69. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature*. 2009;458(7235):206–10. [PubMed: 19078956]
70. Mylvaganam GH, Chea LS, Tharp GK, Hicks S, Velu V, Iyer SS, et al. Combination anti-PD-1 and antiretroviral therapy provides therapeutic benefit against SIV. *JCI Insight*. 2018;3(18).

Key Points:

- Treatment with het-IL-15 has shown promising results in the NHP model, as these studies have shown increased effector function of CD8⁺ T cells and trafficking to B cell follicles within LNs, while also limiting SIV-RNA and SIV-DNA.
- Administration of IL-21 in ART-treated rhesus macaques improved the reconstitution of T_H17 CD4⁺ T cells and mucosal barrier integrity, while decreasing T cell activation and viral persistence.
- ICB has shown promise in reactivating HIV replication, while also restoring function to “exhausted” HIV-specific CD8⁺ T cells. This indicates that ICB could potentially be used in the “shock and kill” approach towards HIV remission.
- Pre-clinical studies using NHPs will be paramount in testing the safety and efficacy of new combined therapies for HIV remission, including the use of cytokines and ICBs.

Figure 1.

(A) Administration of IL-2 and IL-7 results in expansion and circulating CD4⁺ and CD8⁺ T cells, while also inducing transient production of viral RNA in the plasma. (B) Treatment with IL-15 and ALT-803 induces proliferation of Ag-specific CD8⁺ T cells and NK cells, and the Ag-specific CD8⁺ T cells then localize to the B cell follicle. (C) Administration of IL-21 results in decreased T cell activation, while at the same time maintaining T_H17 populations in the mucosal tissue. This results in maintenance of barrier integrity and a reduction to microbial translocation and systemic inflammation.

Figure 2.

(A) PD-1⁺ Memory CD4⁺ T cells have been described to be enriched in integrated HIV-DNA. Blockade of PD-1 with a PD-1-mAb results in transient reactivation of virus, while restoring HIV-specific CD8⁺ T cell function to (B) CTLA-4⁺ Memory CD4⁺ T cells have also been shown to be enriched for HIV-DNA and replication competent virus. Blockade of CTLA-4 with a CTLA-4-mAb results in reactivation of viral production.