HIV AND CANCER IMMUNOTHERAPY: SIMILAR CHALLENGES AND CONVERGING APPROACHES

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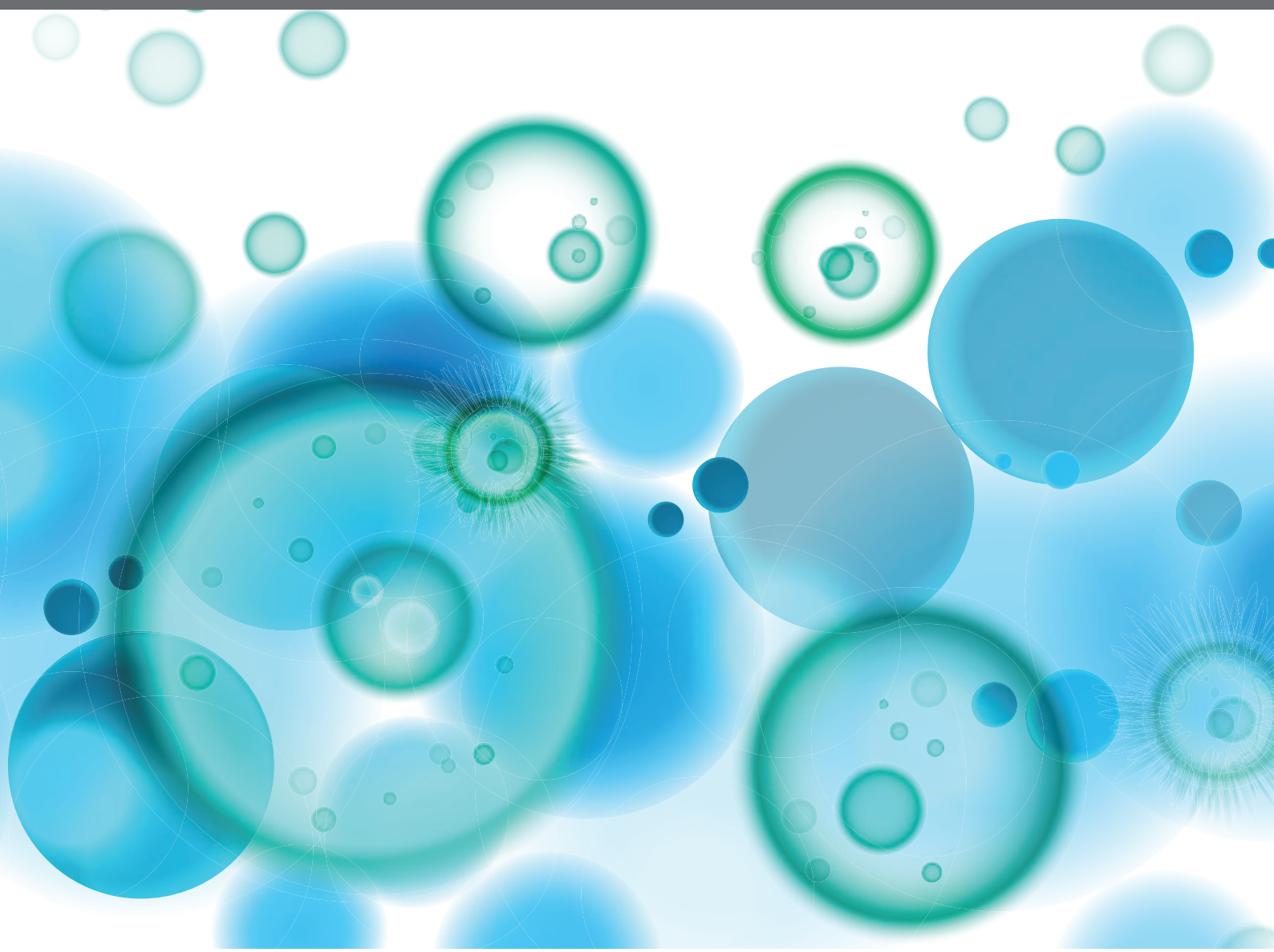

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HIV AND CANCER IMMUNOTHERAPY: SIMILAR CHALLENGES AND CONVERGING APPROACHES

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EDITORIAL
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Editorial: HIV and Cancer Immunotherapy: Similar Challenges and Converging Approaches

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Editorial on the Research Topic

HIV and Cancer Immunotherapy: Similar Challenges and Converging Approaches

INTRODUCTION

Although modern anti-retroviral therapy (ART) permits near-normal life expectancies by suppressing viral replication to clinically undetectable levels in people living with HIV (PLWH) (1), sustained treatment is complicated by complex pharmacological (i.e., adverse events, adherence, resistance) and societal issues (i.e., stigma, cost burden, medical access). Furthermore, ART is incapable of eliminating the latent viral reservoir, which is responsible for recrudescence when therapy is interrupted (2–5). Viral persistence is facilitated by a variety of mechanisms such as the exhaustion of HIV-specific cytolytic T-cells (CTLs) driven by chronic inflammation (6–8); epigenetic modifications to dampen the expression of viral proteins allowing evasion of immunosurveillance (9, 10); the localization of infected cells within immune privileged anatomical sites (11–13); and the survival of long-lived, virus-harboring cells allowing reservoir expansion via homeostatic proliferation (14, 15). Although formidable challenges exist for completing eradicating HIV from infected individuals (a "cure"), there is growing enthusiasm that novel immunotherapy approaches might eventually result in durable control of replication-competent HIV in absence of any therapy (a "remission"). Much of this enthusiasm comes from dramatic progress made in using immunotherapy to treating cancer. This editorial summarizes how the 13 review articles included in this special issue highlight key parallels between HIV and tumor persistence as well as how these similarities inform the development of novel immunotherapy-based strategies toward an HIV cure.

THE PERSISTENCE OF MEMORY

In both HIV and cancer, subsequent pathology arises from a relatively rare, yet difficult to distinguish and persistent subset of cells. In the non-human primate model of HIV infection, the persistent viral reservoir is established within 4–9 days post-infection (16); similarly, very early ART initiation does not induce viral remission in PLWH (17). In a meta-analysis of human cohorts, Etemad et al. propose that preferential infection of transitional memory (T_{TM}) $CD4^+$ T-cells, as opposed to longer-lived central memory or naïve cells, is a key predictor for

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post-treatment control (18) despite weak HIV-specific $CD8^+$ T-cell responses. Intriguingly, Goonetilleke et al. hypothesize that the generation of the long-lived reservoir, particularly in central memory (T_{CM}) and stem-cell memory (T_{SCM}) $CD4^+$ T-cells, can be blunted by inhibiting the IL-7 signaling axis, thereby disrupting the transition and maintenance of $CD127^+$ memory subsets from highly-infected effector $CD4^+$ T-cells (18). Gavegnano et al. explore the use of Jak inhibitors in inhibiting the activity of the anti-apoptotic Bcl-2 protein to reduce cellular lifespans (19, 20). By blocking the formation and maintenance of the viral reservoir in long-lived memory subsets, the authors proposed that a reduction in viral burden will facilitate HIV remission as mimicked in post-treatment controllers.

ESCAPE THROUGH EDITING

Once the viral reservoir is established, HIV-specific $CD8^+$ T-cells are required for viral suppression (21, 22); however, in most infected people, HIV-specific CTLs are incapable of eliminating infected cells (23) indicative of failure in immune surveillance independent of mutational escape or dysfunction (24). This incomplete elimination permits subsequent equilibrium phase sculpting of reservoir-harboring cells by immune pressures, which in cancer models has been termed "immunoediting" (25). Analogous to "antigen loss" in tumors models, Huang et al. explore the novel concept that during ART cells harboring replication-competent virus undergo clonal expansion with subsequent immunoediting; thereby decreasing CTL susceptibility by selecting for BCL-2 expression (26) and integration sites favoring cell division (27, 28). As HIV infection impacts on cellular metabolism and oxidative stress (29, 30), immunoediting may also select for an altered cellular lipid antigen composition that, as summarized by Tiwary et al., in oncology models impinges on chronic inflammation by modulating the macrophage M1 to M2 balance (31) and impairs antigen processing in dendritic cells (32); specifically, CD1d antigen loading for natural killer T-cells (NKT) (33). As a model comparison (Mota and Jones) examine how HTLV-1 generates malignant "repliclones" by an interplay of host- and viral-mediated immunoediting. Therefore, these articles support the notion that HIV CTL escape might be more complex than viral epitope mutations, but rather involve the progressive selection of immunoedited, infected cells resistant to immune surveillance.

WHO WATCHES THE WATCHMEN?

Effective immunosurveillance of HIV-infected cells remains problematic as CTLs exhibit exhausted effector functions arising from chronic inflammation and antigen persistence during the natural course of infection and residual inflammation, driven by microbial translocation in the gut, despite suppressive ART (34, 35). Structural defects in gut integrity cause by HIV further impacts the microbiota distribution (36), which given its ability in cancer models to modulate toxicity (37) and therapy efficacy (38, 39), may represent an attractive therapeutic avenue as proposed by Herrera et al.. In some respects, as describe by Dhodapkar and Dhodapkar, ART-suppressed HIV mirrors pre-clinical malignancy, a prolonged state characterized by early-onset of T-cell exhaustion coupled with the depletion of stem cell memory (40). However, unlike antigen-rich tumor models, curative HIV therapies require that latent virus be reactivated to render infected cells immunogenic and cleared by potent anti-HIV CTLs ("kick and kill") (10, 41). Given their capacity to promote tumor clearance, as detailed by Puronen et al., many immunotherapies are being investigated in HIV cure studies to induce T-cell activation and restore CTL functionality, such anti-PD-1 and anti-CTLA-4 check point inhibitors (CPI) (42–44), and IL-7 and IL-15 cytokine therapy (45, 46). Given emerging data concerning the importance of innate natural killer (NK) cells in the control of HIV and cancers (47, 48), Lucar et al., discuss immunotherapies targeting NKG2a and killer-cell immunoglobulin-like receptors (KIRs) as novel strategies to determine whether dysfunction NK cell states can be rescued. Curative strategies centered around CPIs have revolutionized the treatment of certain refractory cancers by reinvigorating the host immune response; yet, in PWLH it remains to be seen whether antigen burden is a critical determinant of response.

IN CASE OF EMERGENCY—BREAK GLASS

Beyond these strategies, which may above prove too toxic, fail to penetrate tissue, or lack desire specificity, alternative curative approaches utilize adoptive T-cell therapy to redirect CTL responses. Kim et al. describe the re-emergence of chimeric antigen receptor (CAR) T-cells as an attractive immunotherapy strategy given its progressive re-engineering in oncology settings to improve safety, expression, and persistence (49). Although CAR T-cells have attained remarkable remission rates for $CD19^+$ B-cell acute lymphoblastic leukemia (50), significant relapse rates are associated with diminished persistence upon antigen loss/escape, the suppressive tumor microenvironment, and impaired tumor penetration (51). These issue impacting tumor relapse are directly analogous to HIV models vis-á-vis ART-mediated aviremia, the expansion of regulatory T-cells (T_{REGs}) (52, 53), and the exclusion of CTLs from secondary lymphoid tissue (13, 54). Possible strategies to surmount these issues include engineering CAR T-cells to express 4-1BB co-stimulatory domains allowing oxidative metabolism (55); secrete cytokines, such as IL-12 or IL-18 (56, 57); and up-regulate the chemokine receptor CXCR5 to promote homing to the lymphoid B-cell follicle (58) as explored by Mylvaganam et al.. Seemingly, CAR T-cells for HIV applications should be directed against viral proteins to minimize safety concerns and given the lack of reliable biomarkers to identify latently-infected cells. Ergo, CAR T-cells will likely require co-administration with potent latency reactivating agents to promote therapy persistence and reveal cellular targets for clearance. Such combination therapies would benefit from positron emission tomography (PET)-based imaging, as reviewed by Henrich et al., to observe the total-body viral antigen distribution (59, 60) and to gain insights concerning the potential for efficacy in difficult to sample tissues (61, 62).

SUMMARY

Models of cancer and HIV persistence share an interesting paradox: responses promoting self-tolerance when exposed to sustained inflammatory stimuli permit pathological dissemination and escape from immune surveillance. This similarity would suggest common curative approaches via the targeting of immunosuppressive pathways. However, a key distinction is that in cancer the self-immunogen is pervasive; whereas, in ART-treated HIV infection chronic antigenic stimulation arises largely from gut microbial translocation, not from viral proteins. This different in antigen source may represent a key obstacle when translating therapies between cancer and HIV models (63). In designing immunotherapy strategies, it is also important to consider that adverse event outcomes between these models have substantially different tolerances, as HIV is a manageable chronic disease and cancers are invariably fatal. Future trials will be necessary to determine whether these mechanistic insights regarding escape and exhaustion can be successfully adapted to facilitate long-term, ART-free HIV remission.

AUTHOR CONTRIBUTIONS

MP, KD, SD, and RA contributed to formulating the theme for this article collection, recruiting authors, and acting as editors for the submissions. MP and JH wrote the editorial, with contributions, and final edits from all authors.

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HIV, Cancer, and the Microbiota: Common Pathways Influencing Different Diseases

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HIV infection exerts profound and perhaps irreversible damage to the gut mucosal-associated lymphoid tissues, resulting in long-lasting changes in the signals required for the coordination of commensal colonization and in perturbations at the compositional and functional level of the gut microbiota. These abnormalities in gut microbial communities appear to affect clinical outcomes, including T-cell recovery, vaccine responses, HIV transmission, cardiovascular disease, and cancer pathogenesis. For example, the microbial signature associated with HIV infection has been shown to induce tryptophan catabolism, affect the butyrate synthesis pathway, impair anti-tumoral immunity and affect oxidative stress, which have also been linked to the pathogenesis of cancer. Furthermore, some of the taxa that are depleted in subjects with HIV have proved to modulate the anti-tumor efficacy of various chemotherapies and immunotherapeutic agents. The aim of this work is to provide a broad overview of recent advances in our knowledge of how HIV might affect the microbiota, with a focus on the pathways shared with cancer pathogenesis.

Keywords: HIV, cancer, microbiota, immunotherapy, dysbiosis

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INTRODUCTION

A hallmark of treated HIV infection is sustained, low-level viral inflammation. While the cause of this persistent activation of innate and adaptive immunity despite well-controlled HIV RNA replication is not completely understood, it is widely assumed that chronic defects of mucosal immunity are a major contributor (1). HIV targets the mucosa on structural and functional levels (2–4). Arguably, these disturbances will have consequences on the signals required for the coordination of commensal colonization, which may explain the shifts in microbial distributions and metabolic activity of gut microbial communities (5–7). In addition, these abnormalities caused by HIV infection have been shown to result in increased translocation of microbial products from the gut to the circulation in both animal models and HIV-infected individuals (8, 9). It has been repeatedly shown that biomarkers of bacterial translocation positively correlate with markers of T-cell activation, monocyte activation, and proinflammatory cytokines (10). It is widely accepted now that sustained low-level activation of the innate and adaptive immune systems is a major driver of AIDS and non-AIDS-related comorbidities (11–15). Collectively, these observations argue that microbial translocation, a phenomenon intrinsically linked to the gut microbiota, is a driver of inflammation, and adverse outcomes during treated HIV infection.

INFLUENCE OF THE MICROBIOTA ON HIV IMMUNOPATHOGENESIS DURING TREATED INFECTION

The gut microbiota has been associated with HIV immunopathogenesis (5, 16–19). Defining the influence of HIV on the microbiota, however, is more difficult. Studies on the impact of SIV infection in the gut microbiota of non-human primates have found only modest differences in the fecal bacterial communities between SIV-infected macaques compared to uninfected macaques, suggesting that the development of immunosuppression, rather than SIV infection itself, may drive the differences (20, 21). In addition, induction of dysbiosis with vancomycin does not accelerate the progression of untreated SIV infection (22). The effects of HIV infection on microbial diversity appear to be confounded by a number of factors, including the nadir of CD4+ T-cells (23) and the risk factor for HIV acquisition (24, 25). While admittedly there are difficulties dissecting the specific effects of HIV disease on the microbial communities, there is wide consensus that the gut microbiomes of HIV-positive individuals exhibit specific compositional and functional shifts (5, 19, 26–29). Surprisingly, the microbiota associated with HIV infection shares traits with that associated with other proinflammatory conditions, such as the depletion of butyrate-producing bacteria observed in inflammatory bowel disease (30).

It is therefore tempting to assume that so-called "HIV-associated dysbiosis" may be implicated in the sustainment of systemic inflammation in treated HIV disease. Several taxa and their associated pathways (**Figure 1**) have been linked with persistent immune abnormalities (5, 7, 31). The real picture, however, may be far more complex. From an ecological point of view, the components of a rapidly evolving ecosystem will respond to environmental perturbations by adapting their composition and functions to achieve the optimal fitness within their changing habitat (32). For example, the fecal microbiota of people with HIV has been shown to harbor greater abundances of genes related to resistance to oxidative stress, such as the genetic machinery for glutathione metabolism or zeatin biosynthesis pathways (7, 31).

Defining the clinical scope of the changes in gut microbial communities can be challenging because a big proportion of bacteria are dead, dormant, or inactive (33, 34). Expensive and time-consuming techniques are required to measure the proteins and metabolites synthesized by active bacteria. The extent of functional adaptation of microbial communities to the ecological perturbation induced by HIV might influence the different immunologic outcomes achieved during antiretroviral therapy (ART). In fact, HIV infection activates an important fraction of the gut microbiota. Although only 20% of the fecal microbiota is metabolically active in healthy controls, HIV infection is characterized by the activation of up to 50% of microbial communities (35). Among immunological ART responders, the metabolic activity of some taxa (Succinivibrionaceae family) is boosted, acting as anti-inflammatory buffers thanks to the accumulation of proinflammatory mediator. In addition, cannabinoid oleamide and biliverdin (a viral inhibitor) are also accumulated within bacteria and may contribute to health recovery by inhibiting viral replication, stimulating the immune system, and ultimately reducing inflammation. These findings are in sharp contrast to those observed in immunological non-responders whose gut bacteria metabolism is most similar to that of ART-naïve participants. The metabolic activity of their gut bacteria is characterized by the cleavage of the sialic and dolichol components necessary to maintain enterocyte integrity (19).

The Kynurenine Pathway

Indoleamine-2,3-dioxygenase-1 (IDO1) involved in tryptophan catabolism via the kynurenine pathway is correlated with epithelial barrier disruption and bacterial translocation in HIV infection (36). Induction results in the production of kynurenine derivatives with immunosuppressive effects, impairing mucosal immunity, and promoting bacterial translocation and higher mortality (37). In a seminal study, Vujkovic-Cvijin et al. (5) characterized 140 genera significantly correlated with tryptophan catabolism. Some of these taxa were found to encode the genetic machinery that reproduces the same tryptophan catabolism as human IDO1. This finding was further confirmed by metabolomic analysis in gut bacteria via the detection of the kynurenine subproduct 3-hydroxyanthranilate (34). In a subsequent study combining metagenomic and metatranscriptomic data, we showed that HIV-infected individuals exhibited increased anaerobic catabolism of tryptophan via tryptophanase anaerobic fermentation compared with healthy controls (23). This expression was upregulated in the Prevotella, Acidaminococcus, and Clostridium genera. It is likely that the HIV-associated microbiota exerts a strong influence on this critical pathway at the crossroads between metabolism and immunity.

Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs) are the primary fermentation products of gut microbiota from dietary fibers. The most abundantly produced SCFAs include acetate, propionate, and butyrate (38, 39). Butyrate is a regulator of intestinal homeostasis and a modulator of immune cell response. It is involved in the maintenance of enterocyte barrier integrity and mucine production (40), induces transcription of human genes via histone deacetylase inhibition (41), and promotes immunotolerance to commensal bacteria (42). Several studies have demonstrated a decrease in butyrate-producing bacteria, including *Roseburia*, Coprococcus, Faecalibacterium, and *Eubacterium*, in both HIV-treated and ART-naïve individuals, in association with altered SCFAs profiles (17, 43). In patients with ulcerative colitis, depletion of both *Faecalibacterium prausnitzii* and *Roseburia intestinalis* has been proposed to be the hallmark of dysbiosis (44). It is increasingly accepted that the butyrate synthesis pathway supports intestinal inflammation and represents a potential therapeutic target for interventions aimed at mitigating chronic inflammation (45). Propionate and acetate have been less studied in HIV but have been linked to conferring protection against cardiovascular disease and playing other beneficial roles in other diseases (46).

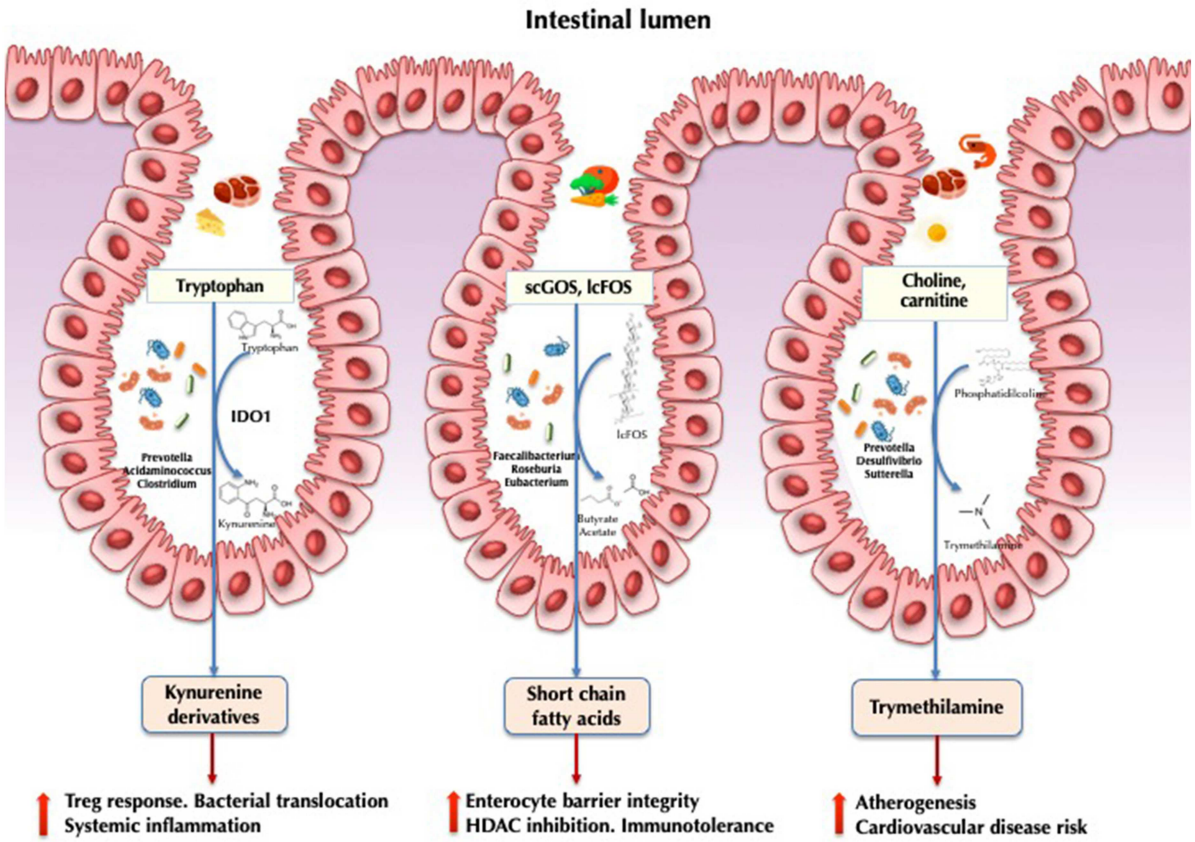

FIGURE 1 | Implications of the gut microbiota in HIV pathogenesis. IDO1, indoleamine 2,3-dioxygenase 1; scGOS, short-chain galactooligosaccharides; lcFOS, long-chain fructooligosaccarides; HDAC, histone deacetylases.

Trimethylamine-N-Oxide

Trimethylamine-N-oxide (TMAO) is a gut microbiota-dependent choline and carnitine metabolite that is responsible for an increased risk of atherogenesis and cardiovascular disease risk (47), particularly in individuals who consume large quantities of meat and possess a specific microbiome signature with enriched proportions of the genus Prevotella (48). This metabolite has also been associated with atherosclerotic plaque burden in HIV in some (49, 50) but not all (51) studies. A recent cohort study comparing the fecal microbiota of HIV-infected individuals with and without ischemic heart disease showed that high TMAO plasma levels was a marker of cardiovascular heart disease and correlated with the fecal abundance of *Phascolarctobacterium, Desulfovibrio, Sutterella,* and *Faecalibacterium* (52).

Microbiota as a Tool for Precision Medicine for HIV

Hopefully, future studies will exploit these connections between microbiota and HIV immunopathogenesis to improve the clinical management of HIV infection. From a diagnostic point of view, one could utilize microbiota to identify individuals at higher risk of HIV acquisition (53–55), to anticipate the responsiveness to pre-exposure prophylaxis strategies with topical antiretroviral drugs (56), and to predict the risk of precancerous anal lesions (57). From a therapeutic point of view, we may gain the ability to manipulate the microbiota to enhance vaccine immunogenicity (58), boost immune recovery after ART initiation (59, 60), and attenuate chronic inflammation and bacterial translocation (61). A number of studies assessing HIV patients' dietary supplementation with prebiotics and probiotics have collectively suggested that dietary supplementation may exert some beneficial immunological effects, particularly in ART-naïve individuals (30, 59, 62–64). However, two recent controlled studies focused on ART-naive (60) and ART-suppressed (65) individuals have failed to detect significant parameters of inflammation, bacterial translocation or immune activation. These findings call into question the utility of these strategies. The first pilot study of fecal microbiota transplantation in HIV failed to demonstrate adequate engraftment of colonoscopy microbiota on the microbiota of the recipients (66). Ongoing studies (NCT02256592 and NCT03329560) are evaluating different modalities of fecal microbiota transplantation. Clinical trials assessing the use of postbiotics—metabolites or cell-wall components released by microbiota—and represent the future landscape of this fascinating field.

INFLUENCE OF MICROBIOTA IN CANCER

Microbiota as a Trigger of Cancer Pathogenesis

Cancer is a multifaceted disease influenced by both genetic and environmental factors. Microorganisms are emerging as one of the contributors to carcinogenesis, and today we know that approximately 20% of the global cancer burden is directly attributable to infectious agents (67). Beyond the neoplasias directly linked to infectious agents, increasing evidence reveals that microbial communities as a whole play a key role in carcinogenesis by altering the balance of host cell proliferation and apoptosis; hindering anti-tumoral immunity; and influencing the metabolism of host-produced factors, ingested food components, and drugs (68, 69).

Barrier failure has been proposed to be the most relevant mechanism for bacterially driven carcinogenesis, resulting in increased host-microbiota interactions (70, 71). The failure of control mechanisms (e.g., barrier defects, immune defects, dysbiosis) is believed to represent the trigger of bacterial-driven carcinogenesis (72), leading to activation of different responses that converge in cell proliferation and cancer development. The microbiome itself represent a functional barrier by suppressing the growth of pathobionts via different mechanisms, including both resource competition and direct interference competition (73). Therefore, dysbiosis has also been associated with cancer (71). Alterations of gut bacteria have been linked to the development of colorrectal cancer (CRC) (74), but also to extraintestinal cancers, including liver (75), breast (76), and lung cancer (77, 78). While lung microbiome investigations are still in their infancy, the lung microbiotas of patients with lung cancer are distinct from those of other patients (e.g., individuals with emphysema) (79). The abundance of several types of bacteria in the lungs—including *Granulicatella*, *Streptococcus*, and *Veillonella*—has been proposed to be a hallmark of lung cancer (80). An association between the abundance of the Koriobacteriaceae family in the lungs and recurrence free survival has been reported (81). Furthermore, the fecal microbiota of individuals with lung cancer is depleted of Bifidobacteria (82), a commensal genus with known anti-tumoral effects. Bifidobacteria appears able to enhance the efficacy of anti-programmed cell death ligand 1 therapy (83).

Microbiota-Associated Pathways Linked to Carcinogenesis

Recent studies of CRC have identified different mechanisms of carcinogenesis. The bacterial driver-passenger model proposes that the colonic mucosa of patients at risk of CRC is colonized by pro-inflammatory bacteria that can produce genotoxins that lead to DNA mutations and increase cell proliferation ("drivers"). These changes facilitate the replacement of the commensal bacteria with opportunistic pathogens ("passengers") with competitive advantage in this niche, which leads to tumor progression (72). From the 1990s onward, various studies have demonstrated an association between CRC and specific colonic bacterial species, which favor the development of cancer through different pathogenic pathways (**Figure 2**) (86). Very impressively, *Fusobacterium nucleatum* and certain co-occurring bacteria have been found not only in primary CRC but also in distant metastases. Antibiotic treatment of mice carrying xenografts of *F. nucleatum*-positive human CRC slowed tumor growth, demonstrating the causal role of this taxon in oncogenesis (87).

Among the carcinogenic mechanisms shown in **Figure 2**, microbial fermentation products of dietary fiber into SCFAs, including butyrate, propionate, and acetate, with known anti-inflammatory properties (85) likely play a major role. Butyrate is one of the primary sources of energy for enterocytes, and it has been associated with the downregulation of the WNT signaling pathway, inhibition of proliferation and migration of neoplastic cells, and apoptosis induction (88). Butyrate also reinforces mucosal health via T_{reg}-cell activation and IL-10 expression (89). Butyrate producers (e.g., *F. prausnitzii*, *Roseburia*, *and Bifidobacterium*) are depleted in CRC patients (69).

Another mechanism related to the catabolism of dietary precursors strongly influenced by the microbiota is the production of the proatherogenic TMAO. While the implications of this derivative of choline metabolism appear clear for cardiovascular disease (47), this pathway has been rarely studied in the field of oncology. One investigation has suggested that alterations in choline metabolism may be associated with a higher risk of CRC (90).

The Microbiota Modulates the Efficacy and Toxicity of Anticancer Therapies

The microbiota can modulate cancer initiation and progression, but it might also influence response to therapy and treatment-related toxicity (91). First, the bioavailability of many oral drugs depends on their biotransformation in the gut by local microbiota and may also indirectly affect the metabolism of systemically delivered drugs via the regulation of xenobiotic metabolism in distant organs such as the liver (92). Second, the immune response plays an essential role in anticancer activity, and the microbiome might affect chemotherapy response via this mechanism. There is evidence that oxaliplatin and cyclophosphamide activity is modulated by gut microbiota by priming myeloid cells for high-level reactive oxygen species (ROS) production (resulting in DNA damage) and enhancing T-helper cell-mediated anti-tumor responses, respectively (93, 94). Chemotherapy-related adverse events can also be managed via microbiome modulation. For example, diarrhea caused by irinotecan toxicity, which is mediated by microbial-produced β-glucuronidases, can be regulated by targeting microbial metabolism (95). The microbiota might also play a role in response and toxicity to radiotherapy. Radiation-related mucosal injury is associated with changes in the microbiome, and germ-free mice have been shown to be resistant to radiation enteritis (91). Lastly, recent pioneering studies have yielded paradigm shifts in our knowledge of the interactions between gut bacteria and cancer therapy. The gut microbiome has been shown to modulate the anti-tumor efficacy in pre-clinical models of various chemotherapies (93, 94) and immunotherapeutic agents (96–99), including antibodies against cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and anti-programmed cell death

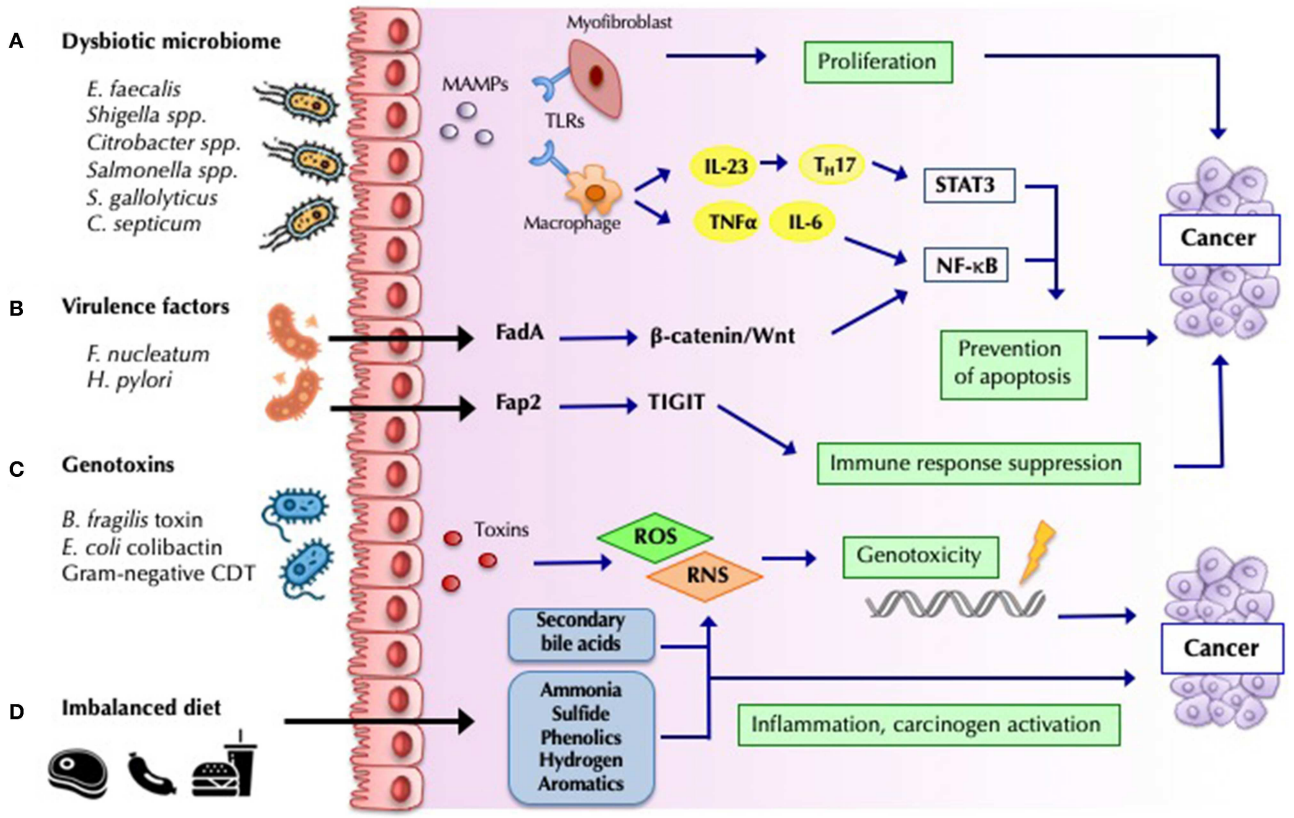

FIGURE 2 | Mechanisms by which bacteria influence cancer development and progression. **(A)** Barrier loss and increased bacterial translocation engages pattern recognition by Toll-like receptors (TLRs) and activation of innate and adaptive responses. The interleukin-23 (IL-23)-IL-17 axis, IL-6, and tumor necrosis factor-α, lead to chronic inflammation mediated by nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) activation, favoring tumor progression (68). **(B)** Bacterial virulence-factors promote carcinogenesis by engaging specific host pathways, which plays a decisive role in many malignancies. *Fusobacterium nucleatum* Fad-A binds host E-cadherin on colonic epithelial cells, and triggers Wnt/β-catenin pathway activation, resulting in increased NF-κB, and ultimately in increased tumor growth (84). Other virulence factor such as *H.pylori* CagA have been widely studied (68). **(C)** Some microorganisms modulate tumorigenesis through specific toxins, which induce host DNA damage. Cytolethal distending toxin (CDT) produced by Gram-negative bacteria, *Bacteroides fragilis* toxin and *Escherichia coli* colibactin constitute some of the most studied toxins identified as potential drivers of CRC (70). **(D)** Dietary residues determine the composition and metabolic activity of the microbiota. An imbalanced high-fat, high-meat, low-fiber diet, lead to a greater exposition to secondary bile acids, and protein fermentation metabolites (such as ammonia, phenols, sulfides, and nitrosamines), which have inflammatory and carcinogenic effects (85).

protein 1 (PD-1) (92). Individuals with metastatic melanoma responding to anti-PD-1 were enriched with *Faecalibacterium* genus in intestinal microbiota; non-responding individuals had a higher abundance of Bacteroidales (97). Another study found an abundance of *Bifidobacterium* in responding individuals; *Ruminococcus obecum* and *Roseburia intestinalis* were associated with a lack of responsiveness (99). The role of the microbiota on treatment response is further supported by striking data showing poorer survival outcomes on patients with metastatic non-small cell lung cancer or renal cell carcinoma receiving antibiotics just before or just after initiation of treatment with immune checkpoint blockade (100). Converging data support a robust interaction between specific bacteria and the systemic immune response (97–99). In subjects with non-small cell lung cancer specific memory CD4+ and CD8+ T-cells against *Akkermansia muciniphila* predicted a longer progression-free survival (98). In subjects with melanoma the abundance of *Faecalibacterium* genus positively correlated with the with a higher frequency of cytotoxic CD8 T-cell infiltration in the tumor bed. Similarly, in mice intratumoral CD8+ T-cell infiltration after anti-PD-L1 treatment correlated the microbiota composition (100).

Is It Possible to Exploit the Microbiome to Improve Clinical Outcomes in Oncology?

Emerging evidence suggests that altering the microbiota might represent a therapeutic avenue for cancer management (101). Modulation of gut microbiota in preclinical models has been shown to enhance therapeutic response (102). Landmark studies have demonstrated that fecal microbiota transplantation from cancer patients who had responded to anti-PD-1 therapy improved the effects of PD-1 blockade in germ-free or antibiotic-treated mice (97–99). Several trials involving patients on immune checkpoint blockade undergoing fecal microbiota transplant

TABLE 1 | Gut microbial signatures associated with clinical outcomes in both HIV and cancer and putative mechanisms.

Bacteria implicated	Pathway/Function	Mechanisms	Biological effect	Clinical consequences	References
↓ *Faecalibacterium prausnitzii* ↓ *Lachnospira* spp. ↓ *Roseburia intestinalis* ↓Ruminococcaceae	SCFA-production	Histone deacetylase inhibition Human gene transcription ↓ antigen presentation ↑ immunotolerance	Immunotolerance Cell proliferation	**HIV**: systemic inflammation. Higher risk of tuberculosis **Cancer**: risk of CRD development (*Roseburia intestinalis*)	(30, 31, 97, 105, 106)
↑ *Gammaproteobacteria* ↑ *Pseudomonas* spp. ↑ *Bacillus* spp. ↑ *Burhloderia* spp. ↑ *Prevotella* ↑ *Acidaminococcus*	Tryptophan catabolism	IDO1 inhibition ↑ immunosuppressive kynurenine derivatives ↓ Th17 cells	Immunotolerance Barrier failure Angiogenesis	**HIV**: bacterial translocation, inflammation, mortality **Cancer**: Overexpressed in tumoral cells (e.g., endometrial cancer, lung cancer) *IDO1 inhibitors under evaluation in both conditions.*	(5, 29, 107–109)
↑ *Bacteroides fragilis*	IL-10 signaling pathway	Polysaccharide A production TLR-2 activation IL-10 expression	Immunotolerance	**HIV**: Systemic immune activation. Periodontitis **Cancer**: anti-tumoral effects. Enhancement of CTLA-4 blockade efficacy	(5, 110–113)
↑ Actinobacteria ↓ Bacteroidetes ↑ Firmicutes ↑ Gammaproteobacteria ↑ Clostridium XIVa ↑ *Faecalibacterium* spp.	Choline metabolism	TMAO production	Endothelial dysfunction Inflammation	**HIV**: carotid atherosclerosis, monocyte activation **Cancer**: malignant transformation, risk of colorectal cancer	(51, 52, 114–117)
↑ Bifidobacteria	Antitumoral immunity	↑ Dendritic cell activation ↑ CD8+ T cell priming and accumulation in the tumor microenvironment ↑ Cross-reactivity with tumor antigens	CTL responses Epithelial cell turnover Immunomodulatory strain-dependent effects	**HIV**: immune recovery under ART **Cancer**: Protection against cancer development. Enhancement of immunocheckpoint blockade efficacy.	(19, 82, 83, 118–120)
↓ *Akkermansia muciniphila*	Chemotaxis	↓ Mucin degradation	Host immune regulation	**HIV**: higher systemic inflammation (sCD14, IP10) and intestinal inflammation (fecal calprotectin) **Cancer**: longer progression free-survival. Enhanced efficacy of PD-1 blockade	(26, 121–123)
↑ *Fusobacterium* spp.	Cell proliferation	TLR-4 signaling.PPAK1 cascade. Nuclear factor KB induction	Cell proliferation and oncogenesis	**HIV**: poor immune recovery after ART **Cancer**: colorrectal cancer development	(17, 124–126)
↑ *Lactobacillales*	Inflammation. Antitumoral immunity	Upregulated IFN-γ, GZMB, and PRF1 expression in CD8+ T-cells	Enhanced antitumor response	**HIV**: greater immune recovery after ART **Cancer**: predictor of enhanced immunotherapy efficacy	(19, 99, 126–128)

ART, antiretroviral therapy; CTL, cytotoxic T-cell mediated; SCFA, short-cain fatty acid; IDO1, indolamine-2,3-deoxygenase-1; LPS, lypopolisaccharide; TMAO, trimethylamine-N-oxidase.

are currently underway, but definitive data are lacking (91). Probiotics have been shown to boost anti-tumor immune responses in mice, but their off-trial use in humans is discouraged because there is still insufficient evidence to implement dietary guidelines or prebiotic administration in the setting of cancer therapy (91). Manipulation of the microbiome in cancer patients might result in novel indications for this intervention, as illustrated by the efficacy demonstrated in the first case series of patients with refractory immune checkpoint inhibitor-associated colitis successfully treated with fecal microbiota transplantation (103). Nearly 40 clinical trials assessing gut microbiota modulation in cancer are ongoing (91). The results of these investigations will inform best strategies and define indications of this therapeutic approach to improve clinical outcomes in oncology.

HIV, CANCER, AND THE MICROBIOTA: CONVERGING PATHWAYS AND RESEARCH AVENUES

Can we learn anything from microbiome studies of HIV-positive patients that may be applicable to cancer? First, the vast majority

of mechanistic studies regarding the influence of the microbiome in HIV are cross sectional in nature (104). The well-known limitations of these studies are magnified by underappreciated confounding factors related to microbiota studies. For example, it took several years for the field to recognize that the increased abundance of *Prevotella* spp. observed in the first studies of HIV-infected individuals (5, 7, 16, 18) was confounded by the lower proportion of men who had sex with men in the control groups (24). Given the particular clinical profile of patients undergoing anticancer treatment, these confounders may be even more pronounced in patients with cancer.

Several pathways strongly influenced by microbiota appear to affect pathogenic mechanisms present in different conditions. Gut microbial signatures associated with clinical outcomes in both HIV and cancer and the putative mechanisms are summarized in **Table 1**. For example, the major butyrate producers *Faecalibacterium prausnitzii* and *Roseburia intestinalis* are depleted in subjects with HIV (17, 43), intestinal bowel disease (44), and CRC (69). Because butyrate production has been shown to promote T_{reg}-cell activation and IL-10 expression (89, 105), the butyrate synthesis pathway is a potential therapeutic target for conditions in which enterocyte barrier integrity and mucosal tolerogenic immune responses are implicated. The kynurenine pathway has been also implicated in both HIV (5) and cancer pathogenesis (129). IDO1 is frequently overexpressed in many malignancies, where it correlates with poor survival and prognosis. Besides its role in immunosuppression, IDO1 promotes cancer development by inducing inflammatory neovascularization, interacting with checkpoint inhibitors, and modulating gut microbiota (130). While it is still too soon to draw conclusions about the therapeutic potential of IDO1 inhibitors for HIV disease and cancer, an increasing number of IDO1 inhibitors are currently in preclinical development or under evaluation in clinical trials (131, 132).

Analyzing gut microbiota from a functional perspective will be crucial to advancing knowledge about the role of the microbiome in the pathogenesis of cancer and understanding its interactions with immunotherapy. While bifidobacteria have not typically appeared to be compositionally relevant in most HIV studies reliant on 16S sequencing, its functional importance is clear when we assess the functional level of the microbiota. For example, while using 16S sequencing we only demonstrated modest changes in gut microbiota structure after a short prebiotic intervention, which did not include changes in the abundance of bifidobacteria (30). Using proteomics we demonstrated a 100-fold increase in the activity of the Bifidobacteriaceae family, which strongly correlated with the thymic output, a surrogate marker of the ability of the immune system to renew the T-cell pool (118). In a study aimed at identifying the bacterial biomarkers of precancerous anal lesions in HIV, *Bifidobacterium* spp. were also the most predictive taxa in stools of anal dysplasia (57). Because *Bifidobacterium* spp. enhance anti-tumor immunity and anti-PD-L1 efficacy (83), it is likely that the importance of this genus will remain underappreciated until researchers evaluate the functional level of the microbiota.

While the microbiome agenda is expanding, it is still unclear whether we can effectively manipulate the microbiome to treat HIV and cancer. Pilot studies analyzing the effects of fecal microbiota transplantation will provide powerful indications of our ability to modify clinical outcomes via microbiota manipulation. In the coming years, we look forward to learning to exploit the potential of the microbiota for precision medicine (e.g., predicting treatment responsiveness or toxicities). Gaining further insights into the mechanisms by which the microbiota influences HIV disease and cancer will help to leverage the microbiome to develop interventions for both conditions.

AUTHOR CONTRIBUTIONS

SS-V conceived the paper. SS-V, SH, and JM-S draft the first version of the manuscript. All authors reviewed and approved the final version.

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Learning From the Exceptions: HIV Remission in Post-treatment Controllers

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Among the top priorities of the HIV field is the search for therapeutic interventions that can lead to sustained antiretroviral therapy (ART)-free HIV remission. Although the majority of HIV-infected persons will experience rapid viral rebound after ART interruption, there are rare individuals, termed post-treatment controllers (PTCs), who demonstrate sustained virologic suppression for months or years after treatment cessation. These individuals are considered an ideal example of durable HIV control, with direct implications for HIV cure research. However, understanding of the mechanisms behind the capacity of PTCs to control HIV remains incomplete. This is in part due to the scarcity of PTCs identified through any one research center or clinical trial, and in part because of the limited scope of studies that have been performed in these remarkable individuals. In this review, we summarize the results of both clinical and basic research studies of PTCs to date, explore key differences between PTCs and HIV spontaneous controllers, examine potential mechanisms of post-treatment control, and discuss unanswered questions and future research directions in this field.

Keywords: HIV, post-treatment controllers, remission, treatment interruption, elite controllers

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INTRODUCTION

Within each medical field, there exist individuals who exhibit extreme responses to medical treatment. As an example, individuals who have an unexpectedly dramatic response to cancer therapy are termed "exceptional responders." These exceptional responders represent an area of intense research interest within the oncology field (1) and have already made important contributions to the understanding of both basic tumor biology and drug development (2). In this review, we focus on a group of exceptional responders within the HIV field, specifically individuals who were treated with antiretroviral therapy (ART) and can subsequently maintain HIV remission even when the ART is discontinued.

HIV infection is characterized by sustained viral replication and progressive decline in CD4 cell counts (3). ART is effective in suppressing viral replication and decreasing HIV-associated morbidity and mortality, but it cannot completely eradicate all HIV-infected cells. Consequently, HIV viral load rebounds rapidly after treatment interruption in most HIV patients (4, 5). However, there are rare individuals, termed post-treatment controllers (PTCs), who are able to suppress the virus for a prolonged period of time after treatment interruption (**Figure 1**). These individuals are considered an ideal example of durable HIV control and have the potential to provide substantial insight into the "natural" mechanisms of functional cure and sustained HIV remission (7).

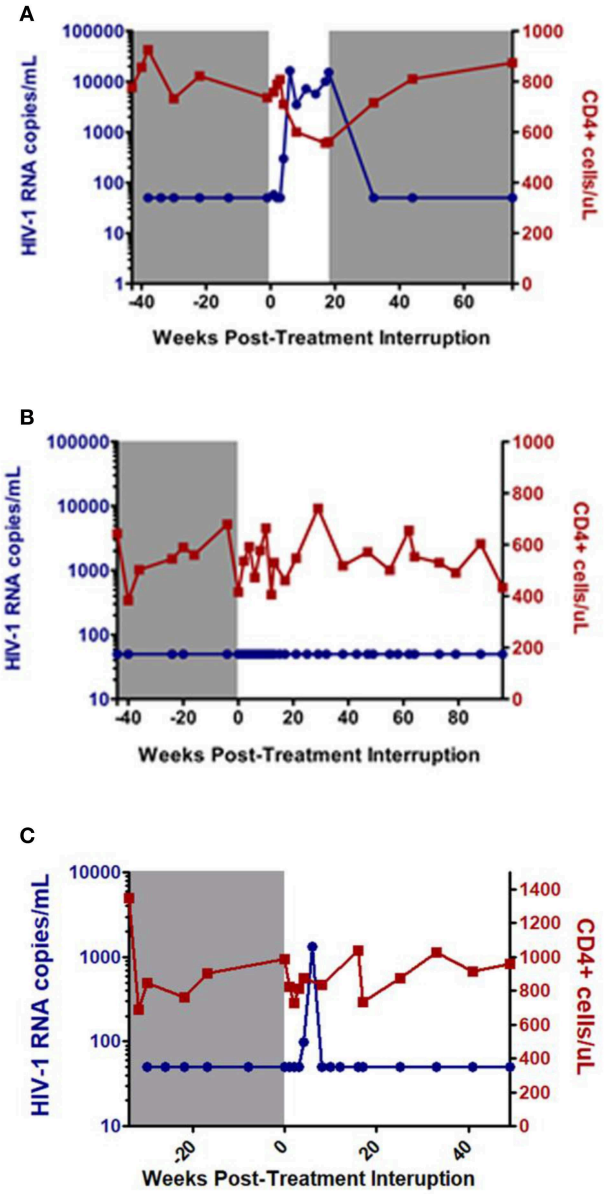

FIGURE 1 | Examples of post-treatment non-controller (NC) **(A)**, and post-treatment controllers **(B,C)**. Gray shaded area represents time on antiretroviral therapy. Adapted from CHAMP study (6).

Interest in ways to induce post-treatment control were initially kindled by a report of an individual who was able to control HIV without ART after undergoing several sequential treatment interruptions (8) and in an in-depth report of 14 early-treated PTCs reported in the VISCONTI study (7). There have been a number of subsequent studies of PTCs with a wide range of reported frequency amongst those who discontinue ART (6, 7, 9–19). This variation in reported frequency of PTCs may be attributed to different baseline characteristics of the populations in which these studies were done, as well as the heterogeneous definitions applied for defining this rare group of HIV patients (18). In this review, we will summarize the most recent findings on the clinical and immunological characteristics of PTCs, differentiate them from HIV spontaneous controllers (SCs), and discuss the role of PTCs in the search for strategies toward HIV remission and cure.

POST-TREATMENT CONTROLLER DEFINITIONS

Since the initial description of the post-treatment controller phenotype, a number of observational studies and interventional clinical trials have been performed to investigate the characteristics of this rare group of patients and to determine the mediators of post-treatment control. However, the heterogeneities in study designs have made it challenging to compare studies and to gain a clear grasp of the PTC population. For example, the definition of post-treatment control has differed dramatically between studies. Some studies have considered virologic rebound to be a plasma viral load above 50 HIV-1 RNA copies/ml after treatment interruption, while others have used a threshold of 400 HIV-1 RNA copies/ml or 1,000 HIV-1 RNA copies/ml for this purpose (**Table 1**, **Supplementary Table S1**). The duration of viral control after treatment interruption has also differed dramatically between studies and ranged from a median of 6 month to more than 2 years (7, 9–32). Furthermore, the loss of viral control was also defined differently between previous studies. Some considered 2 consecutive viral loads above 50 HIV-1 RNA copies/ml to indicate the loss of post-treatment control (8–10), while others considered 1–4 consecutive viral loads higher than 400 HIV-1 RNA copies/ml as the definition for viral rebound post-treatment interruption (7, 12, 16–18). Of note, the largest PTC study to date has been the Control of HIV after Antiretroviral Medication Pause (CHAMP) study, which identified 67 PTCs through the pooled analysis of 14 clinical studies from the AIDS Clinical Trials Group (ACTG) and other North American cohorts (6, 14, 20–32). In this study, the PTCs were defined as individuals who maintained viral loads $\leq$400 copies/mL at two-thirds or more of time points for $\geq$24 weeks post treatment interruption (6).

DEMOGRAPHIC CHARACTERISTICS OF PTCs

The median age of PTCs in these studies ranged from 27 to 46 years old. The majority of PTCs identified were male, likely reflecting the sex distribution of the clinical trial participants (6, 7, 9, 11, 12, 15–18). Intriguingly, there have been reports that female gender may be associated with a higher chance of post-treatment HIV control (10) and spontaneous control (33, 34), highlighting the need for studies focusing on female participants of treatment interruption trials. In addition, the majority of PTCs have been reported by studies from North America and Europe (6, 7, 9–12, 15–18) and little is known about PTCs from outside of those regions. In an analysis of SPARTAC trial participants who initiated ART during early HIV infection, individuals with delayed viral rebound could be

TABLE 1 | Post-treatment controller (PTC) frequency after treatment interruption reported from previously published studies.

References	Cohort	Timing of ART	PTC, Total, *N*	PTC Frequency (%)	VF threshold copies/ml	PTC duration
Hocqueloux et al. (9)	ANRS	Early	5	15.6	>50	75 months (median)
Goujard et al. (10)	ANRS PRIMO	Early	14	8.5	>50	4.5 years (median)
Lodi et al. (11)	CASCADE	Early	11	5.5	>50	24 months
Saez-Cirion et al. (7)	VISCONTI	Early	14	15.3	>400	89 months (median)
Stohr et al. (12)	SPARTAC	Early	4	2.4	>400	164–202 weeks
Van Gulck et al. (15)	Secondary Controllers	Chronic	4		>1,000	At least 6 months
Assoumou et al. (16)	ANRS SALTO	Chronic	7	4.2	>400	12 months (7 patients) 36 months (4 of the 7 patients)
Calin et al. (17)	ULTRASTOP	Early Chronic	1	10	>400	56 weeks
Perkins et al. (18)	NHS	Chronic	4	4.2	>400	267–1,058 days
Fidler et al. (19)	CASCADE	Early	22	2.8	>50	24 months
Namazi et al. (6)*	CHAMP	Early & Chronic	67	13 (Early) 4 (Chronic)	>400	24–804 weeks

**The CHAMP study includes participants from 8 AIDS Clinical Trials Group (ACTG) studies [ACTG 371 (20), A5024 (21), A5068 (22), A5102 (23), A5130 (24), A5170 (25), A5187 (26), and A5197 (27)], the Montreal Primary HIV Infection Cohort (Montreal PIC) (28), the Seattle Primary Infection Program (SeaPIP) (13, 29), the University of California San Diego Primary Infection Cohort (UCSD PIC) (14), a National Institutes of Health (NIH) therapeutic vaccine trial (30), the University of California San Francisco (UCSF) OPTIONS study (31), and the Ragon HIV Controllers cohort (32).*

ART, Anti Retroviral Therapy; VF, Viral Failure.

identified from participants enrolled in South Africa and Uganda (35). Furthermore, African participants tended to have lower pre-ART viral load and integrated HIV DNA levels, and after treatment interruption, Africans appeared to experience a longer duration of viral remission than non-Africans in the SPARTAC study (12, 36). These results provide a strong rationale for additional studies of PTCs from Africa and other regions to assess the impact of race and HIV subtype on barriers to HIV remission.

CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS

Historically, the majority of PTCs have been identified in studies of patients who initiated ART during early HIV infection (7, 9–12, 20, 26, 29–31, 37). However, PTCs have also been identified in participants who were treated during chronic HIV infection (15, 16, 18, 21, 22, 25, 27, 38). The CHAMP study directly compared the frequency of post-treatment control between individuals who initiated ART during early and chronic HIV infection. This study found that individuals who were treated during early infection were far more likely to meet the PTC criteria after treatment interruption compared to those treated during chronic infection (13 vs. 4%, $P < 0.01$, **Figure 2**) (6).

At the time of treatment interruption, CD4 cell counts for the PTCs were generally quite high with a median of 720 to 1,429 cells/mm^3 amongst the studies (7, 9–12, 15–19). After ART discontinuation, PTCs can exhibit a range of viral load dynamics with a subset demonstrating persistent viral load suppression (**Figure 1B**) while others experience early viral rebound before subsequently regaining viral control (**Figure 1C**). In the CHAMP study, ∼45% of PTCs had early viral load peaks ≥1,000 HIV-1 RNA copies/mL and 33% had early viral load peaks ≥10,000 HIV-1 RNA copies/mL amongst those with intensive weekly viral load monitoring (6).

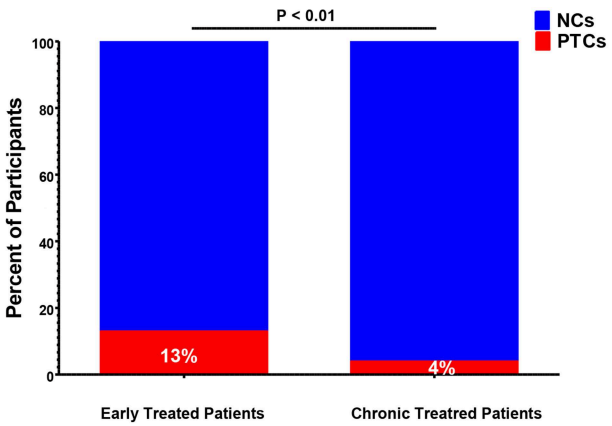

FIGURE 2 | Frequency of post-treatment controllers (PTCs) identified in early- vs. chronic-treated participants of the CHAMP study (6). NCs, post-treatment non-controllers.

The comparison of previously published PTC studies has also been difficult due to heterogeneity in the inclusion of PTCs with varying duration of viral control. To place the PTC studies in context, the median time of HIV rebound after ART interruption for post-treatment non-controllers (NCs) is ∼3–4 weeks and only a small proportion of non-controllers are able to maintain viral suppression to 12 weeks or beyond (4). The VISCONTI study was one of the earliest and most comprehensive of the PTC studies (7). The inclusion criteria for the 14 VISCONTI participants were individuals who were treated during early HIV infection and maintained viral suppression <400 HIV-1 RNA copies/mL for at least 2 years after ART interruption. To assess the durability of HIV remission, the CHAMP study used a more inclusive definition of post-treatment control (viral

suppression for 24 weeks). In this analysis, the median duration of post-treatment control was a little over 2 years and the proportion of PTCs who remained virologically suppressed in years 1–5 were 75, 55, 41, 30, and 22%, respectively (6). These results show that post-treatment control is not always durable and that PTCs will require continued clinical and virologic monitoring. These results highlight the heterogeneity in the post-treatment controller phenotype, with some individuals losing control within 1 year and others maintaining viral suppression for more than 10 years (6, 7). While the latter group may be the best model of sustained HIV remission, uncovering factors that lead to the loss of viral control in PTCs may also provide insight on the mechanisms behind their HIV remission. It should be noted though, that the rates of viral suppression reported in the PTCs are in the absence of any additional interventions and that strategies to augment key HIV-specific immune responses have the potential to improve the durability of post-treatment control.

COMPARING SPONTANEOUS AND POST-TREATMENT CONTROLLERS

Without ART, most HIV-infected individuals will have high levels of HIV-1 RNA and experience progressive absolute CD4+ T-cell decline, clinical immunodeficiency, and death (39). However, a small proportion of those infected with HIV can spontaneously maintain very low levels of plasma viral load without the use of antiretroviral therapy (ART) (40, 41). The existence of these HIV spontaneous controllers (SCs), also known as elite controllers (ECs), represented the first indication that the goal of drug-free HIV remission is possible. Although these SCs have low or even undetectable viremia by conventional viral load assays, they generally harbor replication competent virus and have evidence of ongoing viral replication and evolution (42–45). Through robust genetic and functional studies, the most consistent mediator of spontaneous HIV control appears to be through the effects of cytotoxic CD8 T lymphocyte (CTL) responses (46, 47), and the protective effects of certain HLA alleles, such as HLA B*27 and B*57 (48–50). Similar to the PTCs, SCs appear to be a heterogeneous population of individuals with respect to the level and durability of HIV control (51, 52). While some SCs can maintain viral loads <50 copies/ml in absence of ART (i.e., elite controllers [ECs]) (41, 53). Viremic controllers (VCs) can maintain a less robust level of viral suppression, with detectable viral loads below 2,000 HIV-1 RNA copies/mL in the absence of ART (54).

However, even amongst the ECs, there is evidence of heterogeneity in immune responses (49, 55), and a subset will lose viral control and experience immunological and clinical progression over time (56–58). Low Gag-specific CD8 T cell response, high levels of inflammatory cytokines and high viral diversity have been reported as factors that predict loss of viral control in ECs (51).

Due to the rarity of individuals undergoing treatment interruption, PTCs have for a long time not been recognized as a separate entity from SCs. While it is possible that some PTCs treated during early HIV infection may have achieved spontaneous control in the absence of ART, there are now several lines of evidence that PTCs are indeed distinct from HIV SCs: (1) CTL responses have been found to be far weaker in PTCs compared to SCs (7); (2) Unlike SCs, PTCs do not appear to be enriched in protective HLA alleles (3, 10, 59), with the VISCONTI study reporting a high frequency of HLA alleles previously associated with less favorable clinical outcomes (7); (3) PTCs frequently present with symptomatic acute retroviral syndrome and have pre-ART viral loads that are similar to that of non-controllers, but significantly higher than that of HIV SCs (6, 7); and (4) Results from both the SPARTAC and CHAMP studies have demonstrated an ART-specific effect as early ART initiation significantly increases the chances of achieving post-treatment control (6, 35). Together, these findings support the concept that PTCs are largely distinct from SCs and represent individuals who would not have been able to achieve HIV remission without the period ART.

MECHANISMS AND PREDICTORS OF POST-TREATMENT CONTROLLERS

While the exact mechanism behind the ability of PTCs to maintain HIV remission remains unclear, there is evidence for an unusual degree of reservoir restriction and relatively weak HIV-specific CTL activity. In prior studies of ART-treated individuals, the HIV reservoir is primarily maintained within memory CD4 T cells, especially those of central memory (T_{CM}) and transitional memory (T_{TM}) cells (60). In prior treatment interruption studies, smaller total and active HIV reservoirs before treatment interruption have been associated with delayed HIV rebound after treatment interruption. Specifically, lower levels of pre-treatment interruption HIV proviral DNA have predicted delayed viral rebound (16, 61), as has lower levels of cell-associated HIV RNA (4, 30). In PTCs, levels of HIV DNA and cell-associated RNA have also been found to be low in some studies (10, 15) but not others (38). In the VISCONTI analysis, the predominant cellular subset contributing to the HIV reservoir has been reported to be the T_{TM} cells (7), similar to that found in other early treated patients (62) and suggest that the low frequency of HIV infection within the longest-lived CD4 T cells (naïve and central memory) may contribute to post-treatment control. In studies of SCs, there have been reports that the HIV reservoir is also restricted within the T_{CM} cell subset (63), although this has not been replicated in other studies (7). In ART-treated individuals, the vast majority of HIV proviral DNA are defective and until recently, the proviral landscape within PTCs had not been investigated. In an analysis of ACTG PTCs, Sharaf et al. reported near-full length proviral sequencing results showing that PTCs had an ∼7-fold smaller HIV reservoir compared to NCs prior to the ATI, but that some PTCs had relatively large fractions of intact proviruses (64). In a separate case report, post-treatment control could be maintained despite the presence of a clonally-expanded population of HIV-infected cells harboring replication-competent virus (65). Overall, these results demonstrate that PTCs have a restricted HIV reservoir, especially within longer-lived cellular subsets, which may contribute to their ability to maintain HIV remission. Additional studies are needed to

explore the role of viral fitness (15), clonal expansion and the integration sites of intact proviruses in HIV remission.

Primate studies have also provided insight on strategies for delaying viral rebound. In particular, early ART therapy restricts the seeding of SIV reservoirs and lead to delayed timing of viral rebound (66, 67). Similarly, early initiation of ART has been associated with a significantly increased chance of achieving post-treatment control both within CHAMP study and others (6, 19). Prior studies of early ART treatment have found that it is effective in dramatically reducing the size of the HIV reservoir (68–71). In addition, early ART may preserve HIV-specific T cell responses (72–74). However, the VISCONTI study and others have shown that HIV-specific CD8 T cell responses in PTCs are weak compared to either SCs or viremic individuals (7, 75, 76). These results are consistent with reports that pre-ART viral loads are generally quite high in PTCs (6, 7) and that they do not tend to harbor protective HLA alleles (7, 38, 59, 75). However, other studies have not found significant differences in T cell responses between PTCs and SCs (15). In addition, there are reports from the VISCONTI study that early HIV treatment in PTCs preserves robust poly-functional CD4+ responses to HIV (77). Finally, there have been several reports that early ART initiation in infants may also lead to long-term HIV remission (76, 78, 79). In the first reported case, known as the "Mississippi baby," the infant initiated ART 30 h after delivery until 18 months of age. ART remission was achieved without detectable HIV-specific antibody or T cell responses (78), but viral rebound occurred ∼2 years after ART discontinuation (80). In the second case, the infant became infected despite 6 weeks of Zidovudine prophylaxis after delivery and initiated ART at 3 months of age. ART was discontinued between 5 and 7 years of age and viral control has been documented for ∼12 years despite several transient viral blips, a detectable replication-competent reservoir, and weak HIV-specific CD8+ T cell responses (76). The final report is that of a child who initiated 40 weeks of ART at day 61 after delivery as part of the Children with HIV Early antiretroviral therapy (CHER) trial (81, 82). The child has maintained viral suppression for almost 9 years after ART discontinuation, with detectable HIV DNA and residual viremia, low level of HIV-specific antibody and weak T cell response (79). Importantly, none of these children harbored the protective HLA class I alleles B*27 or B*57 associated with spontaneous viral control and levels of immune activation during HIV remission were low in all three children (76, 78, 79). These cases also highlight that post-treatment control in children can occur with a range of ART initiation times (between 30 h and 2–3 months after delivery), HIV subtypes (B, H, and C in the three cases, respectively), and duration of ART (10 months to 6 years) (76, 78, 79). Although these studies support the possibility of HIV remission in early-treated children, the frequency of post-treatment control appears to be rare as only 1 of 227 children in the CHER trial achieved this outcome (79) and smaller studies of treatment interruption in children have failed to detect any PTCs (83).

Early ART initiation has also been shown to preserve HIV-specific humoral immunity by preserving memory B cell numbers and function (84, 85). There are reports from a small case series that PTCs may harbor high levels of autologous neutralizing antibodies (15), although that has not been replicated in other studies (8, 75).

KNOWLEDGE GAPS AND UNANSWERED QUESTIONS

Among the top priorities of the HIV field is the search for therapeutic interventions that can lead to sustained ART-free HIV remission (41). Understanding the mechanisms and predictors of post-treatment control would represent a key step toward that goal as PTCs represent a realistic model for the functional cure of HIV infection. Only in the past few years have interest heightened in the study of PTCs and a host of important questions remain unanswered. First, it has become clear that early initiation of ART is not only associated with personal health and public health benefits but may also lower the barrier to HIV remission and post-treatment control. However, the optimal timing of ART during early HIV infection is unknown. It is interesting to note that the vast majority of PTCs in the VISCONTI and CHAMP studies initiated ART during Fiebig stages III-V (6, 7) and that a small treatment interruption study of individuals who initiated ART during Fiebig I did not identify any PTCs as all individuals demonstrated rapid viral rebound (86). While extremely early initiation of ART will limit the extent of HIV reservoir seeding (87), additional research is needed to assess whether a slight delay in ART initiation allows for the further maturation of the HIV-specific immune response that may be important for post-treatment control.

As noted above, there is increasing evidence that PTCs do not appear to mediate HIV suppression through the same CTL and HLA-mediated mechanisms as SCs. While important, the favorable genetic profiles of SCs have not been easily translatable to therapeutics and the elucidation of the mechanisms of control in PTCs may have a greater impact on the design and evaluation of the next generation of HIV therapeutics. Studies of the HIV reservoir in PTCs have revealed the restricted size of the reservoir, including the intact proviral genomes (64). This, however, does not fully explain post-treatment control, especially given our experience in hematopoietic stem cell transplant participants who can dramatically lower their peripheral reservoir size, but are unable to maintain HIV remission (88). Additional studies are needed to assess potential differences in the distribution of infected cell types (7), cellular transcription environment, integration sites, and other factors that could contribute to the maintenance of a "deeper" state of viral latency (89).

Finally, little is known about the clinical implications of post-treatment control. While SCs can maintain low or undetectable viremia in the absence of ART, the ongoing viral replication and immune response in SCs may be associated with adverse consequences, including the progressive loss of CD4+ T cells in some individuals, increased T cell activation and inflammation (90–93). Chronic immune activation and systemic inflammation has been associated with poor clinical outcomes in non-controllers (94–97) but also in SCs, who are reported to have an increased risk of cardiovascular disease (98) and hospitalization (58), although the extent of this risk is still a matter of some uncertainty (99, 100). There is some evidence that PTCs may

not exhibit the same heightened levels of immune activation as SCs (7, 10), but additional studies are needed to confirm these findings and to assess the long-term clinical implications of sustained HIV remission.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

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Have Cells Harboring the HIV Reservoir Been Immunoedited?

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Immunoediting is an important concept in oncology, delineating the mechanisms through which tumors are selected for resistance to immune-mediated elimination. The recent emergence of immunotherapies, such as checkpoint inhibitors, as pillars of cancer therapy has intensified interest in immunoediting as a constraint limiting the efficacy of these approaches. Immunoediting manifests at a number of levels for different cancers, for example through the establishment of immunosuppressive microenvironments within solid tumors. Of particular interest to the current review, selection also occurs at the cellular level; and recent studies have revealed novel mechanisms by which tumor cells acquire intrinsic resistance to immune recognition and elimination. While the selection of escape mutations in viral epitopes by HIV-specific T cells, which is a hallmark of chronic HIV infection, can be considered a form of immunoediting, few studies have considered the possibility that HIV-infected cells themselves may parallel tumors in having differential intrinsic susceptibilities to immune-mediated elimination. Such selection, on the level of an infected cell, may not play a significant role in untreated HIV, where infection is propagated by high levels of cell-free virus produced by cells that quickly succumb to viral cytopathicity. However, it may play an unappreciated role in individuals treated with effective antiretroviral therapy where viral replication is abrogated. In this context, an "HIV reservoir" persists, comprising long-lived infected cells which undergo extensive and dynamic clonal expansion. The ability of these cells to persist in infected individuals has generally been attributed to viral latency, thought to render them invisible to immune recognition, and/or to their compartmentalization in anatomical sites that are poorly accessible to immune effectors. Recent data from *ex vivo* studies have led us to propose that reservoir-harboring cells may additionally have been selected for intrinsic resistance to $CD8^+$ T cells, limiting their elimination even in the context of antigen expression. Here, we draw on knowledge from tumor immunoediting to discuss potential mechanisms by which clones of HIV reservoir-harboring cells may resist elimination by $CD8^+$ T cells. The establishment of such parallels may provide a premise for testing therapeutics designed to sensitize tumor cells to immune-mediated elimination as novel approaches aimed at curing HIV infection.

Keywords: HIV, cancer, latent reservoir, immunoediting, immunotherapy

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INTRODUCTION

The cancer immunoediting hypothesis proposes that the immune system sculpts tumor immunogenicity even as it protects the host against the development of cancer. This occurs through a dynamic process consisting of three stages—elimination, equilibrium, and escape. Tumor elimination is the process through which the cancer immunosurveillance network is assembled, and drives the rapid elimination of tumor cells as they acquire somatic mutations. Equilibrium represents the period of immune-mediated clinical latency that follows the incomplete elimination of potentially cancerous cells, where the immune response and tumor engage in a cycle of tumor cell elimination, followed by selection and outgrowth of mutants escaped from immune pressure. The final stage involves the escape of tumor cells from immune control, resulting in the unrestrained outgrowth of the tumor. Cancer immunoediting was first reported in mouse models of cancer, where immunodeficient mice showed earlier and greater penetrance of carcinogen induction and spontaneous cancer development compared to wild-type mice (1–6). A substantial body of evidence now shows that this process is also prevalent in humans [reviewed in (1, 2, 7)]. Of particular importance, it has been shown that $CD8^+$ T cells play an important role in cancer immunoediting, especially in cancers that acquire resistance to the adaptive immune response (8–10). In this Hypothesis and Theory article, we draw attention to the similarities between immunoediting in cancer and HIV, highlighting established and hypothetical parallels between tumor escape and the persistence of HIV-infected cells, and their potential implications on future applications of HIV cure strategies.

Immunoediting in Cancer Evolution

Over the past several decades, there has been increased appreciation that adaptive and innate immunity can help sculpt the mutational landscape of cell lineages constituting tumors during cancer evolution and progression (3, 9–14), in some cases even before they are macroscopically detectable (15, 16). Observational studies have revealed that when either mice or patients are immunodeficient in adaptive immunity, incidence of certain types of cancer, including viral-induced cancers, increases (17–19). The overall process of how tumors are sculpted by adaptive and innate immune responses is referred to as cancer immunoediting (and less commonly, immune surveillance or immunoselection). While most studies of immunoediting have focused on T cell mediated immunoediting, a growing number of studies provide evidence highlighting the role that Natural Killer (NK) cells may play, particularly for tumor cells that have lost class I major histocompatibility complex (MHC) cell surface presentation (see below) (20–23).

The initial studies proposing the existence of immunoediting were largely drawn from studies of chemically induced mouse tumors in interleukin-2 receptor common subunit and VDJ recombinase (RAG) mutant mice that are immunodeficient in T cells, B cells, and NK cells (15, 16, 24). However, more recent studies evaluating the landscape of the specific mutations carried by individual tumors paired with the host patient HLA alleles provide additional evidence that tumor-specific changes in MHC-mediated antigen presentation affect tumor growth in humans (25, 26). All homeostatic nucleated human cells (except for certain testicular cell types that are immune-privileged) are decorated by class I MHC molecules on the cell surface membrane referred to as HLA. These molecules present proteasome degraded cytosolic 8–11 amino acid peptides to $CD8^+$ cytotoxic T cells (CTLs) for recognition. Briefly, different dendritic cell populations (DCs) that encounter tumor cells can act as antigen presenting cells and present tumor antigens in the context of class II MHC [reviewed in (1, 2, 7)]. This cross-presentation by DCs expands and activates $CD8^+$ cells, as well as $CD4^+$ helper T cells that promote $CD8^+$ cytotoxic T cell expansion.

Class I MHC HLA is encoded by three genes (HLA-A, -B, and -C) and is highly polymorphic. Different allelic combinations of HLA-A, -B, and -C, create significant diversity between individuals as to which antigens can be presented to $CD8^+$ T cells. Typically, early in tumor development, cancer cells retain their HLA, and can be recognized and eliminated by immune cells if they present mutated host proteins (referred to as neoantigens). Additionally, cancer cells may over-express homeostatic antigens found in "normal" tissues (e.g., Mucin I (MUC1), or the HER2 growth factor receptor), that can have varying degrees of effect on central (thymic) tolerance. Recent studies (25, 26) show that, for human tumors paired with their patient host HLA from The Cancer Genome Atlas (TCGA), neoantigens with higher predicted HLA-neoantigen binding affinities, indicative of a higher likelihood of presentation to $CD8^+$ T cells, were significantly more likely to experience mutations that decrease the HLA affinity of the targeted neoantigens. Additionally, these studies revealed that recurrent oncogenic mutations, such as KRAS or BRAF or IDH1 (collectively present on >35% of all solid tumors as well as many hematological tumors), have low predicted HLA-binding affinities. Thus, these paired tumor-host studies provide important new evidence that immunologically invisible human mutations are under an evolutionary selective pressure.

As mentioned above, cancer immunoediting is typically delineated into three stages: elimination, equilibrium and escape (9, 10, 20). Elimination is the first phase, whereby pre-malignant cells are killed by adaptive and innate immune cells patrolling normal tissues. This has been studied in mouse models, where both adaptive (T cell) (4, 8) and innate (NK cell) immunity (27–29) have important roles. For transformed cancer cells that evade elimination, perhaps starting even at the single-cell stage, cancer cell consortia form and enter the equilibrium stage. During the equilibrium stage, adaptive and innate immune cells kill some, but not all, tumor cells, leading to an evolutionary process whereby the epigenetic and somatic mutation landscape of tumor cells is sculpted. Consequently, although tumors may not appear to grow

Abbreviations: CTL, Cytotoxic T lymphocyte; HIV, Human Immunodeficiency Virus; ARV, Antiretrovirals; ART, Antiretroviral therapy; QVOA, Quantitative viral outgrowth assay; PCR, Polymerase chain reaction; TCGA, The Cancer Genome Atlas; TME, tumor microenvironment.

macroscopically, the "mutanome" of cancer clonal lineages that together comprise cancer consortia continuously evolve to promote immune escape. A common model to study this evolutionary process during equilibrium is colorectal cancer, as it is (a) often a relatively slow growing tumor, (b) a subset are hypermutators and have highly elevated mutations rates from DNA mismatch repair or DNA polymerase Delta/Eplison that can be tracked sequentially and (c) there are distinct histopathological stages that occur during its progression (e.g., normal colorectal epithelial crypts, aberrant crypt foci, dysplasia, carcinoma *in situ*, polyps and adenomas, frank carcinoma and metastases). Recent studies evaluating the landscape of tumor mutations during the evolution of colorectal cancers provide evidence that specific Single Nucleotide Variant (for example KRAS), small insertion/deletion (for example APC), and structural variants (e.g., TP53 loss), evolve, both as these lesions remain in equilibrium and also expand during progression (30–34).

Tumor cells that have acquired the pre-requisite mutations necessary to overcome immune pressure during equilibrium then enter the escape stage. The phenotypic changes required to reach this stage rely on a variety of factors, ranging from the geography of the tumor, to whether the cancer is liquid or solid. In solid tumors, an important step for immune escape is the development of an immunosuppressive microenvironment, known as the tumor microenvironment [TME, reviewed here (35, 36)]. This microenvironment is generally characterized by the secretion of immunosuppressive cytokines such as IL-10 and TGF-Beta [reviewed in (37–40)], nutrient scarcity imposed on immune effector cells by the ability of cancer cells to scavenge macronutrients from their environment (41), generation of a hypoxic environment that inhibits tumor infiltration and killing by T cells, B cells, and NK cells (42), and the promotion of a extracellular matrix that both enhances tumor cell growth while inhibiting immune cell penetration (43). While TMEs are not present in liquid cancers, similarities remain in how these cancerous cells escape from elimination, including: (1) the absence of a strong tumor antigen (44, 45), (2) the downregulation/loss of MHC-class I expression levels or co-stimulatory molecules (46, 47), (3) upregulation of exhaustion markers [e.g., CTLA-4, PD-L1, (45, 48)] (4) or the development of apoptosis resistant phenotypes due to increased expression of pro-survival proteins [e.g., BCL-2, MYC, STAT3, and 5, reviewed here (43)].

Interestingly, some of these characteristics that facilitate the escape of liquid cancers are similar to those seen in people living with HIV—Nef downregulation of MHC-I leads to low antigenicity of infected cells, and viral epitopes rapidly mutate in response to immune pressure, and escape immune recognition. Furthermore, our recent work has highlighted the inherent resistance of HIV-infected cells to immune-mediated elimination during suppressive anti-retroviral (ARV) therapy (49, 50). The following sections will highlight the potential mechanisms through which these phenotypes may arise, and discuss how the immunoediting of HIV-infected cells may occur.

Treated vs. Untreated HIV Infection As Distinct Arenas for Immunoediting

In the absence of ARV therapy, HIV infections are characterized by three stages—acute infection, chronic infection, and AIDS. Acute infection encompasses the first 4–8 weeks of infection, and is characterized by rapidly rising viral loads, often to $>10^6$ copies/mL, and steep declines in the numbers of $CD4^+$ T cells, both in circulation and in tissues (51). At ∼6 weeks post-infection, robust HIV-specific $CD8^+$ T cell responses develop that capably suppress HIV viremia to a set point that is typically 2–3 logs below peak (52, 53). While $CD8^+$ T cells may control viral replication through a number of mechanisms (54–56), a key mode of action is the direct recognition and elimination of infected cells by $CD8^+$ CTLs (57–60). This viral load set point is the primary characteristic of the second stage of HIV infection, known as the chronic phase, and represents the equilibrium between ongoing viral replication, viral immune evasion, and elimination of infected cells by the host immune response [reviewed in (61)]. Individuals with higher viral load set points progress more rapidly than individuals with lower set points to the final stage of HIV infection (62); where HIV eventually overcomes immune pressure in the large majority of individuals, leading to the onset of AIDS (**Figure 1**).

The current treatment for HIV is antiretroviral therapy (ART), which can durably suppress viremia to levels that are undetectable by clinical tests, and halt progression to AIDS for as long as treatment is maintained. However, using ultra-sensitive PCR methods, it has been shown that low-levels of virus production do persist in the majority of individuals (63), and are not reduced even if ART regimens are intensified (64, 65). Additionally, anywhere from 4 to 10% of people on ART may display levels of persistent viremia that are detectable by standard assays (50–500 copies/mL), even in the absence of drug resistance (66). Although there is strong evidence that ongoing cycles of viral replication do not occur during ART (67–69), uncertainty remains as to why HIV-specific $CD8^+$ T cell responses do not seem to eliminate all infected cells that are producing viral particles. Lastly, upon ART cessation, viral loads rapidly rebound within a few weeks in the majority of individuals (70). This occurs despite the pre-existence of robust HIV-specific T cell responses which, though diminished in magnitude relative to untreated infection, are sustained at readily-detectable levels in most ART-suppressed individuals (71–74). While these studies seemingly highlight the limitations of $CD8^+$ T cells in controlling and eliminating HIV-infections, multiple studies have unambiguously established the importance of $CD8^+$ T cells in viral suppression (52, 57, 58, 75–78). Indeed, in non-human primate studies, $CD8^+$ T cells are necessary for maintaining viral suppression of SIV during the course of both natural infections and ART (54, 79). These contrasting results raise important questions about why certain HIV-infected cells are efficiently eliminated by HIV-specific $CD8^+$ T cell responses, while others persist, and may even continue generating viral particles during ART. While viral latency is known to play a critical role in HIV persistence, we will draw on insights from tumor immunoediting to propose additional cell-intrinsic mechanisms by which HIV

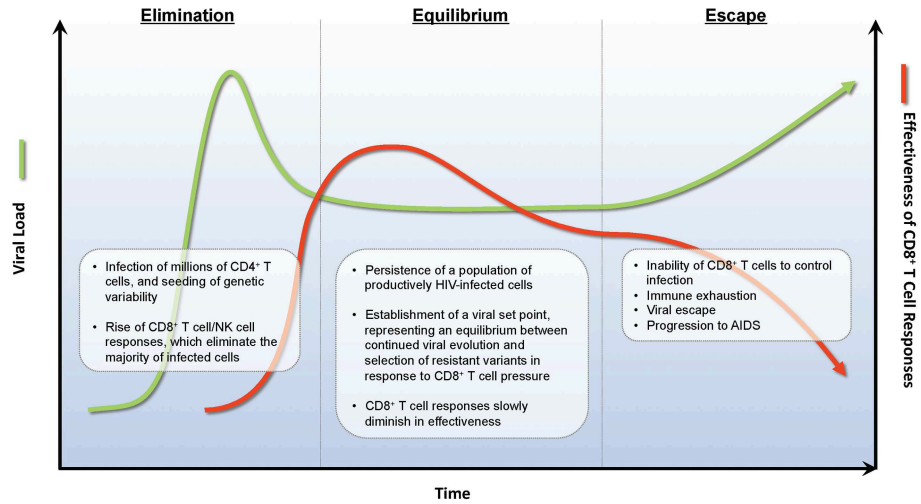

FIGURE 1 | Immunoediting during natural infection. During the acute phase of infection, HIV rapidly expands infecting new target $CD4^+$ T cells. Approximately 2 weeks post-infection, HIV-specific $CD8^+$ T cell responses develop and eliminate many infected $CD4^+$ T cells decreasing viral burden by ~1 × 10^{2-3} RNA copies/ml of plasma. A viral set point is reached when virus replication and $CD8^+$ T cell elimination of infected cells reaches an equilibrium. During the equilibrium phase, ongoing rounds of viral replication and $CD8^+$ T cell elimination provides evolutionary pressure to select for viral variants that are not recognized by $CD8^+$ T cell responses. A combination of viral escape variants and $CD8^+$ T cell exhaustion eventually leads to viral escape and progression to AIDS.

reservoir-harboring cells may resist elimination by $CD8^+$ T cells, and thus pose the question: have cells harboring the HIV reservoir been immunoedited?

IMMUNOEDITING OF THE VIRUS DURING THE COURSE OF UNTREATED HIV INFECTIONS

A critical distinction in our discussion is between immunoediting of the virus during the course of untreated infections (which is a well-characterized phenomenon, although not typically branded as immunoediting), and the more novel idea that immunoediting may also occur on the level of reservoir-harboring cell physiology, particularly in the context of ART. The current section will focus on the former, which largely consists of the interplay between viral evolution and escape in response to $CD8^+$ T cell pressure. While HIV infections are generally established by one to five "transmitter/founder" viruses (59, 80, 81), the high error rate of HIV reverse transcriptase (~1 point mutation per reverse transcription event, and a recombination frequency of ~2.8 crossovers) gives rise to a vast number of HIV "quasispecies," each with varying degrees of replicative fitness (82–87). These mutations often incur a fitness penalty on the virus, as evidenced by the fairly homogenous makeup of viral sequences prior to $CD8^+$ T cell pressure, despite the high mutation rate of HIV reverse transcriptase (88–91), and the presence of secondary compensatory mutations that arise in response. Despite these fitness costs, multiple lines of evidence have shown the importance of these mutations for viral replication, as they modify epitopes targeted by the host immune response and allow subdominant viral quasispecies to escape from immune recognition (75, 92–95). This mechanism of immune escape is well-documented in many longitudinal studies of HIV-infected individuals, where dominant $CD8^+$ T cell responses can be matched to changes in the amino-acid sequence of targeted viral epitopes, leading to poor HLA presentation and the outgrowth of new HIV quasispecies (57, 96). Such escape on the level of viral epitope recognition is paralleled by the phenomenon of "antigen loss" in tumors—for example, the loss of MART-1 antigen in melanoma patients after adoptive transfer of MART-1 specific T cells (97, 98), or the loss of CD19 following CD19 targeted CAR T cell therapy for acute myeloid leukemia (99) [reviewed in (100)]. Thus, immunoediting on the level of viral sequence diversity has been well-established in HIV infection, and this "immune escape" is analogous to the phenomenon of tumor "antigen loss." Antigen loss, however, is just one facet of tumor immunoediting, inspiring us to consider whether other mechanisms may also have parallels in HIV.

DOES IMMUNOEDITING AT THE LEVEL OF INFECTED CELLS OCCUR IN INDIVIDUALS ON ART?

Immunoediting in untreated HIV infections involves the equilibrium between $CD8^+$ T cell responses and HIV, followed by the eventual escape of HIV from $CD8^+$ T cell killing in the majority of individuals. However, viral replication to a degree that allows for evolution does not occur during suppressive antiretroviral therapy (67–69), preventing the development of new HIV escape mutations in response to $CD8^+$ T cell mediated immune pressure. Instead, infected cells are thought to persist and evade the immune response during ART by hiding in a non-immunogenic quiescent, or latent, state. Infected cells that do not undergo this transition are largely eliminated: HIV DNA levels

experience an 86% decline within the first year following ART initiation before stabilizing (101–103), while HIV RNA levels in the plasma drop precipitously over the first 7–10 days post-ART, with a half-life of 6 h, followed by a second phase of slower viral decay with a half-life of 14 days (104, 105). Infected cells that survive this selection and persist are remarkably stable, with a minimal half-life of at least 44 months as measured by total or intact HIV DNA, or by quantitative viral outgrowth assays (QVOAs) assessing the number of cells infected with replication competent proviruses (106–108). This suggests that the persistent reservoir would require at least 73 years to naturally decay in the majority of people living with HIV.

It is important to note that although HIV-specific $CD8^+$ T cell responses decay sharply upon ARV initiation, in parallel with frequencies of HIV-infected cells, they are still readily detectable by *ex vivo* assays (ex. ELISPOT) in the large majority of individuals on long-term suppressive ART (71). The main paradigm for how infected cells persist during ART, despite the existence of $CD8^+$ T cell responses, is that the reservoir "hides" from the immune system; this occurs primarily by maintaining a state of viral latency, but also through sequestration in anatomical sites that are poorly accessible to $CD8^+$ T cells, such as lymph node follicles (109, 110). While these are indisputably important mechanisms of persistence, we propose that interactions between reservoir-harboring cells and $CD8^+$ T cells are also likely to occur at some frequency in individuals on long-term ART (see Is Immune Selection Pressure Exerted on Infected Cell Clones During ART?, below), providing the potential for the shaping of the landscape of reservoir harboring cells in ways which may parallel tumor immunoediting.

Immunoediting is an evolutionary process, and thus will occur over time when the following three requirements are met: (i) reproduction, (ii) selective pressure, and (iii) heritable variation (14). The mechanisms by which these criteria are met in tumor cells are described above. Here, we make the case that these ingredients are also present in the persistent HIV reservoir, defined as follows: (i) reproduction—clonal expansion of HIV reservoir-harboring cells, (ii) selective pressure—ongoing immune recognition and clearance of certain reservoir-harboring cells, and (iii) heritable variation—genetic or epigenetic features of reservoir-harboring cells that confer differential susceptibility to immune recognition and clearance.

Reproduction—Expansion of Clones of HIV-Infected Cells During ART

A major hallmark of cancer is the ability of cancer cells to promote continued expansion, even in a nutrient scarce environment, or lack of external stimuli. These hallmarks are a result of mutations in oncogenes (i.e., *MYC)*, which promote growth, or tumor suppressor genes (i.e., *p53*), which may inhibit cell division, repair DNA damage, or induce apoptosis if cellular functions become deregulated. In liquid cancers, the deregulation of c-*myc*—e.g., translocation from chromosome 8–14 in Burkitt's lymphoma (111)—generates abnormally high levels of MYC expression, resulting in enhanced cell cycle progression and cell growth (112). Conversely, p53 induces cell cycle arrest and apoptosis in the presence of cellular stress signals such as nutrient deprivation or DNA damage, and mutation of this gene allows cancer cells to continually proliferate under otherwise genotoxic conditions (113). Together, these gene mutations allow cancer cells to engage in constant clonal proliferation.

In contrast to cancer cells, the HIV-reservoir is thought to largely reside in long-lived resting memory $CD4^+$ T cells, where the expansion and/or division of these cells are generally driven by either recognition of cognate antigen, or cytokine-induced homeostatic proliferation (114). Until recently, it was generally thought that an HIV-infected cell would be incapable of expanding in numbers, as cell division was thought to be inextricably linked to viral expression—which in turn, it was thought, would lead to death through viral cytopathic effects or immune-mediated elimination (102, 103). However, multiple studies have since demonstrated the ability of infected cells to proliferate *in vitro*. Hosmane et al. observed in QVOAs (115) that increasing numbers of cells producing replication competent viruses were found as $CD4^+$ T cells were subjected to additional rounds of activation by mitogens (116), suggesting that cell activation and division are not intrinsically linked with reactivation of latent proviruses. Furthermore, a study by Bui et al. observed sustained levels of HIV RNA in a culture supernatant over 21 days, following activation with PMA/ionomycin, including sequences matched to replication competent viruses found in QVOAs. As these assays were performed in the presence of ARVs, these results demonstrate that production of replication competent viruses in reservoir-harboring cells does not necessarily lead to cell death (117).

The fact that HIV-infected $CD4^+$ T cells can clonally expand *in vivo* was unambiguously established by the observation that 40–60% of all cells harboring proviruses had genomic integration sites that were identical to those of at least one other infected cell (118–121). Since HIV integrates into the genome without targeting specific sequences, it is extraordinarily improbable that the same integration site would occur independently in two separate cells, indicating instead that these cells clonally expanded from a common infected-cell ancestor. As the integration site loop amplification assay used to determine proviral integration sites (120) only amplifies a small portion of the 5′ and 3′ ends of the provirus, it was unclear whether these expanded clones contained intact proviruses, vs. the defective proviruses that make up the large majority of proviruses in individuals on long-term ART (ex. containing deletions, hypermutations, or other mutations that render them replication incompetent) (122, 123). It thus initially seemed that a simple potential explanation for how these cells could divide, without dying from cytopathic effects or immune elimination, was that they may contain defective proviruses—a subset of which are incapable of expressing virus or viral antigens (124). However, multiple studies have since provided evidence indicating that a subset of these clonally expanded populations can harbor intact, replication competent proviruses (125–128). These studies utilized QVOAs to isolate viral RNA from single viruses, and then assessed their clonality on the basis of viral sequences. It was inferred by phylogenetic and statistical approaches that these clonal proviruses almost certainly arose as the result of clonal

expansion of the host cell, as opposed to the seeding of multiple infections by a single massive infection event.

More recently, a novel assay (matched integration site and proviral sequencing, MIP-seq) was developed to determine near full-length proviral sequences and the corresponding integration site simultaneously (129). This assay utilized a limiting dilution of proviral templates, followed by multiple displacement amplification to generate multiple copies of the proviral template and surrounding DNA, which could then be used for both full-length sequencing and integration site analysis. This approach definitively demonstrated that clonally expanded cells could indeed harbor intact proviruses (129). Moreover, the authors observed that these intact proviral sequences matched the sequences of viruses that had grown out in previous QVOAs from these same individuals. Thus, clonal expansion provides a mechanism through which the "replication with heritability" criterion of evolution may be fulfilled, accounting for the expansion of certain infected cell clones while others are eliminated.

Is Immune Selection Pressure Exerted on Infected Cell Clones During ART?

Clonal expansion of HIV reservoir-harboring cells occurs in a setting where the overall size of the reservoir is relatively stable (106, 108). This implies that the death or elimination of some infected cells must occur on an ongoing basis, to counterbalance clonal expansion. A recent study examined this, by analyzing clonal composition of replication competent reservoir viruses (from viral outgrowth assays) longitudinally in 8 study participants. Wang *et al* found that while most of the clonal proviral populations were found at each time point throughout the course of the study, their proportional makeup of the total population differed at each time point (130). The authors observed a similar variation in the makeup of HIV clones found in the plasma of these participants, and concluded that populations of infected cell clones likely persist, but change in proportion relative to each other ("wax and wane") over time. There are three possible and non-mutually exclusive explanations of these population dynamics: (i) stochastic effects—either random fluctuations of *in vivo* prevalence or in sampling, (ii) driven by the physiology of the $CD4^+$ T cells themselves—ex. Expansion of a given clone driven by exposure to its cognate antigen, and iii) driven by fitness differences with respect to a selective pressure imposed on the infected cell.

In the oncology setting, a key determinant of whether or not a cancer cell clone will be subject to immune selection pressure is whether it possesses neoantigens that can be recognized as foreign by the immune system. In the case of an HIV reservoir-harboring clone, foreign antigens exist in the form of provirus-encoded viral genes. Moreover, these viral gene products are known to be immunogenic—in particular Gag, Pol, and Nef—and, in untreated infection, stimulate high magnitude T cell responses in the majority of infected individuals. In considering whether a reservoir-harboring clone is subject to immune selection, the key question is therefore the degree to which these gene products are expressed in an individual on ART.

In the large majority of individuals, cell-associated HIV RNA remains detectable at relatively low, but stable, levels in *ex vivo* $CD4^+$ T cells even after years of suppressive ART (131). While the presence of viral RNA cannot be equated with protein expression, given that blocks to translation can exist at various levels, including splicing (132), and nuclear export (133), the transcriptional level data also are not counter-indicative of the possibility that antigen expression may occur at some level in individuals on ART. The direct assessment of HIV antigen expression in individuals on ART is limited by the much poorer sensitivity of protein vs. RNA detection assays, given the low frequency of infected cells. However, some studies have reported the detection of HIV proteins in *ex vivo* T cells from individuals on long-term ART (134). One way to infer whether ongoing interactions occur between the immune system and HIV in individuals on ART is to study the decay of HIV-specific immune responses in this context.

The maintenance of effector immune responses is dependent upon the presence of antigen (135–138). In addition to being a general tenet of immunology, this is supported by several lines of evidence in HIV. The first comes from the study of rare individuals who, without ongoing ARV therapy, exhibit extraordinary control over HIV infection as defined by undetectable plasma viremia by a single copy assay, extremely low to undetectable HIV DNA levels, and difficult to isolate replication-competent virus (139). These extremely low to absent levels of HIV were associated with the loss of HIV-specific antibody responses (sero-reversion), and with low to undetectable HIV-specific $CD8^+$ or $CD4^+$ IFN-γ responses in *ex vivo* PBMCs. *In vitro* stimulation did however result in the proliferation of HIV-specific T cells and subsequent antiviral activity, suggesting that cells had been present in a memory state. In contrast, while ART-treatment initially results in the decay of HIV-specific T cell responses with a half-life of 38.8 weeks for ∼2 years (140), these then appear to stabilize, as HIV-specific T cell responses are readily detectable in *ex vivo* assays (ex. IFN-γ ELISPOT) in the large majority of individuals—even those who have been on treatment for over a decade (71). Similarly, while HIV-specific antibody responses wane upon initiation of therapy, ART-treated individuals do not sero-revert [with the exception of some individuals who initiate therapy very early (141)]. The second line of evidence for ongoing interactions between the immune system and HIV comes from the observation that the magnitudes of HIV-specific Ab responses correlate directly with frequencies of HIV-infected cells (HIV DNA) in individuals on long-term ART (142). Similarly, we have observed that T cell responses directed against the early HIV gene product Nef correlated directly with HIV DNA in this cohort (with Ab and T cell responses also correlating with each other) (71). While additional longitudinal studies are needed, these data are consistent with an ongoing interaction between HIV-infected cells and the immune system, including $CD8^+$ T cells. Finally, it is interesting to note that the two individuals who achieved long-term remission of HIV through bone marrow transplantation—the "Berlin patient" and the "London patient"—also sero-reverted, and the London patient lost HIV-specific T cell responses (143, 144), though the ablation of the recipient

immune systems does complicate the applicability of these cases to the current argument. Thus, while additional study is needed, we propose that the preponderance of evidence supports some level of ongoing interaction between the immune system and HIV-infected cells in individuals on long-term ART. If this occurs, it would satisfy the second criteria for the evolutionary process of immunoediting to occur—namely, selective pressure.

How Might Reservoir-Harboring Clones Possess Heritable Variation in Susceptibility to Immune Clearance?

In the tumor immunoediting model, heritable variations generally arise during the cell replication cycle due to the failure of DNA mismatch repair enzymes to fix mutations, or when the cell fails to undergo apoptosis following a chromosomal break or translocation. Mutations that confer a selective advantage—through enhanced cell proliferation, resistance to apoptosis, and/or resistance to immune mediated elimination—are passed on to progeny cells, which will continue accumulating mutations that improve their survival or proliferative capabilities. Two major pathways that are mutated in many cancers, are those involved in MHC-I expression and BCL-2 overexpression, paralleling observations in reservoir harboring cells: the HIV protein Nef downregulates MHC-I expression, while Tat can upregulate BCL-2 expression. Here, we propose three potential sources of heterogeneity in the susceptibility of a given reservoir-harboring cell to immune-mediated elimination in ART-treated individuals: (i) virus intrinsic factors, (ii) host cell intrinsic factors (iii), and proviral integration sites.

Virus Intrinsic Sources of Heterogeneity in Susceptibility to CTL

Virus intrinsic mechanisms include variation in targeted epitopes that affect sensitivity to $CD8^+$ T cell recognition, as discussed in section Immunoediting of the Virus During the Course of Untreated HIV Infections (above), as well as variable activity of viral immune evasion activity. As an example of the latter, it has been recently demonstrated that viruses reactivated from the reservoirs of ARV-treated individuals can vary greatly in their abilities to downregulate HLA-C through the actions of HIV-Nef (145). Of the three mechanisms of heterogeneity proposed here, these virus intrinsic mechanisms are the most well-established, and thus will not be a principle focus of this Hypothesis and Theory article.

Host Cell Intrinsic Sources of Heterogeneity in Susceptibility to CTL

With respect to host cell intrinsic mechanisms, it is known that various $CD4^+$ T cell subsets display natural heterogeneity in their intrinsic susceptibility to $CD8^+$ T cell-mediated killing. Effector and transitional memory $CD4^+$ T cells are more susceptible to elimination than central memory $CD4^+$ T cells (146), where the majority of the latent reservoir is thought to reside in (147). Another study observed that the $CD4^+$ T cells of elite controllers were intrinsically more susceptible to $CD8^+$ T cell mediated elimination than those from progressors, suggesting that $CD4^+$ T cell sensitivity to killing may play a role in disease outcomes (148). Although the mechanisms underlying this heterogeneity within the $CD4^+$ compartment are not well-understood, multiple mechanisms of resistance are known in other cell types. CTL protect themselves from this killing process by inactivating perforin through Cathepsin B or CD107a expression (149, 150). Similarly, macrophages and dendritic cells avoid being killed by expressing serine protease inhibitors that degrade granzyme B (151–154). BCL-2 can also confer resistance to CTL further downstream in both the perforin/granzyme B and FasL/Fas pathways by sequestering Bid, thus preventing mitochondrial membrane permeabilization by tBid (155, 156). In recent work, we have identified one mechanism by which HIV reservoir harboring cells are disproportionately resistant to CTL killing: through the over-expression of the prosurvival factor BCL-2 (50). Interestingly, previous studies have also described a disparate role for BCL-2 in the survival of reservoir-harboring cells through prevention of apoptosis mediated through Casp8p41, an HIV-protease cleavage product of procaspase-8 (157–159). While this is a fairly nascent area of research, barring the null hypothesis—which is that all $CD4^+$ T cells are precisely equal in their susceptible to killing—it stands to reason that any heritable variation in susceptibility to CTL will influence which infected cells survive to form the persistent reservoir, and thus the subsequent sensitivity of the reservoir to immune-mediated clearance.

Proviral Integration as a Potential Source of Heterogeneity in Susceptibility to CTL

Likely the most provocative of our proposed sources of heterogeneity in sensitivity to CTL is the potential role of proviral integration sites. As a retrovirus, a defining step in the lifecycle of HIV is integration of the proviral DNA into the host genomic DNA. After reverse transcription generates a double stranded cDNA of the viral RNA, the reverse transcription product is shuttled into the nucleus via the nuclear pore complex as part of a pre-integration complex (PIC). Once inside the nucleus, Integrase (IN) resects 2 nucleotides from both $3'$ ends of the viral DNA molecule, binds the target genomic DNA, then makes a 5-nucleotide staggered cut in the host DNA allowing for transfer of the viral DNA onto the host genome where host enzymes, DNA polymerase and ligase, fill in the gaps and irreversibly ligate the two DNA strands together—now designated the HIV provirus [reviewed in (160)]. While HIV integration occurs across the human genome, the chromosomal location of integration is not completely random. *In vitro* studies have shown that HIV preferentially integrates into actively transcribed genes, gene-rich regions, intronic regions, and largely avoids promoter regions (161). Preferences for these sites are largely mediated by cellular cofactors that bind IN and possess chaperone-like and chromatin tethering activity, most notably, the transcriptional activator LEDGF/p75 (162, 163). While LEDGF/p75 plays an important role in guiding chromosomal integration, it is not a necessary factor as loss of LEDGF/p75 showed no decrease in the overall frequency of HIV integration, but instead resulted in an altered proviral landscape (164).

In vivo studies of patients on long-term ART corroborated *in vitro* findings with a preference for HIV integration into transcriptionally active genes, and principally within introns (165–167). *In vivo* studies have also identified large, clonally expanded populations of HIV-infected cells with integration sites within genes controlling cell growth and division (120, 121). Of note, multiple patients have been identified with integrations in the BACH2, MKL2, and STAT5B genes. *In vitro* infections of primary cells demonstrated integrations throughout BACH2 and MKL2 with equal distribution of chromosomal orientation. However, large clonally expanded proviral sequences from patients on long-term ART were all in the same orientation as gene transcription and found only within a specific subset of introns (121). While the exact mechanism of survival is not completely understood, it is believed that BACH2 and MKL2 gene expression may be driven by the HIV LTR promoter [reviewed in (161)]. The existence of these clonal integrations within genes associated with cell growth in patients on long-term ART strongly suggests a role in maintaining the persistent reservoir through the induction of clonal expansion. However, to our knowledge, there are currently no studies extensively evaluating the impact that the site of HIV integration may have on maintaining the persistent reservoir by providing a mechanism of resistance to immune recognition and clearance.

Our hypothesis that HIV proviral integration sites may alter susceptibility of target cells to CTL recognition and elimination was inspired by findings related to immunoediting in cancer. Immunotherapies have recently achieved remarkable success in the treatment of certain types of cancer, but exhibit variability in responses across patients (168). A recent study of patients undergoing anti-PD-1 therapy (pembrolizumab) for metastatic melanoma who experienced cancer relapse after tumor regression, found that a majority of relapsing cancer cells contained somatic mutations in genes associated with interferon receptor signaling (JAK1 and JAK2) or antigen presentation (B2M) (169). These cancer cells were therefore less responsive to IFN-γ or had reduced MHC-I surface expression, leading to escape from immune-mediated control. Additionally, a number of groups have employed high-throughput CRISPR screens to identify genes controlling susceptibility/resistance to immune clearance (170, 171). Using a large-scale CRISPR screen of a melanoma cell line, Patel et al. found that disruptions in antigen processing/presentation and IFN-γ signaling pathways resulted in decreased $CD8^+$ T cell effector functions (170). The top hits identified in the CRISPR screen were compared back to the TCGA database where it was demonstrated that identified mutations in these genes naturally occur in human cancers. Thus, the acquisition of resistance to CTLs by tumors can underlie poor responses to immunotherapy.

Integration of the HIV genome into cellular genes has parallels with cancer-induced mutations or CRISPR-mediated disruptions, leading us to posit that HIV integration into genes essential for immune recognition and signaling could reduce $CD8^+$ T cell killing of those cells, thereby resulting in an immunoedited subset of survivor cells enriched for integrations in those genes. A few important differences, however, exist between CRISPR-mediated gene disruptions and those caused by HIV proviral integration. First, CRISPR gRNA libraries are developed to specifically target exonic regions, resulting in loss-of-function mutations. As discussed previously, the vast majority (93–96%) of HIV integrations occur within introns (165–167). The impact of a ∼9 kb intronic insertion containing an LTR promoter, or of a truncated defective provirus, depends upon a number of factors, and could plausibly increase, decrease, or not at all impact gene expression and/or protein function. Second, HIV only integrates into a single locus of a given gene whereas CRISPR-mediated cleavages typically disrupt both alleles of the target gene. Therefore, HIV integration into a single allele may not impact overall protein function. However, a number of genes exhibit haplo-insufficiency, whereby a single copy of the gene product is not sufficient to support normal gene function, and thus disruption in a single allele may disrupt normal gene function; either wholly or on a nuanced level [reviewed in (172)].

While it is possible that the site of HIV integration may impact the susceptibility of an individual cell to recognition and/or elimination by $CD8^+$ T cells—thereby providing a means of immunoediting—further research is needed to determine if this is indeed a genuine HIV-induced survival mechanism. There are quite daunting challenges involved in testing this hypothesis: (i) The fact that most proviral integrations in ARV-treated individuals are associated with defective proviruses—many of which are non-antigenic—comprise a source of "noise," since only the minority of antigen-expression competent proviruses would potentially be subject to immune selection. Thus, bulk integration site analysis would be expected to miss any selection for integration sites that affect immune susceptibility. (ii) There is extensive complexity inherent in both the vast landscape of potential unique integration sites across the genome, and the divergent impacts that any potential integration could have in terms of gain/loss of function, or more exotic effects such as the generation of novel chimeric proteins (173). This will likely make it much more difficult to discern patterns than simple CRISPR loss of function mutations. (iii) Any selection on the level of integration sites would occur on the backdrop of differential susceptibility to CTL on virus- or host-cell intrinsic levels. As a simple example, an infected cell with an integrated provirus that contains escape mutations to autologous $CD8^+$ T cell responses would be exempt from any putative selection on the level of integration sites, and thus would confound analysis if not accounted for. Despite these challenges, the question of whether or not integration sites affect immune susceptibility may be addressable if one were to effectively harness novel approaches to obtaining integration sites in conjunction with whole provirus sequences; and to apply sophisticated analytical approaches. Inspiration for how this might be approached can be drawn from the study of cancer immunotherapy resistance—ex. the TIDE (Tumor Immune Dysfunction and Exclusion) computational framework, which draws on transcriptomic signatures from 33,000 samples taken across 189 studies to predict immune checkpoint blockade responses and derive insights into immunotherapy resistance mechanisms (174). While the outcomes of such efforts in the setting of HIV may indeed be to find that integration sites

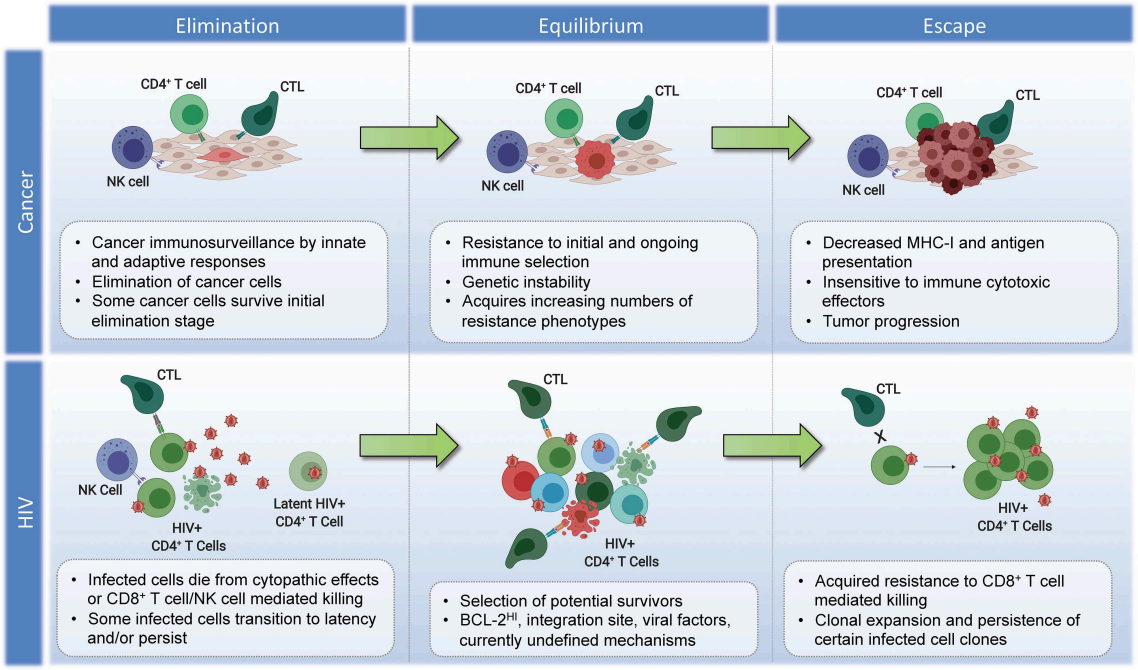

FIGURE 2 | Parallels in immunoediting: comparing cancer and HIV during suppressive ART. Cancer–Elimination: Innate and adaptive immune cells work together to destroy developing tumors before they become clinically apparent, which play critical roles in cancer immunosurveillance. Highly antigenic tumor cells are recognized and eliminated by increased antigen presentation and IFN-γ, NKG2D, TNF, IL-12, TRAIL, Perforin, and Granzymes. Equilibrium: Tumor cells that survive elimination may enter the equilibrium phase. T cells, IL-12, and IFN-γ work in tandem to maintain the tumor cells in a state of functional dormancy. Tumor cells are in a state of genetic instability, and acquire an ever-increasing number of mutations to resist immune pressure. Escape: Tumor cells surviving the equilibrium phase of the cancer immunoediting process enter the escape phase, where tumor growth is no longer blocked by immunity. Tumor cell evasion generally occurs in cases with poor antigen presentation, and increased tumor-derived immunosuppressive cytokines, ligands, and inhibitors of T cell responses (see section Immunoediting in Cancer Evolution). Tumor cells escaped from immune pressure can grow unchecked, resulting in clinically apparent disease progression. HIV–Elimination: Seeding of millions of infected cells, each with a unique viral and host cell signature. The majority of infected cells die from viral cytopathicity or immune-mediated elimination following ART initiation. Some infected cells persist. Equilibrium: low-level/episodic antigen presentation allows for ongoing selection of infected cells. Some infected cell clones are eliminated, while others persist and expand. The overall number of infected cells remains stable. Escape: Expansion of infected cell clones with characteristics that enhance their resistance to immune recognition and/or elimination.

have no bearing on susceptibility to CTL, the alternative result would both have important implications for efforts to cure HIV infection, and would comprise a potential source of fundamental immunological insights—with the potential to cross-fertilize our understanding of cancer immunoediting. The proposed parallels between the immunoediting of tumors and the persistent HIV reservoir are summarized in **Figure 2**.

IMPLICATIONS OF A PERSISTENT RESERVOIR THAT HAS BEEN IMMUNOEDITED

The potential ongoing selection of certain infected cell populations *in vivo* during suppressive ART has many implications for current cure approaches, and may help explain the differential outcomes of these strategies *in vitro* vs. *in vivo*. One particularly prevalent approach, termed "kick-and-kill," combines latency reversing agents to initiate viral transcription, ARVs to prevent viral spread, and effectors to eliminate reactivated virus-harboring cells. While applications of kick-and-kill initially had shown great promise in primary cell models of latency (175), these approaches have, thus far, not measurably reduced the latent reservoir in multiple clinical trials (176–182). Multiple studies have also attempted to apply kick-and-kill approaches in further *in vitro* or *ex vivo* models, but have not definitively shown reductions in the natural, replication competent reservoir (49, 179, 183, 184), suggesting that there are intrinsic differences in susceptibility to $CD8^+$ T cell killing between natural and model reservoirs.

One possibility is that the remaining infected cells comprising the latent reservoir may be adapted to survive the host immune response, as our group has provided evidence that cells harboring the latent reservoir may be intrinsically resistant to $CD8^+$ T cell killing (49). We combined maximal T cell activating agents, such as stimulation using anti-CD3/CD28 antibodies or PMA/ionomycin, with autologous HIV-specific $CD8^+$ T cell clones targeting non-escaped epitopes, and still failed to detect decreases in the number of replication competent proviruses by QVOA. We then harvested the replication competent proviruses that grew out in the QVOA, which are individual clonal lineages due to the limiting dilutions utilized in QVOAs,

and super-infected activated $CD4^+$ T cells from the same donor. We then co-cultured these newly infected cells to the same HIV-specific $CD8^+$ T cell clones as before, and observed elimination of nearly all the infected cells. These contrasting results of efficient $CD8^+$ T cell elimination of infected cells during productive infections, but inability in eliminating latently infected cells, suggests that there likely are host-cell associated factors that impact survival of latently infected cells.

Drawing from the tumor immunoediting literature, we identified overexpression of the pro-survival protein BCL-2 as one potential mechanism of resistance—which can act to antagonize perforin/granzyme killing by sequestering truncated BH3-only domain members of the BCL-2 family (185). Using cells from individuals on long-term ART, we observed that reactivated HIV reservoir-harboring cells from *ex vivo* $CD4^+$ T cells over-expressed BCL-2 relative to uninfected cells (50). In contrast, we did not observe over-expression in *ex vivo* HIV infected cells from ART-naïve individuals—suggesting that this was a unique feature of long-term reservoirs. The addition of the BCL-2 antagonist ABT-199 to combinations of HIV-specific $CD8^+$ T cells and latency reversal agents resulted in partial eliminations of *ex vivo* reservoirs from ARV-treated individuals. We propose that these results comprise proof-of-principle for the idea that reservoir-harboring cells may be selected for resistance to $CD8^+$ T cells, but would suggest that BCL-2 over-expression may be just one of many mechanisms yet to be discovered.

Other long-term implications of the potential immunoediting of persistently HIV-infected cells during suppressive ART are unclear. In a paired submission to this same issue, we discuss in detail a model of virus- and host-coordinated immunoediting of a retrovirus that causes cancer: adult T cell leukemia/lymphoma arises in ∼5% of individuals living with the Human T cell leukemia virus type 1 (HTLV-1), although the development of malignancy can take 40–50 years (186, 187). The HTLV-1 specific immune response acts in concert with cancer immunosurveillance, driving the proliferation of immortalized immune-evading infected clones with identical integration sites that may acquire properties through years of equilibrium that can drive malignancy. Virus- and host-coordinated immunoediting sculpts the selection of a single clonal population to become malignant after decades of latency and clonal expansion (188). Although HIV does not persist through the classical escape phase and is not known to cause T cell malignancy, the immunoediting of HIV-infected cells that persist through ART may drive the selection of clonal populations of cells arising from a single integration site, which persist indefinitely. We argue that this persistence may represent the escape phase for people living with HIV on ART, particularly for clonally expanded cells harboring replication competent HIV that remain refractory to immunosurveillance and survive for years. The fate of these cells remains unknown, and understanding the mechanisms of their survival will ultimately inform their capacity to be purged.

SUMMARY

The recent revolution in cancer immunotherapy has underscored the potential for the human immune system to combat tumors, and shone a spotlight on the diverse mechanisms by which cancers can acquire cell-intrinsic immune resistance. Using sophisticated Omics approaches, and cutting-edge technologies, it has been revealed that both the genetic and epigenetic features of a given tumor cell can influence its intrinsic sensitivity to immune recognition and elimination. This variation serves as the basis for an evolutionary process known as clonal selection, which leads to the escape of tumors that have been immunoedited. Some mechanisms of immunoediting may be therapeutically targetable—e.g., IFN-γ treatment to augment antigen processing and presentation, or PD-L1 blockade for tumors that overexpress this co-inhibitory ligand (189). In contrast, the field of HIV persistence has generally not considered the idea that reservoir-harboring cells themselves may differ intrinsically in their susceptibilities to CTL, focusing instead on the roles of virus expression/latency, and on aspects of CTL functionality. Here, we have attempted to build a case for the potential role of cell-intrinsic immunoediting in the persistence of the HIV reservoir; including preliminary evidence supporting this model, suggested mechanisms for how this may arise, and a discussion of how this theory can be further evaluated. In moving forward, we propose drawing on the concepts, technologies, and methodologies that have been developed to study tumor clonal section and immunoediting to accelerate progress toward understanding the nature of HIV persistence, and how this may be overcome to cure infection.

DATA AVAILABILITY

No datasets were generated or analyzed for this study.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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A Natural Impact: NK Cells at the Intersection of Cancer and HIV Disease

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Despite efficient suppression of plasma viremia in people living with HIV (PLWH) on cART, evidence of HIV-induced immunosuppression remains, and normally benign and opportunistic pathogens become major sources of co-morbidities, including virus-induced cancers. In fact, cancer remains a primary cause of death even in virally suppressed PLWH. Natural killer (NK) cells provide rapid early responses to HIV infection, contribute substantially to disease modulation and vaccine protection, and are also major therapeutic targets for cancer immunotherapy. However, much like other lymphocyte populations, recent burgeoning evidence suggests that in chronic conditions like HIV, NK cells can become functionally exhausted with impaired cytotoxic function, altered cytokine production and impaired antibody-dependent cell-mediated cytotoxicity. Recent work suggests functional anergy is likely due to low-level ongoing virus replication, increased inflammatory cytokines, or increased presence of MHC^{low} target cells. Indeed, HIV-induced loss of NK cell-mediated control of lytic EBV infection has been specifically shown to cause lymphoma and also increases replication of CMV. In this review, we will discuss current understanding of NK cell modulation of HIV disease, reciprocal exhaustion of NK cells, and how this may impact increased cancer incidences and prospects for NK cell-targeted immunotherapies. Finally, we will review the most recent evidence supporting adaptive functions of NK cells and highlight the potential of adaptive NK cells for cancer immunotherapy.

Keywords: HIV, cancer, natural killer, innate immunity, immunotherapy

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NK CELLS HAVE THERAPEUTIC POTENTIAL TO ENHANCE CONTROL OF BOTH HIV AND HIV-RELATED CANCERS

While efficient suppression of plasma viremia by combination antiretroviral therapy (cART) has substantially decreased mortality of people living with HIV (PLWH), burgeoning evidence suggests a higher occurrence of a vast range of comorbidities linked to long-term treatment and aging among PLWH, including cancers. The incidence of AIDS-defining cancers such as Kaposi Sarcoma, Non-Hodgkin lymphoma, and cervical cancer, has substantially decreased with access to cART. However, cART-treated PLWH still have a higher susceptibility to non-AIDS defining cancers (NADCs) compared to the general population, and NADCs currently represent a major cause of mortality among PLWH (1, 2). In particular, lymphomas, including Burkitt and classical Hodgkin lymphomas, have been reported at a significantly higher frequency in PLWH,

yet many other cancers associated with infections (i.e., anus, oropharynx, liver) and some cancers associated with cigarette smoking (i.e., lung, kidney) were also found to be elevated among PLWH (3). Several mechanisms have been proposed to explain the predisposition of cART-treated PLWH to NADCs (4). Nevertheless, as cART treatment only partially prevents HIV-induced chronic inflammation and immune senescence, it is very likely that immune dysregulation in PLWH is an important determinant of NADCs and explains why most cancers predominantly found in PLWH are related to viral infections (4, 5).

NK Cell Subpopulations

Natural killer (NK) cells are large granular leukocytes that play a central role in the control of viral infections and neoplasms. Human NK cells are defined as $CD3^{neg}CD56^{pos}$ lymphocytes (6) and can be subdivided into functionally distinct subpopulations based on expression levels of CD56 and CD16 (7). $CD56^{bright}CD16^{neg}$ NK cells have a high proliferation potential and the ability to secrete a large amount of cytokines, notably IFN-γ in response to IL-12, with limited cytotoxic functions (8), while $CD56^{dim}CD16^{pos}$ NK cells display strong cytolytic activity as well as a significant capacity to secrete cytokines upon triggering of activating receptors (6, 9). In addition, a subset of $CD56^{neg}CD16^{pos}$ NK cells appears to expand in chronic viral infections including HIV and might represent an exhausted/anergic subset of NK cells (10–12).

Our understanding of human NK cells has essentially been acquired while studying peripheral blood NK cells, yet it is now clear that subsets other than $CD56^{bright}$ and $CD56^{dim}$ NK cell subpopulations can be found in peripheral tissues. Tissue-resident NK cells differ from circulating NK cells and are found not only in secondary lymphoid organs but also in many peripheral tissues including the uterus, lung, and liver where they represent up to 50% of lymphocytes (13–15). Findings from recent studies have allowed reliable identification of tissue-resident NK cells based on their expression of CD69, CD49a, or CD103, three markers functionally involved in the retention of lymphocytes in tissues. Besides the uterus, lung and liver, NK cells have been characterized in many additional tissues such as the intestinal mucosa, skin, and kidneys. However, in a majority of older studies it is not clear if those NK cells represent tissue-resident NK cells, NK cells circulating between tissues and blood, or innate lymphoid cells (ILCs). Indeed, ILCs can express markers associated with NK cells such as CD56, NKp46, or NKp44, and it was only lately appreciated that a deeper analysis of expressed transcription factors and produced cytokines is required to discriminate NK cells and ILCs. Until recently, NK cells were even considered as part of ILC group 1 due to the common innate lymphoid progenitors. However, NK cells are now distinguished from other ILCs because of their unique development and cytotoxic functions (16). In summary, tissue-resident NK cells likely play a crucial role in select tissues or organs involved in cancer and HIV disease, yet due to the scarcity of data on the contribution of tissue-resident NK cells in HIV infection or cancer development, herein we will focus primarily on circulating NK cells.

NK Cell Function

NK cells can efficiently discriminate between transformed or virally-infected cells and normal cells without the need for prior sensitization, and have the capacity to kill abnormal cells before adaptive immunity develops, thereby containing viral replication or tumor development. NK cells can clear cellular targets by a number of different mechanisms, including (i) exocytosis of cytotoxic granules containing perforin and granzyme that results in cell lysis, (ii) signaling through Fas ligand or TRAIL death receptors which induces apoptosis, (iii) release of cytokines with potent anti-viral and anti-tumor activities, and (iv) antibody-dependent cellular cytotoxicity (ADCC), triggered through binding of the FcγRIIIA receptor (CD16) on NK cells by the constant (Fc) domain of IgG antibodies. NK cells also play major roles in tuning and controlling adaptive immune responses (17).

NK Cell Receptors

Unlike other lymphocytes, NK cells lack antigen-specific receptors but lyse target cells following the integration of inhibitory and activating signals. These signals are generated by an arsenal of germline encoded cell surface molecules, with effector functions taking place when activating signals overcome inhibitory ones (18). The major NK cell receptors, which allow NK cells to discriminate between "self" and a variety of pathological cell states belong to three main categories: (i) natural cytotoxicity receptors (NCRs) such as NKp46, NKp30, and NKp44, which can bind to several viral or tumor-associated molecules (19, 20), (ii) NKG2A/C/E-CD94 heterodimers and NKG2D homodimers, which are c-type lectins binding to the non-classical Human Leukocyte Antigen E (HLA-E) molecule and stress-induced ligands, respectively, and (iii) the killer-cell immunoglobulin-like receptors (KIRs), which primarily recognize HLA class Ia (HLA-Ia) and Ib (HLA-Ib) molecules and related surface molecules (21).

The classical HLA-Ia group includes the highly polymorphic and ubiquitously expressed HLA-A, -B and -C antigens. Non-classical HLA-Ib antigens comprise HLA-E, -F, and -G molecules which are expressed in a tissue-specific manner, display low genetic diversity, and limited peptide repertoire (22). While the biological function and clinical relevance of most HLA-Ia and -Ib antigens have been investigated in detail, HLA-F was only recently recognized for its important immune-regulatory functions in cancer (23–26) and potentially in HIV infection (27). Besides their role in mediating recognition and elimination of unhealthy cells, a direct interaction between inhibitory KIRs and their HLA class I ligands during NK cell development is necessary for NK cells to acquire self-tolerance and functionality through an education process termed "licensing." Besides NK cell licensing, which involves engagement of self-HLA class Ia molecules by their inhibitory ligand, non-classical HLA class I as well as non-HLA class I molecules also contribute to NK cell education (28).

While NKp30, NKp46, NKG2D, and NKG2C are expressed at relatively comparable levels on circulating $CD56^{dim}$ and $CD56^{bright}$ NK cells, other major NK cell receptors are differentially expressed on distinct subsets of NK cells (11).

Peripheral blood CD56^{bright} NK cells have been proposed to represent a mixture of immature NK cells that are direct precursors of CD56^{dim} NK cells (29, 30), and mature NK cells, including CD56^{dim} NK cells that have upregulated CD56 and lost CD16 upon activation in peripheral tissues (31). Immature CD56^{bright} NK cells lack expression of KIRs, which are sequentially acquired during the differentiation process into mature CD56^{dim} NK cells, a process that occurs in parallel with a progressive decrease in NKG2A expression and acquisition of the marker of terminal differentiation CD57 (32). NKp44 is usually not expressed on peripheral blood NK cells and up-regulated upon IL-2- or IL-15-mediated NK cell activation (33).

Other groups of receptors have received attention because their expression on NK cells is modulated in HIV and/or cancer and impacts NK cell function. These include Signaling Lymphocyte Activation Molecule (SLAM)-related receptors such as 2B4 (34–37) that displays co-stimulatory functions on NK cells and binds to CD48, or sialic acid-binding immunoglobulin-type lectins (Siglec), which are HLA class I-independent inhibitory receptors that recognize sialic acid-containing carbohydrates (38, 39). T cell immunoglobulin and mucin-domain containing-3 (Tim-3), which can binds to galectin-9, carcinoembryonic antigen cell adhesion molecule 1 (Ceacam1), high-mobility group box 1 (HMGB1) or phosphatidylserine (PtdSer), is another immunoregulatory molecule highly expressed on NK cells with relevance for NK cell function in both HIV and cancer (40–47). NK cells also express members of the immunoglobulin (Ig) superfamily such as the activating receptor DNAM-I (48–51), which has been shown to recognize CD112 (PVR) and CD155 (Nectin-2), two ligands expressed on tumor cells.

NK Cell Control of Cancers and HIV Infection

NK cells were originally defined as immune cells capable of lysing tumor cell lines. Since then, their capacity to kill primary cancer cells *in vitro* as well as their ability to prevent growth and metastasis of certain tumors *in vivo*, principally hematological cancers, has been clearly established (52–54). In particular, protection against development of cancer has been associated with higher NK cell cytotoxicity (55) and increasing evidence has highlighted the implication of NK cells in defense against leukemia. Importantly, in the context of hematopoietic stem cell transplantation (HSCT), it has been demonstrated that allogeneic NK cells from the donor can prevent relapse of myeloid leukemia via graft-vs.-leukemia effect (56, 57). However, thus far clinical trials aimed at harnessing NK cell anti-tumor activity have shown marginal therapeutic efficacy (58–61), with beneficial effects reported mainly against hematologic malignancies (62). Development of therapeutic strategies to enhance NK cell activity against tumor cells *in vivo* has therefore become a major field of investigation.

Besides NK cell anti-metastatic properties, numerous studies have emphasized the early and pivotal role of NK cells in the control of HIV infection. Notably, particular KIR genes expressed in conjunction with their HLA ligands are associated with significantly slower HIV disease progression and lower viral set-point (63, 64), elite control of HIV (65), and protection against disease acquisition (66, 67). In particular, activating KIR3DS1 has been associated with delayed HIV disease progression in individuals with specific HLA-B alleles since a first study by Martin et al. (63), yet a ligand for KIR3DS1 was only recently described, underscoring the relevance of HLA-F in regulating immunity to HIV (27). Indeed, HLA-F open conformers (OCs), which constitute heavy chains not bound to β_2-microglobulin, can be recognized by several KIRs but have the highest affinity for KIR3DS1 (27, 68). HLA-F OCs trigger polyfunctional responses by KIR3DS1^{pos} NK cells, which efficiently suppress HIV replication *in vitro*. HLA-F is expressed on activated CD4^{pos} T cells and may act as a marker of cellular stress in specific conditions including viral infections and cellular transformation.

Control of HIV infection has also been associated with NK cells displaying potent cytotoxic function and IFN-γ expression after stimulation (69) as well as with polyfunctional CD8α^{pos} NK cells (70). Moreover, it has been demonstrated that NK cells expand in the peripheral blood during early acute HIV infection, can inhibit HIV replication *in vitro*, and can mediate *in vivo* immune pressure in infected individuals, resulting in viral escape (71–77). Finally, indirect NK cell-mediated ADCC has been linked to vaccine-induced protective immunity against HIV infection (78), elite control of HIV (79–81) and slower HIV disease progression (82, 83). Therefore, in cART-treated PLWH, therapeutic interventions targeting NK cells might result in improved control of HIV and other viral infections as well as in decreased incidence of cancers.

ABERRANT EXPRESSION OF KEY NK CELL RECEPTORS MAY CONTRIBUTE TO DECREASED CONTROL OF PRE-CANCEROUS CELLS IN PLWH

NK cell-mediated immunosurveillance is decreased in PLWH, mostly as a long-term consequence of chronic HIV infection. While administration of suppressive cART partly restores NK cell properties, NK cells undergo many HIV-associated functional and phenotypic alterations, which are likely to severely impair NK cell-mediated control of viruses as well as of pre-cancerous cells.

Engagement of the well-described NCRs, NKG2D, and CD16 receptors represent major pathways to promote potent NK cell activation and cytotoxic responses. In both chronic HIV infection and cancer, NK cell recognition of abnormal cells through those activating receptors is defective, mainly as a result of chronic exposure to the respective ligands, which results in persistent down-modulation of NCRs, NKG2D, and CD16 on NK cells. In this section, we will review known effects that malignancies and HIV infection have on the expression of key NK cell receptors (**Figure 1**, left panel). It is important to note that a simplified definition of NK cells as CD3^{neg}CD56^{pos} lymphocytes or different gating strategies to identify the major NK cell subsets represent a caveat of some older studies, precluding any definite conclusions on phenotypic alterations specifically affecting individual NK cell subsets.

NCRs represent a particularly important family of activating receptors in NK cell-mediated elimination of tumor cells, with a few tumor-associated ligands described for those molecules thus

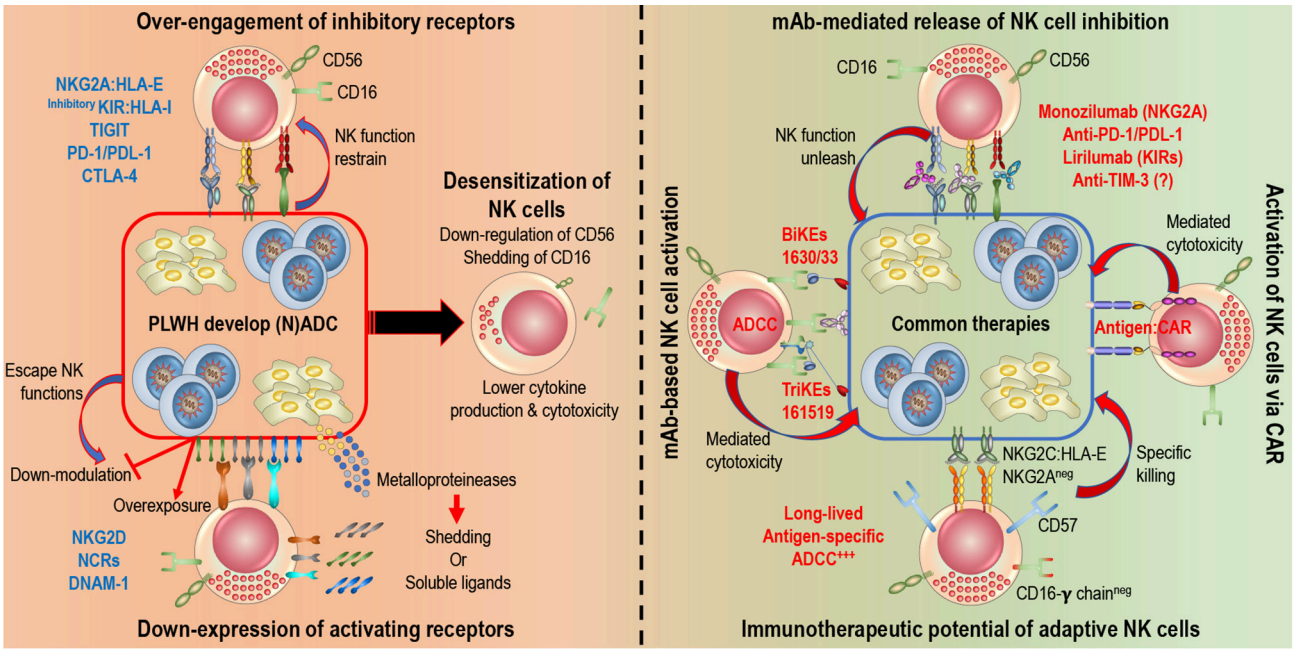

FIGURE 1 | Rescuing and harnessing NK cell potency in PLWH developing cancers. **Left**: HIV-infected and cancer cells share common NK cell escape mechanisms. 1. Over-engagement of inhibitory receptors (i.e., NKG2A, inhibitory KIRs, PD-1...) blocks killing abilities of NK cells. 2. Down-modulation (blocking expression of ligand or shedding of ligand) or over-exposure (constant expression of ligands or release of soluble ligands) induce down-expression of activating receptors (NKG2D, NCRs, DNAM-1…) on NK cells. **Right**: Novel immunotherapies are being develop to harness NK cell potency and target HIV-infected and cancer cells. 1. Monoclonal antibodies (mAb) release engagement of inhibitory receptors, unleash NK cell cytotoxicity and engage Fc receptors (CD16) to induce ADCC. Several clinical trials are in progress. 2. Engineered proteins, Bi-specific or Tri-specific Killer engagers (BiKEs or TriKEs) and Chimeric Antigen Receptors (CARs), act as a link between NK cells and target cells to induce cytotoxicity. BiKEs or TriKEs induce ADCC by engaging CD16 receptors and bind to antigen on target cells. 3. Adaptive features of NK cells, defined by a higher expression of CD57, NKG2C, and/or absence of FcR-γ, could be harnessed to elicit specific killing of target cells.

far (19). Accordingly, strategies to escape immune recognition by NCRs have been reported in both HIV infection and cancer, and have been associated with dysfunctional NK cells expressing lower levels of NCRs than NK cells from control subjects in many studies. Upon HIV infection, a population of dysfunctional $CD56^{neg}CD16^{pos}$ NK cells expands at the expense of the $CD56^{dim}CD16^{pos}$ NK cell subset and is progressively eliminated with cART treatment. In PLWH, decreased NK cell expression of NKp30 and NKp46 receptors has been reported, and appears to be a characteristic of $CD56^{neg}CD16^{pos}$ NK cells, reducing their cytokine production and cytotoxicity, notably against tumor target cells, as well as their ability to interact with other immune cells (84–86). Similarly, decreased NK cell cytotoxicity in patients with acute or chronic myeloid leukemia (AML or CML) correlates with lower levels of NKp30 and NKp46 expression on NK cells compared to healthy individuals (87–89). NCRs downregulation on NK cells is induced by cell-to-cell contact with AML blasts and linked to poor survival in AML patients (87), whereas high levels of NKp30 and NKp46 expression on NK cells at AML diagnostic are predictive of better outcomes (90, 91). In AML, high expression of the immunosuppressive glycoprotein CD200 on tumor cells has been shown to directly impair NK cell anti-tumor responses and is associated with downregulated expression of NKp44 and NKp46 receptors on NK cells (92).

As overexposure to their ligands promotes decreased NCRs expression on NK cells, it is not surprising that shedding of NCR ligands is a hallmark of tumor escape, underscoring further the importance of this family of receptors in anti-metastatic NK cell functions. The A disintegrin and metalloproteinase ADAM-10 and ADAM-17 can cleave B7–H6, a ligand for NKp30, from the surface of tumors, likely leading to reduced NKp30 expression on NK cells surrounding the tumor (93, 94). Shedding of NKp30 ligands has also been described in chronic lymphocytic leukemia (CLL), in which exosomal expression of BAG6 mediates NK cell activation, whereas soluble BAG6 suppresses NK cell cytotoxicity (95). Galectin-3 is another molecule released by tumor cells that can serve as ligand for NKp30 and prevent NK cell activation (96). As another immune escape mechanism, catabolites specifically generated in tumor microenvironments, such as L-kynurenine, can also directly down-modulate NKp46 expression on NK cells (97). Whether HIV infection-associated NCR ligands are shed from the surface of infected cells remains to be fully determined, but likely contributes to impaired NCR^{pos} NK cell function in HIV infection.

Altogether, these data suggest that fully restoring and even enhancing NCR-mediated signaling in NK cells might be crucial to efficiently control pre-cancerous cells in PLWH. Of note, B7–H6 is the only NCR ligand expressed on tumors that has been characterized so far. Identification of NCR ligands

specifically expressed in cancer or HIV infection would represent a milestone in the development of therapeutic interventions aimed at maintaining NCR-mediated NK cell function in PLWH. Finally, given the crucial role played by NCRs in regulating NK cell function in both blood and tissues, therapeutic interventions to enhance tumor surveillance by NK cells and targeting NCR signaling are currently being explored (20).

NKG2D is one of the most important NK cell activating receptor in terms of recognition and elimination of abnormal cells expressing stress-induced ligands. Similarly to NCRs, tumors and HIV evolved immune escape mechanisms to specifically circumvent NKG2D-mediated recognition by NK cells. In HIV infection, reduced NKG2D expression on NK cells and dampened NK cell function have been linked to elevated levels of the soluble form of its major histocompatibility complex I-related chains A (MICA) ligand in patient sera (98). MICA is likely released by HIV-infected $CD4^{pos}$ T cells based on their increased expression levels of matrix metalloproteinases MMP-2 and -7, a family of enzymes previously described for their role in proteolytic shedding of NKG2D ligands in human tumors (99, 100). UL16 binding proteins (ULBP) also serve as ligands for NKG2D and their expression is induced on HIV-infected cells (34), yet levels of ULBP-1 and -2 is down-modulated by the HIV accessory protein Nef, thereby dampening NKG2D-mediated NK cell cytotoxicity (101).

Tumor progression has been associated with lower levels of NKG2D (as well as NKp30 and NKp46) expression on NK cells from patients with cervical cancer (102), and defective NK cell function owing to NKG2D downregulation has been linked to high-risk myelodysplastic syndrome (MDS) (103). Shedding of NKG2D ligands also plays a central role in tumor escape. In AML patients, chronic exposure to MICA/B decreases expression of NKG2D on NK cells (104) and the concentration of NKG2D soluble ligands in the peripheral blood correlates with reduced NK cell cytotoxicity in AML and CML (105). MICA is released in multiple myeloma (106, 107), and MICA/B as well as ULBP-6 are shed from leukemic cells (108). NKG2D ligand shedding is also involved in Hodgkin lymphoma, in which lymph node stromal cells express proteases that shed MICA and ULBP-3 from the surface of the lymphoma cells (109). Thus, NKG2D and its well-described ligands represent additional promising therapeutic target to enhance immunosurveillance by NK cells in PLWH. Accordingly, it has been recently demonstrated that antitumor responses by NK cells can be efficiently promoted by antibodies against MICA by blocking MICA/B shedding and coating MICA-expressing tumor cells, rendering them susceptible to ADCC (110).

Function of additional NK cell receptors is modulated by HIV infection and play an important role in NK cell responses to cancerous cells, including the activating receptor DNAM-1 that is expressed on the majority of peripheral blood NK cells (111–115). The CD155 ligand for DNAM-I has been shown to be present on HIV-infected T cells and, although discrepant results were obtained based on the cell culture model used, some studies found CD155 to be counter-regulated by the HIV proteins Nef and Vpu, thereby preventing NK cell activation (50, 51, 116). Many tumors also express ligands for DNAM-1, triggering NK cell cytokine production and cytotoxicity (117, 118). Tumor escape from DNAM-1 has been described and associated with DNAM-1 downregulation on NK cells isolated from patients with cancer (119–124).

Siglec receptors, and particularly Siglec-7 and -9, have also gained a lot of attention in the past decade for their involvement in immune evasion of tumor and virus-infected cells. Siglec-7 and Siglec-9 are constitutively expressed on all peripheral blood NK cells and on a mature subset of cytotoxic $CD56^{dim}$ NK cells, respectively (125). Reduced Siglec-7 expression marks a subset of dysfunctional NK cells that appears in early stages of HIV infection, prior to downmodulation of CD56, in subjects with elevated HIV replication, and also characterizes the dysfunctional $CD56^{neg}$ NK cell subset in chronic HIV infection (126, 127). Interestingly, an association between downregulation of Siglec-7 and dysfunction of NK cells has also been described in HIV-2 infection (128).

Siglec-7 and -9 ligands are widely expressed on distinct tumor cells and shield them from $Siglec\text{-}7^{pos}$ and $Siglec\text{-}9^{pos}$ NK cells (125). Siglec-10, another member of the Siglec family expressed by NK cells, is associated with decreased survival and impaired NK cell function in hepatocellular carcinoma (129). Therefore, targeting Siglec molecules on NK cells, or their ligands on malignant cells, might prove an attractive immunotherapeutic strategy to augment NK cell antitumor immunity (130). Supporting this hypothesis, a $Siglec\text{-}7^{neg}$ NK-92 cell line exhibited high cytotoxicity against leukemia cells *in vitro* (131).

Overall, interactions between ligands and major activating receptors on NK cells are impaired in both HIV and cancer, with some common underlying mechanisms such as cleavage of membrane-bound receptor molecules by zinc-dependent endopeptidases such as MMPs and ADAMs. Therefore, drugs that prevent shedding of NK cell-activating ligands or receptors may enhance protection against development of cancer in PLWH. Several inhibitors of the metalloproteinase ADAM17, for instance, have already entered clinical trials and are being tested in combination with other therapeutics against cancer (132). Metalloproteinase inhibitors would also prevent CD16 shedding form the surface of NK cells, a mechanism that naturally occurs following CD16 ligation (133, 134) yet is dysregulated in HIV infection and cancer, thereby decreasing ADCC activity and cytotoxicity against HIV-infected or tumor cells. Moreover, increased levels of inhibitory receptors such as inhibitory KIRs or TIGIT on NK cells further contribute to decreased NK cell functions in PLWH (135, 136). Finally, unresolved inflammation is a hallmark of chronic HIV infection and is widely accepted to elicit malignant transformation of cells and carcinogenesis (137, 138). Several inflammatory mediators, such as TNF-α, IL-6, tumor-derived transforming growth factor β (TGF-β), and IL-10 have been shown to play a role in carcinogenesis. For instance, TGF-β is a cytokine endowed with immune-suppressing and anti-inflammatory properties that plays a key role in promoting NK cell dysfunction and is found elevated in both the tumor microenvironement and plasma of PLWH. In addition, TGF-β has been shown to elicit production of vascular endothelial growth factor by NK cells, thereby promoting tumor

growth along with other cytokines chronically found elevated in PLWH (137, 139).

Altogether, these observations show that the ability of NK cells to eliminate tumor cells is impaired by the tumor microenvironment and further constrained in HIV infection, and that NK cell dysfunction in cART-treated PLWH may significantly contribute to their enhanced susceptibility to develop malignancies.

RECENT ADVANCES IN DEVELOPMENT OF NK CELL-BASED STRATEGIES FOR THE TREATMENT OF CANCER

NK cell-based immunotherapies rely on enhancement of endogenous NK cell activities in the tumor microenvironment or on adoptive transfer of NK cells with improved function. Strategies so far have included blockade of inhibitory NK cell receptors or immunosuppressive processes in the tumor microenvironment as well as enhancement of NK cell activation via cytokine stimulation or chimeric receptor expression (140). In this section, we will focus on strategies that could be of particular benefit in PLWH for elimination of HIV as well as HIV-associated cancers (**Figure 1**, right panel).

mAb-Mediated Release of NK Cell Inhibition

Autologous NK cells are oftentimes suppressed by self HLA class-I molecules expressed on tumor cells that bind to inhibitory CD94/NKG2A or KIR. This can be circumvented by adoptive therapy of allogeneic NK cells with a KIR-HLA class I mismatch. Alternatively, release of inhibitory signals using mAbs that target HLA class I-binding NK cell inhibitory receptors represent another strategy to enhance NK cell antitumor functions. This approach might be particularly beneficial in PLWH, as HIV infection results in downmodulation of the major activating NK cell receptors.

NKG2A is a c-type lectin that has been shown to mediate NK cell suppression in both HIV infection and cancer. In particular, elevated expression of HLA-A has been linked to poor control of HIV (141). This deleterious effect is mediated by NKG2A^{pos} NK cells that are functionally suppressed by increased levels of HLA-E; whose expression is directly regulated by the availability of HLA class I-derived peptides. Whether increased HLA-A also correlates with poor outcome in cancer remains to be determined. Overexpression of HLA-E by tumor cells has long been proposed as a mechanism of escape from the action of NK cells (142). For instance, enhanced expression of HLA-E in hepatocarcinomas is driven by IL-10 released in the tumor micro-environment and is associated with enhanced NKG2A expression, a profile that correlates with NK cell exhaustion/anergy, as measured by low IFN-γ intracellular production upon stimulation with IL-12, and with a poorer prognosis (143). Failure to achieve remission in AML patients has been linked to impaired function of NK cells that upregulated NKG2A (144), and expression of HLA-E in multiple myeloma cells decreases NK cell cytotoxicity (145). Accordingly, efficacy of a specific IgG4 mAb that targets NKG2A (Monalizumab) is currently being assessed in various tumor settings along with other mAbs (61). Promising results were obtained in phase II trials in combination with the anti-EGFR antibody Cetuximab in head and neck cancers (146). Interestingly, Monalizumab targets both T cell and NK cell responses, promoting effector T cell responses in combination with anti-PDL1 and enhancing NK cell effector functions, including ADCC. Whether therapeutic blockade of HLA-E:NKG2A interaction, potentially in combination with PD-1 signaling blockade, could significantly improve control of HIV remains to be evaluated. NKG2A also significantly contributes to NK cell education in the early stages of NK cell ontogenesis. Accordingly, administration of Monalizumab has been suggested to promote NK cell alloreactivity against malignant cells when administered early after haplo-HSCT, thereby circumventing the need for a KIR-mismatched donor (147).

Inhibitory KIRs represent another interesting target for immunotherapies. For instance, Lirilumab, an IgG4 mAb that targets KIR2DL1/2/3 and KIR2DS1/2 has been evaluated in several clinical trials in combination with different mAbs in AML (phase II NCT02399917), MDS (phase II NCT02599649), lymphoma (phase II NCT01592370), and CLL (phase I NCT02481297). However, long-term use of inhibitory KIR blocking agents might lead to desensitization of NK cells (60). Finally, the recent discovery of HLA-F OCs' ability to bind KIRs, and particularly KIR3DS1 that has a widespread influence in human diseases including HIV, has made HLA-F a target of significant interest for therapies to enhance anti-tumor function of NK cells that might be particularly relevant for PLWH with malignancies.

Numerous antibody-based immune checkpoint inhibitors currently under investigation target the interaction of PD-1 or CTLA-4 and their cognate ligands on tumor cells, in order to boost the power of tumor-specific CD8^{pos} T cells. In particular, clinical studies assessing the blockade of PD-1 or its ligand PD-L1 reported potent therapeutic efficacy against several cancers such as melanoma and non-small cell lung cancer. Selective PD-1 expression on CD56^{dim}CD57^{pos} mature NK cells in some but not all healthy individuals has been reported (148) and associated with functional defects (149). However, overall expression and functional relevance of those markers on NK cells in health and disease is still unclear, and recent studies suggest that blockade of CTLA-4 and PD-1 might enhance NK cell anti-tumor activity mostly via indirect mechanisms (150). Interestingly, PD-1 also mediates T-cell exhaustion in chronic HIV infection, and dual immune checkpoint blockade targeting PD-1 and IL-10 significantly enhances NK cell function through reversal of adaptive immune exhaustion in PLWH (151). Therefore, immunotherapeutic interventions targeting PD-1 may augment NK cell responses against both HIV and tumors in PLWH.

Another immune checkpoint inhibitor currently tested in clinic is a mAb targeting Tim-3, a receptor associated with exhaustion in T cells. Tim-3 has been proposed to mark mature NK cells, with chronic Tim-3 upregulation being associated with NK cell dysfunction, yet the precise impact of TIM-3 expression on NK cell function require further investigations (152). While Tim-3 has been shown to be upregulated on NK cells in various tumors, studies dissecting the effects of Tim-3

blockade on NK cell function in cancer settings have yielded mixed results (150).

Finally, even though it represents a promising approach for the treatment of cancer, administration of mAbs targeting regulatory immune checkpoint molecules has been associated with toxicities known as immune-related adverse events (irAEs) (153). irAEs are mainly caused by the release of inhibitory mechanisms that normally constrain the immune response, leading to various local and systemic autoimmune responses. Clinical benefit of immune checkpoint therapy is also restricted to a subset of patients. Several mechanisms of resistance to immune checkpoint inhibition have been described (154, 155). Notably, cancer therapy based on administration of mAbs promotes the induction of antibodies against such humanized mAbs and it is not clear yet whether such antibodies do or do not play a role by neutralizing the effects of the therapy. However, the potential of such antibodies to induce hypersensitivity reactions need to be considered.

mAb-Based NK Cell Activation

Antibody therapy that targets activating NK cell receptors is another strategy that has shown efficacy in certain malignancies. Elotuzumab, an antibody that targets SLAMF7, directly activates NK cells and can simultaneously induce ADCC by coating multiple myeloma cells, which express SLAMF7. The ability of a therapeutic mAb to induce ADCC results in potent NK cell activation and led to the design of bi-specific and tri-specific killer cell engagers, BiKEs and TriKEs, respectively. These single-chain variable fragment recombinant reagents can bind the tumor cells and NK cells via CD16 to induce direct killing via ADCC. This technique has been used in clinical trials where Hodgkin target cells expressing CD30 were linked to CD16 expressed on NK cells (156). The anti-CD16XCD33 BiKE activation can override the inhibitory signals mediated by ligation of inhibitory NK cell receptors and their HLA class I ligands expressed on AML (157) and MDS (158) targets. However, BiKEs do not promote *in vivo* proliferation and survival of NK cells. To overcome this issue, TriKEs were manufactured to engage the IL-15 receptor and are evaluated in different tumor pathologies (159–161). Use of therapeutic mAbs with potent ADCC activity may lead to substantial benefit in PLWH who present high frequencies of NK cells with enhanced antibody-dependent activation, as described in the last section.

Activation of NK Cells via CAR

A new tool for immunotherapy is chimeric antigen receptor (CAR)-engineered NK cells. CAR are artificial receptors composed of an extracellular antibody-derived tumor antigen binding domain as well as transmembrane and intracellular domains for activating signal transduction (162). Thus far, CAR T cells have been developed and successfully employed in the treatment of hematological malignancies. However, use of CAR-T cells has been limited as therapy for solid tumors and triggered numerous severe side effects in clinical trials that can be overcome with the use of CAR-NK cells. These include graft-vs.-host disease, cytokine release syndrome, and off-target toxicities. Moreover, CAR-NK cells can also eliminate tumor cells in a CAR-independent manner through recognition of ligands expressed on tumor cells by a range of activating receptors such as NKp30, NKG2D, DNAM-I, providing another advantage to use CAR-NK cells over CAR-T cells for cancer immunotherapies (163, 164). However, safety and efficacy of CAR-NK cells in humans need to be fully evaluated as only few clinical trials have been using CAR-NK cells up to now. One issue pertaining to CAR-NK cells is their limited *in vivo* persistence. To circumvent this restriction, a phase II trial is currently assessing the persistence and anti-tumor activity of IL-15- and caspase-9 suicide gene-transduced CD28-CAR-NK cells in B cell lymphoma (NCT03056339). Alternatively, CAR expression in adaptive NK cell subsets discussed in the next section may overcome expansion and persistence issues while simultaneously boosting anti-tumor activity. Finally, implementation of CAR-based strategies optimized for NK cells is warranted. For instance, induced pluripotent stem cell (iPSC)-derived NK cells transduced with novel CAR constructs that include NK cell-specific signaling domains instead of CD3ζ signaling-based domains are being evaluated and may significantly enhance their potency (165). Importantly, while CAR-T cell-based clinical trials have failed to provide clinical benefit and HIV viral suppression in PLWH, advanced CAR strategies that are developed specifically for NK cells in the cancer field can benefit PLWH as they could be applied to efficiently redirect NK cell functions toward HIV-infected cells (166).

Immunotherapeutic Potential of Adaptive NK Cells

While NK cells are classically viewed as non-specific effector cells of the innate immune system, a vast amount of independent studies has demonstrated that subsets of murine, non-human primate and human NK cells are capable of adaptive immune functions, including antigen-dependent expansion and long-lived immunological memory (167, 168). Adaptive NK cell-based immunotherapies may circumvent many of the limitations inherent to the various strategies tested thus far to harness anti-tumor functions of conventional NK cells.

The best characterized adaptive NK cell subset in humans is the one driven by HCMV infection, originally identified as a population of NK cells expressing high levels of the activating CD94/NKG2C receptor and the marker of terminal differentiation CD57, which expand upon HCMV infection or reactivation and can persist for years at high frequency in HCMV-seropositive individuals (169–173). Corroborating the adaptive features of this NK cell subset, it was recently shown that expansion and differentiation of this CD94/NKG2C^{pos} NK cell subset is driven by the HCMV UL40 peptide presented by HLA-E, the ligand for NKG2C (174).

The CD94/NKG2C^{pos} NK cell population largely overlaps with an FcεRIγ adaptor protein-deficient memory NK cell subset with enhanced antibody-dependent functions (FcγRΔg NK cells) that has more recently also been characterized in HCMV-seropositive subjects (175–184) and rhCMV-positive macaques (185). Adaptive characteristics of FcγRΔg NK cells include a distinctive epigenetic signature close to that of memory CD8^{pos} T

cells, endowing these adaptive NK cells with specialized functions such as enhanced responses to CD16 cross-linking, potent IFN-γ production to selective stimuli and reduced activation by innate cytokines.

Interestingly, adaptive CD94/NKG2C^{pos} NK cells proliferate not only in response to CMV reactivation or infection in patients receiving hematopoietic transplantation (169, 172, 186–188), but also upon *de novo* infection with different viruses including HIV and upon HCMV reactivation in PLWH (171, 189, 190). Several reports strongly suggest that HCMV-associated adaptive NK cells improve control of HIV infection. Higher frequencies of CD94/NKG2C^{pos} NK cells during primary HIV infection are linked to lower viral set points, are predictive of higher CD4^{pos} T cell counts and of an overall better outcome in treated PLWH (191, 192). In contrast, individuals with NKG2C gene deletions are more susceptible to HIV infection and once infected may have accelerated disease progression (193). Finally, in HCMV-seropositive PLWH, CD94/NKG2C^{pos} NK cells exhibiting adaptive signatures of FcγRΔg NK cells present conserved effector functions (190). The beneficial effect of adaptive CD94/NKG2C^{pos} NK cells has also been demonstrated in cancer settings. HCMV reactivation has been linked to longer relapse-free survival in patients with hematological malignancies receiving allogeneic hematopoietic cell transplantation (194). More specifically, expansion of adaptive NKG2C^{pos}CD57^{pos} NK cells upon HCMV reactivation after HCT is associated with reduced leukemia relapse (195, 196). Of note, specific phenotypic signatures have been associated with this NK cell adaptive subset and include lack of NKG2A expression. As a result, these cells are intrinsically insensitive to tumor-mediated suppression through HLA-E. Therefore, HCMV-associated adaptive NK cells represent an attractive subset of NK cells that could be exploited instead of conventional NK cells to limit cancer incidence in PLWH, particularly in combination with tumor-targeting therapeutic antibodies that efficiently promote NK cell-mediated ADCC.

NK cell memory has been described against multiple viral, bacterial, and tumor antigens, and can also be induced by brief exposure to specific cytokines. Indeed, NK cells can differentiate into cytokine-induced memory-like (CIML) NK cells that display enhanced effector functions after a short pre-activation with a combination of IL-12, IL-15, and IL-18 followed by a prolonged rest period (197). Re-stimulation of CIML NK cells using leukemia target cells, cytokines or FcγRIIIa ligation is associated with increased responsiveness that can be retained for several weeks following their initial pre-activation (197–202). CD56^{bright} and CD56^{dim} NK cells both have the potential to differentiate into CIML NK cells (197). Potent effector functions of CIML NK cells have been linked to expression of the high-affinity IL-2 receptor αβγ (IL-2Rαβγ), demethylation of the conserved upstream non-coding enhancer region of the IFN-γ gene, recruitment of anergic unlicensed NK cells, enhanced antibody-mediated functions and release from KIR-mediated inhibition (198, 200, 201, 203). Therefore, superior functionality of CIML NK cells is not affected by prior licensing through HLA class-I molecules. Compared to control NK cells, CIML NK cells have been shown to express higher levels of CD56, CD94, NKG2A, NKG2D, NKp46, CD25, NKp30, NKp44, CD62L, CD27, TRAIL, perforin and granzyme B, and lower levels of CD16, whereas NKG2C expression was found similar between control and CIML NK cells (197, 199).

The long-lived properties of CIML NK cells have tremendous potential to be exploited for cancer immunotherapy, and preliminary results from a first-in-human phase 1 clinical trial have shown that NK cells pre-activated with IL-12, IL-15, and IL-18 can expand *in vivo* and exert robust responses against leukemia targets, leading to remission in a subset of AML patients (199). A better understanding of the mechanisms behind CIML NK cell responses may lead to novel strategies to further enhance their antitumor function. For instance, recent studies suggested that targeting the interaction between SEMA7A, a potent immunomodulator expressed by cytokine-activated NK cells, and integrin-β1 might provide a novel immunotherapeutic approach to potentiate antitumor activity of CIML NK cells (204).

Strikingly, burgeoning evidence also supports the existence of true antigen-specific memory NK cells in humans (174, 177, 205), including a recent report of human HIV-specific memory NK cells (168). While further studies are warranted to fully characterize human antigen-specific NK cells and define the mechanisms underlying NK cell memory formation and maintenance, it is possible that adaptive NK cells that can specifically recognize tumor-associated antigens and efficiently eliminate cancerous cells develop in cancer patients. Vaccines including components to boost tumor-specific NK cells or infusion of expanded tumor-specific NK cells represent attractive avenues for the development of novel therapeutic interventions.

Overall, the immunotherapeutic potential of adaptive NK cells is expected to exceed that of conventional NK cells as they may overcome some of the major limitations faced in NK cell-based cancer therapies that have been evaluated so far in pre-clinical or clinical studies. For instance, adaptive NK cells can be expanded *ex vivo*, are long-lived and persist *in vivo*, are less sensitive to regulatory T cells-mediated suppression (206) or myeloid-derived suppressor cell inhibition (207) and can achieve significantly enhanced antibody-dependent functions (194) or antigen-specific cytotoxicity (168). Importantly, HCMV-dependent adaptive NK cells are increased 7-fold (181) and confer protection in PLWH (191, 192). Therefore, exploitation of adaptive NK cells may represent an attractive strategy to efficiently prevent or treat malignancies in PLWH.

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OL and SJ contributed to writing of specific sections. RR and SJ edited the final version of the manuscript.

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Blocking Formation of the Stable HIV Reservoir: A New Perspective for HIV-1 Cure

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Recent studies demonstrate that the stable HIV-1 reservoir in resting CD4$^+$ T cells is mostly formed from viruses circulating when combination antiretroviral therapy (ART) is initiated. Here we explore the immunological basis for these observations. Untreated HIV-1 infection is characterized by a progressive depletion of memory CD4$^+$ T cells which mostly express CD127, the α chain of the IL-7 receptor (IL-7R). Depletion results from both direct infection and bystander loss of memory CD4$^+$ T cells in part attributed to dysregulated IL-7/IL-7R signaling. While IL-7/IL7R signaling is not essential for the generation of effector CD4$^+$ T cells from naïve cells, it is essential for the further transition of effectors to memory CD4$^+$ T cells and their subsequent homeostatic maintenance. HIV-1 infection therefore limits the transition of CD4$^+$ T cells from an effector to long-lived memory state. With the onset of ART, virus load (VL) levels rapidly decrease and the frequency of CD127$^+$ CD4$^+$ memory T cells increases, indicating restoration of effector to memory transition in CD4$^+$ T cells. Collectively these data suggest that following ART initiation, HIV-1 infected effector CD4$^+$ T cells transition to long-lived, CD127$^+$ CD4$^+$ T cells forming the majority of the stable HIV-1 reservoir. We propose that combining ART initiation with inhibition of IL-7/IL-7R signaling to block CD4$^+$ T cell memory formation will limit the generation of long-lived HIV-infected CD4$^+$ T cells and reduce the overall size of the stable HIV-1 reservoir.

Keywords: CD4$^+$ T cell, HIV-1, memory, latency, reservoir, IL-7, CD127

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KEY POINTS

- Both the long-lived defective and replication competent HIV-1 reservoirs in CD4$^+$ T cells form near the time of ART initiation.
- The replication competent HIV-1 reservoir in CD4$^+$ T cells is stable under ART.
- Memory CD4$^+$ T cells which mostly express the IL-7 receptor (IL-7R) α chain, CD127 are profoundly depleted during untreated HIV-1 infection.
- HIV-1 infection disrupts IL-7/IL-7R signaling and CD4$^+$ T cell memory formation.
- CD4$^+$ T cell memory formation and IL-7/IL-7R signaling is restored following ART initiation.
- Blocking IL-7/IL-7R signaling limits CD4$^+$ T cell memory generation.
- Blocking IL-7/IL-7R signaling at ART initiation may limit the transition of HIV-infected cells to long-lived memory CD4$^+$ T cells, decreasing the overall size of the stable HIV-1 reservoir.

THE HIV-1 RESERVOIR IS ESTABLISHED AROUND THE TIME OF ART INITIATION

Untreated HIV-1 infection is characterized by continual viral replication and evolution. However, two papers by independent groups, combining HIV-1 sequencing approaches and longitudinal sampling of persons living with HIV (PLWH), before and after ART initiation, concluded that the majority of the HIV-1 reservoir is formed around the time of ART initiation (1, 2). Brodin et al. used an Illumina™ deep sequencing approach to compare p17*gag* sequences in plasma virus RNA (vRNA) collected longitudinally for at least the first 5 years after diagnosis but before ART (pre-ART) to proviral DNA isolated from peripheral blood mononuclear cells (PBMCs) after at least 2 years of suppressive ART. In this study of 10, mostly HIV-1 clade B-infected Swedish individuals (9 male, 1 female), phlyogenetic analysis found that ∼60% of the post-ART DNA sequences were most similar to RNA variants that were present in the plasma just prior to ART initiation (1).

The HIV-1 DNA reservoir is dominated by defective proviruses (3–5), therefore Brodin et al.'s study did not provide information on the timing of establishment of the stable *replication competent* reservoir, which is a primary source of rebounding virus following ART interruption. This question was addressed by Abrahams, Joseph *et al*., who used samples from nine HIV-1 clade C-infected South African women to compare pre-ART HIV-1 RNA (longitudinally sampled from the plasma during 2.7–6.9 years of untreated infection) to replication competent HIV-1 in resting CD4+ T cells obtained post-ART (after 4.7–6.1 years of ART) (2). Briefly, MiSeq with PrimerID (6, 7) was used to sequence five regions of the HIV-1 genome (*gag*, *nef*, and three regions in *env*) in pre-ART vRNA. In addition, resting CD4+ T cells (CD25-CD69-HLADR-) isolated post-ART were cultured after stimulation (PHA, IL-2, and allogenic PBMC) and the PacBio platform was used to generate 5′ and 3′ half genome sequences from the HIV-1 RNA produced by the resting CD4+ T cells. Phylogenetic analyses of these sequences revealed that a median of 78% of outgrowth viruses were most genetically similar to viruses circulating in the year before ART. Together these studies show that both the defective (3) and replication competent HIV-1 reservoirs (2) form near the time of ART initiation.

These independent observations, made in distinct clinical cohorts, are most simply explained by ART indirectly increasing the half-life of cells harboring integrated virus resulting in a stable reservoir. Given that most studies agree that virus evolution does not occur on ART (1, 8–10), the work of Abrahams, Joseph and colleagues identifies a narrow time window immediately after therapy initiation in which the majority of the stable/long-lived HIV-1 reservoir is established. This suggests that strategies to *limit the formation* of the stable HIV-1 reservoir could be combined with ART initiation, when patients are receiving intense clinical care. Preventing generation of long-lived latently infected CD4+ T cells should result in a smaller HIV-1 reservoir, providing a less intractable target for curative approaches. Reducing the size of the HIV-1 reservoir may also reduce ongoing immune senescence and HIV-1 co-morbidities experienced by PLWH on ART.

Here, we propose that establishment of the HIV-1 reservoir at the time of ART initiation is driven by the restoration of IL-7/IL-7R signaling that increases CD4+ T cell transition to long-lived memory cells (**Figure 1**). In this review, we discuss how untreated HIV-1 infection disrupts CD4+ T cell homeostasis and how homeostasis is subsequently restored on ART, consistent with ART facilitating the establishment of the majority of the stable HIV-1 reservoir in long-lived CD4+ T cells. We propose that a novel approach to complement existing HIV-1 therapies is to minimize establishment of the HIV-1 reservoir at ART initiation by blocking the IL-7/IL-7R-mediated CD4+ T cell memory transition until viremia is cleared and the immune environment transitions to a less inflammatory state.

ART-SUPPRESSED INDIVIDUALS HARBOR A STABLE HIV-1 RESERVOIR

ART is highly effective at stopping new rounds of HIV-1 infection, with regimens including integrase strand transfer inhibitors (INSTIs) achieving viral control (<50 copies/ml) in >65% of PLWH within 4 weeks (12) and increasing to >80% of individuals within 12 weeks (12, 13). Viral RNA decay is at least bi-phasic, with an initial steeper decline reflecting the loss of HIV-1 infected cells that are short-lived ($t^1/_2$–hours to days) and a second slower decline reflecting loss of longer-lived HIV-1 infected cells ($t^1/_2$–weeks to years) (14–18). In most individuals, peripheral CD4+ T cell counts increase significantly within 4 weeks of ART initiation and are restored to levels comparable with uninfected individuals in ∼12 months (13). ART also significantly reduces virus-driven immune activation (19), though levels of cellular activation and some proinflammatory cytokines remain higher than in HIV-1 seronegative individuals [reviewed in (20)]. Individuals with durably suppressed viral load on ART in North America and Europe now enjoy near-normal lifespans (21, 22). Yet, with the notable exception of two case reports of long-term remission (23–25), HIV-1 infection cannot be cured using current regimens.

In durably suppressed (>12 months) individuals, the barrier to HIV-1 cure is a rare population of HIV-1 infected cells that are not producing virions (are therefore impervious to the host immune system) but can reactivate to produce replication competent virus. Collectively, these cells are referred to as the latent HIV-1 reservoir (26) and, following interruption of ART, are the source of virus rebound. HIV-1 rebound is consistently observed after 1–12 weeks of ART withdrawal, irrespective of whether ART was initiated in the first weeks–months of infection or years later in chronic infection (27–29). Rebounding virus is typically oligoclonal, suggesting reactivation of >1 latently infected cell (28, 30).

The best-characterized portion of the latent reservoir resides in CD4+ T cells (31, 32). In ART-treated people, >95% of HIV-1 proviruses encode a viral genome that is replication incompetent (4, 5). However, replication competent proviruses can be reactivated following mitogenic stimulation of cells

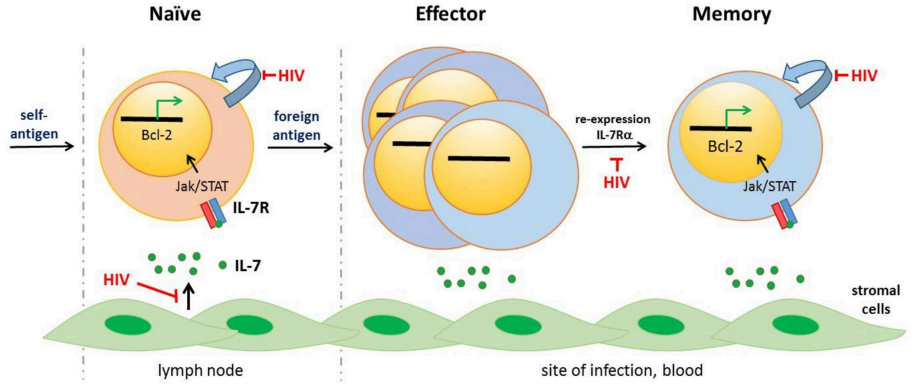

FIGURE 1 | CD4+ T cell lineage differentiation is impaired following HIV-1 infection. IL-7, that is mostly produced by stromal cells, binds the IL-7 receptor (IL-7R) comprising CD132 and IL-7Rα (CD127) initiating signaling pathways including as Jak/STAT5 signaling and expression of anti-apoptopic genes, including Bcl-2. Naïve CD4+ T cells, selected against self-antigens, express the IL-7R. IL-7/IL-7R signaling is necessary for homeostatic turnover of naïve T cells (curved blue arrow). Following priming by foreign antigens, CD4+ T cells undergo significant transcriptional and phenotypic changes, including downmodulation of the IL-7R. These effector CD4+ T cells express activation markers (not shown), undergo rapid division and exit the lymph node to home to the site of infection. Most effector CD4+ T cells undergo apoptopic death; a subset however re-express the IL-7R and induce expression of anti-apoptopic genes. These memory cells are long-lived, undergoing slow mitotic division/homeostatic proliferation. HIV-1 infection drives ongoing expansion of effector CD4+ T cells. In addition, dysregulated IL-7/IL-7R signaling resulting from HIV-induced immune activation impairs several stages of CD4+ T cell lineage—naïve CD4+ T cell survival, generation of long-lived memory CD4+ T cells and homeostatic proliferation of naïve and memory CD4+ T cells. Other cytokines, notably IL-2 and IL-15, contribute to CD4+ T cell differentiation and homeostasis [not illustrated, reviewed in (11)].

in vitro and quantified by measurement of viral RNA or viral antigen. Finzi et al. showed that in most individuals resting CD4+ T cells (not expressing markers of cellular activation) produce replication competent HIV-1 following mitogenic stimulation (33). The frequency of cells harboring inducible, replication competent virus is very low, ~1 infected cell/10^6 resting CD4+ T cells (33) [range: 0.01–10 infected cells/million resting CD4+ T cells (34)] but is remarkably stable in ART-suppressed individuals. Two independent longitudinal studies showed that the half-life of the measured reservoir is 44 months (34, 35). These data can best be explained by ART-suppressed individuals harboring a small population of HIV-1 latently infected CD4+ T cells that are long-lived and/or undergo homeostatic proliferation and undergo occasional stochastic reactivation. Note, while replication competent virus can also be recovered from resting CD4+ T cells in untreated infection, the frequency of infection is >2 logs higher and correlates directly with plasma virus load (15), suggesting that these resting CD4+ T cells harbor contemporaneous viruses (similar to those in the plasma) and do not represent a stable reservoir.

To date, efforts to cure HIV-1 in ART-treated participants have been unsuccessful. Early ART treatment both in non-human primates 3 days after infection with a simian immunodeficiency virus (SIV) (36) and HIV-1-infected people (37–39) has not prevented reservoir formation. In addition, latency reversing agents that seek to force HIV-1 reactivation have induced transient blips of detectable virus in the plasma, but have not impacted the size of the stable replication competent reservoir (40). The intractable nature of the HIV-1 reservoir has led to support for "functional cure" strategies that do not entirely eradicate HIV-1 but rather constrain viral rebound following ART interruption (28). Given that strategies to purge or suppress the stable HIV reservoir have had limited success, it is worth considering whether it is possible to limit the size of the reservoir by blocking its formation.

THE IL-7/IL-7Rα (CD127) PATHWAY IS A CRITICAL REGULATOR OF CD4+ T CELL HOMEOSTASIS

In both humans and animal models, the primary T cell response to infection is dominated by, antigen-specific T cells that are strongly proliferative (Ki67+), express activation markers including CD69 and CD25 (in humans, HLA-DR), but mostly are short-lived. As the acute antigen load decreases, the bulk of primary activated T cells undergo apoptotic death and the effector T cell pool contracts (41, 42). A subset of cells survive (42, 43), having undergone changes that enable them to become long-lived and persist in the absence of antigen. These memory cells retain proliferative potential including homeostatic proliferation (41, 42, 44–46). Memory T cells are responsible for mediating ongoing immune surveillance. In humans, both vaccine studies and BrdU (synthetic nucleoside) labeling of proliferating T cells [reviewed in (47)] have identified subpopulations of memory T cells with half-lives as long as 9 years (48, 49). In response to secondary antigen stimulation, memory T cells exhibit rapid proliferation and give rise to both short-lived effector memory and terminally differentiated effector T cells. In humans who are exposed to many different pathogens, including chronic infections like HIV-1 in which antigen stimulation is ongoing, both short-lived effector and long-lived memory T cells co-circulate.

Short- and long-lived antigen-specific T cells can be further delineated based on their homing capability, anatomical location, phenotype and function which collectively reflect lineage

differentiation. Stem cell-like (TSCM) and central (TCM) cell phenotypes harbor a high proportion of long-lived cells whereas transitional (TTM), effector (TEM) and terminal effector (TEMRA) cell populations are more short-lived and express higher levels of activation markers (50).

Maintenance of the equilibrium between naïve, effector and long-lived memory T cells (and their lineage subsets) is termed T cell homeostasis. IL-7 is a common γ-chain cytokine that together with IL-2 and IL-15 regulate homeostasis of both $CD4^+$ and $CD8^+$ T cells, as well as other lymphocytes. IL-7 is constitutively expressed by stromal and epithelial cells in the thymus, lymphoid tissue and bone marrow, and regulates multiple stages of the T cell life cycle including thymopoeisis, memory cell maturation (44), survival (51), and homeostatic proliferation (52–55). IL-7 is not, however, required for the initial primary expansion of activated effector T cells (53).

IL-7 signals through the IL-7R heterodimer which consists of the common gamma chain (CD132), shared by IL-2 and IL-15 receptors, and IL-7Rα (CD127), which confers specificity to IL-7. IL-7/IL-7R engagement induces JAK/STAT signaling which regulates expression of proliferative and anti-apoptopic genes, including increased expression of the Bcl2 anti-apoptotic gene family, promoting cell survival (54, 56). IL-7 binding also reduces CD127 expression on T cells through both transcriptional and post-transcriptional mechanisms. CD127 is expressed at high levels on naïve T cells (52) and TSCM (57) but is downregulated on activated effector T cells (50, 53, 58–60). As cellular activation decreases and T cells transition to long-lived memory, CD127 is re-expressed (44, 50, 53, 61) (**Figure 1**). In healthy humans with no overt infection, 60–90% of circulating memory ($CD45RO^+$) CD4 T cells in the blood [unpublished observations (62, 63)] and 60–80% of resident memory $CD4^+$ T cells ($CD69^+CD4^+CD45RO^+$) in lymphoid, lung, and gut tissues are CD127hi (64). Consistent with CD127 expression patterns and cellular half-lives, Bcl2 expression is high in naïve and memory $CD4^+$ T cells but lower in effector T cells (52, 65, 66).

The dynamic nature of CD127 expression on $CD4^+$ (and $CD8^+$) T cells reflects that IL-7 levels are not regulated by production but by consumption (67). Downmodulation of CD127 in response to IL-7 binding allows available IL-7 to be shared by the greatest number of cells (68, 69). When T cell homeostasis is dysregulated and lymphopenia occurs (e.g., following myeloablative chemotherapy or $CD4^+$ T cell depletion following HIV infection), IL-7 consumption declines and serum IL-7 levels increase (70). This excess drives rapid expansion of both $CD127^+$ naïve (52) and memory T cells (70) promoting restoration of lymphocyte levels.

CD127+ MEMORY CD4+ T CELLS HARBOR REPLICATION COMPETENT HIV-1

HIV-1 infects T cells via $CD4^+$ and a co-receptor (CCR5 or CXCR4). In untreated infection, HIV-1 infection occurs mostly in effector memory and not naïve $CD4^+$ T cells, in part because memory, particularly activated memory (CD127lo), $CD4^+$ T cells express higher co-receptor levels (63, 71). By contrast, naïve $CD4^+$ T cells typically lack CCR5 (72). Characterization of $CD4^+$ T cells harboring latent HIV in ART-suppressed individuals is very challenging due to the low frequency of circulating infected long-lived cells. Primary cell models of HIV latency, in which a much higher frequency of $CD4^+$ T are infected have proved informative. In superinfected aggregate cultures of tonsils, $CD127^+CD4^+$ T cells were infected with HIV but did not support viral gene expression (73), suggesting these, $CD127^+$ $CD4^+$ T cells may promote HIV latency. In another primary cell model of HIV-1 latency, in which $CD4^+$ T cells were derived from PBMC, CD127 expression was highly associated with latent infection (74). Shan and colleagues also employed primary cell models to show latent HIV infection (as opposed to productive infection), preferentially occurs at the transition of $CD4^+$ T cells from an effector to a memory state (75). Transcriptional reprogramming of $CD4^+$ T cells from the effector to memory state, which was marked by high CCR5 expression, facilitated HIV-1 integration but not subsequent HIV-1 gene expression, thereby promoting latency (75). In summary, HIV-1 $CD127^+$ $CD4^+$ memory T cells may be more likely to harbor persistent HIV-1 with establishment occurring at the transition of activated effector (CD127lo) to longer-lived memory (CD127hi) $CD4^+$ T cells.

HIV-1 INFECTION DYSREGULATES CD4+ T CELL HOMEOSTASIS

HIV-1 infection creates multiple challenges for $CD4^+$ T cell homeostasis, most obviously reflected in the absolute loss of $CD4^+$ T cells in untreated infection The cytopathic effects of direct $CD4^+$ T cell infection alone do not explain this loss of $CD4^+$ T cells, suggesting indirect mechanisms (76). A major contributor to $CD4^+$ T cell depletion in acute and chronic infection is generalized immune activation driven by unabated HIV-1 viremia that can reach 10^8 copies/ml during acute infection and typically remains $>10^4$ copies/ml in chronic infection (77). These unusually high and sustained antigen levels in turn, induce sustained elevation of activation and exhaustion markers on $CD4^+$ T cells (78, 79). This is associated with diminished IL-2 release by $CD4^+$ T cells (58, 80), increased peripheral turnover of both naïve and memory $CD4^+$ T cells and critically, failure to generate long-lived memory $CD4^+$ T cells (81–84).

Dysregulated IL-7/IL-7R signaling in HIV-1 infection (85) has been proposed by several groups as a critical link between HIV-1-driven immune activation and bystander $CD4^+$ T cell loss (86–88). Firstly, activation-induced lymphodepletion increases serum levels of IL-7 (89), combines with other proinflammatory cytokines, such as IL-1β [which is elevated in the lymphoid tissues of HIV infected individuals (88)], to increase turnover of antigen-specific $CD4^+$ T cells favoring the generation of short-lived CD127lo/-activated effector T cells (84, 90, 91). Although circulating IL-7 levels rise, IL-7 bioavailability in the lymphoid tissues is significantly decreased following infection due to TGF-β1-mediated collagen deposition that results in the loss of IL-7-producing fibroblast reticular cell (FRC) networks (92, 93).

This is proposed to directly contribute to the increased apoptosis and loss of naïve $CD4^+$ T cells that mostly reside in lymphoid tissue (93, 94).

The overall effect of these changes is impairment of both the generation and maintenance of long-lived $CD4^+$ T cells in viremic individuals. Several groups have reported both significantly lower frequencies of $CD127^+CD4^+$ T cells as well as lower CD127 expression levels on $CD4^+$ T cells in untreated HIV-1 infection (50, 62, 86, 89, 95, 96). Notably, expression of the CD132 common γ chain remains normal on $CD4^+$ T cells in infected individuals, suggesting a specific impact on IL-7 signaling on $CD4^+$ T cells (97). Down-regulation of CD127 on T cells correlated significantly with both depletion of absolute levels of $CD4^+$ T cells and also with increased concentration of serum IL-7. The decreased CD127 expression was associated with lower cellular levels of Bcl-2 and with the poorer survival of T cells in the presence of IL-7 *in vitro* (86). $CD127^+CD4^+$ T cells also exhibited increased rates of apoptosis in untreated infection relative to healthy controls suggesting in untreated infection, CD127 expression on memory cells is not itself sufficient to maintain cell survival in the face of uncontrolled HIV-1 viremia (89, 98). By contrast, HIV-1 infected individuals who exhibited long term non-progression had higher $CD127^+CD4^+$ T cell frequencies than HIV-1 infected typical progressors (62, 99) and in some individuals, decreased CD127 expression on $CD4^+$ T cells preceded subsequent loss of virus control (62).

The loss of $CD127^+CD4^+$ memory T cells in untreated HIV infection is reflected in lower antigen-specific $CD4^+$ T cell responses to chronic infections. Frequencies of Cytomegalovirus (CMV) (100) and *Mycobacterium tuberculosis* (*M.tb*)-specific $CD4^+$ T cell responses (101) are significantly lower in HIV infected individuals relative to healthy individuals and, despite clearly detectable HIV-specific $CD8^+$ T cell responses, little to no HIV-specific $CD4^+$ T cell proliferation is detectable in untreated HIV infection (100). Generally, immune responses to vaccination (humoral and cellular) are lower and less durable in HIV infected individuals compared to healthy individuals, suggesting weaker $CD4^+$ T cell help [reviewed in (102, 103)]. In one study, vaccination with the experimental vaccine Modified Vaccinia Ankara (MVA) expressing the *M.tb* antigen, 85A was compared in HIV uninfected, HIV viremic ($CD4^+$ count >300 cells/mm^3) and ART-suppressed individuals. Consistent with the formation of long-lived T cell memory being limited during untreated HIV-1 infection, vaccine-induced oligofunctional $CD4^+$ T cell responses at peak and over the course of the following year were significantly lower in untreated HIV-1 infected participants relative in uninfected and HIV infected, ART-treated study participants (101).

In summary, uncontrolled HIV-1 infection skews the memory $CD4^+$ T cell response to a short-lived effector phenotype with lower frequencies of long-lived memory $CD4^+$ T cells, suggesting either or both impaired effector to memory transition of $CD4^+$ T cells or a failure to maintain long-lived memory $CD4^+$ T cells. Dysregulated IL-7/IL-R signaling appears central to these changes.

ART RESTORES THE $CD4^+$ T CELL MEMORY TRANSITION

As described above, as a pathogen is cleared the population of activated, short-lived effector T cells contracts and quiescent, longer-lived pathogen-specific memory cells emerge. A similar phenomenon but on a broader scale, impacting both HIV-specific and non-specific CD4 T cells, is observed in the weeks to months following ART initiation.

Successful ART (91) rapidly reduces viremia. Immune activation is significantly reduced, but not fully abrogated, possibly because of residual low-level viremia. Elevated turnover of $CD4^+$ T cells is decreased to levels that are comparable with healthy controls (81, 104) within 12 weeks of ART (39, 77). Both absolute $CD4^+$ T cell counts and $CD127^+$ $CD4^+$ memory T cell frequencies increase to levels observed in uninfected individuals (105), though CD127 expression levels on $CD4^+$ T cells remain lower (106). With restoration of absolute $CD4^+$ T cell levels, IL-7 in the serum decreases and IL-7 mediated STAT-5 phosphorylation, which is elevated in memory $CD4^+$ T cells in untreated infection (107), is normalized (108). Functionally, memory $CD4^+$ T cells exhibit improved IL-2 release, HIV-1-specific $CD4^+$ T cell responses increase in frequency (100, 109) and $CD4^+$ T cell responses to vaccination improve (101). By comparison, immunological non-responders to ART, in whom viremia is controlled but absolute $CD4^+$ T cells counts are not fully restored, have higher serum IL-7 levels and lower $CD127^+CD4^+$ T cells compared with immunological responders (105, 106, 110). Altogether, in most people ART largely restores $CD4^+$ T cell homeostasis, including $CD4^+$ T cell transition from effector to long-lived memory T cells.

THERAPEUTIC IMPLICATIONS OF ART-MEDIATED RESTORATION OF $CD4^+$ T CELL HOMEOSTASIS

While current curative strategies (eradication or functional) against HIV-1 largely target the established stable HIV-1 reservoir in durably suppressed individuals, we propose that strategies to limit the seeding of long-lived latently infected cells at the time of ART will likely decrease the size of the reservoir. We propose targeting the IL-7/IL-7R pathway by specifically blocking CD127 signaling on $CD4^+$ T cells in early ART to delay restoration of the $CD4^+$ T cell memory transition (**Figure 2**).

Monoclonal antibodies (MAb) that antagonize the IL-7Rα (111) are in investigation for treatment of a range of autoimmune diseases and inflammatory conditions, including diabetes (112), multiple sclerosis, rheumatoid arthritis (113, 114), and inflammatory bowel disease (115). The aim of these approaches is to suppress aberrant memory $CD4^+$ T cell responses (116). A single intravenous administration of anti-CD127 antagonist antibody resulted in inhibition of antigen-specific memory $CD4^+$ T cell responses and decreased chronic inflammation in primates that was sustained for 11 weeks (111). In diabetes studies in mice, CD127 blockage decreased T helper 1 (TH1) IFN-γ-producing $CD4^+$ T cells in secondary lymphoid

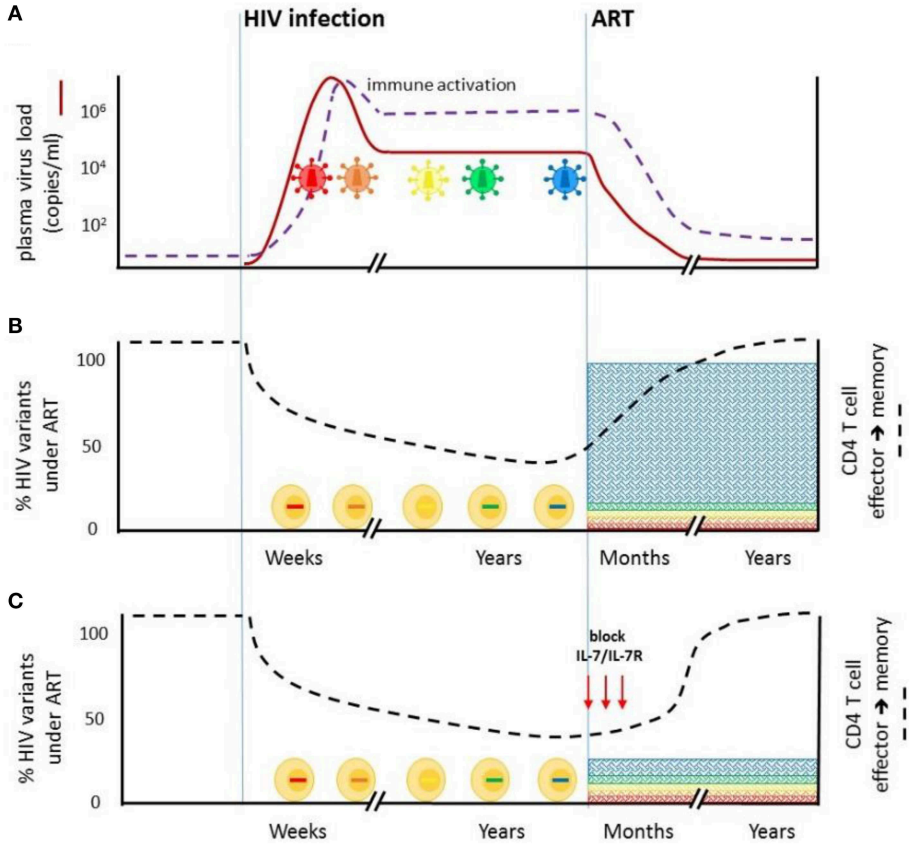

FIGURE 2 | Blockade of IL7/IL-7R signaling at the time of ART initiation may limit the size of the stable HIV-1 reservoir. **(A)** HIV-1 infection is characterized by extensive viral replication (red line) and virus evolution (colored virions). Virus-driven immune activation (purple dotted line) is observed throughout untreated infection. ART rapidly reduces plasma virus levels. Immune activation is also significantly reduced but not fully abrogated. **(B)** HIV-1 infection impairs $CD4^+$ T cell effector to memory transition (black dotted line), which is restored on ART. Abrahams et al. (2) showed that on average, 80% of the replication-competent virus in $CD4^+$ T cells from durably ART-suppressed individuals is derived from virus present in plasma in the year prior to ART initiation. Viruses that were circulating earlier in untreated infection comprise a minority of the latent reservoir. **(C)** Blocking IL-7/IL-7R signaling at the time of ART initiation, aimed at delaying ART-mediated restoration of $CD4^+$ T cell effector to memory transition, may limit the entry of viruses circulating immediately prior to ART into the HIV reservoir.

tissues (117) and elevated PD-1 expression on autoreactive $CD4^+$ T cells, limiting long-memory responses (118). In other murine studies, anti-CD127 antibodies blocked memory $CD4^+$ T cell proliferation and blunted vaccine-induced immune responses (119).

In humans, a single intravenous administration of an αCD127 antagonist mAb was well-tolerated (120) with both full receptor occupancy and inhibition of IL-7/IL-7 signaling (*ex vivo* STAT5 phosphorylation) observed for over 21 days following dosing. Transient depletion of $CD19^+$ B cells and limited changes to T cells, including T regulatory cells (discussed below), were observed during the 24 weeks of participant follow-up. Expression of the activation/exhaustion marker, PD-1 on T cells did not consistently change following dosing. In summary, anti-CD127 immunotherapy is a promising approach to limit $CD4^+$ T cell memory immunity that in clinical studies to date is supported by pharmokinetic and safety data (120).

While we propose blocking CD127 in combination with ART initiation to limit reservoir establishment, others had proposed IL-7 treatment in PLWH on ART as a strategy to improve $CD4^+$ and $CD8^+$ T cell immunity to HIV-1, particularly in individuals who do not regain normal $CD4^+$ T cell counts after virologically successful ART. Consistent with other IL-7 immunotherapy studies (121, 122), treatment of durably ART-suppressed individuals successfully increased circulating $CD4^+$ and $CD8^+$ T cell counts for several months after last dosing (123–126) by increasing expression of pro-survival genes (127). However, the effect on the HIV reservoir was also to increase the frequency of $CD4^+$ T cells containing HIV-1 (124, 128), possibly by CD95-mediated proliferation (129). These results complement our hypothesis that that $CD4^+$ T cell homeostasis mediated by IL-7/IL-7R signaling is critical for the establishment and maintenance of the long-lived latent HIV-1 reservoir.

INSTIs are now widely included as a first-line therapy against HIV-1 largely because of good tolerability (130). INSTI containing regimens produce ∼1 log greater decrease in VL within the first 10–15 days following ART initiation (18). This more rapid decrease in antigen levels produces proportionate

and earlier decreases in cellular immune activation (131) that are likely to result in earlier T cell memory restoration, arguably within days. Accordingly, we propose that blocking of T cell memory formation to prevent HIV-1 seeding of the reservoir should begin very early, possibly alongside ART initiation and continue short-term until all productively infected $CD4^+$ T cells are cleared; that is the participant is no longer viremic (**Figure 2**). In the pre-INSTI era, ART regimens increased $CD127^+CD4^+$ CM T cells within 1 month of ART initiation (132). Detailed studies describing the kinetics of $CD4^+$ T cell memory restoration in the weeks-months following INSTI-ART initiation are however needed to better inform dosing strategies.

IL-7 is also critical for memory T cell homeostatic proliferation. While this perspective prioritizes employing IL-7R blocking to delay CD4 T cell restoration at ART initiation, CD127 antagonism may have application in limiting homeostatic proliferation of latently infected cells under durable ART (8). Here, bi-specific antibody approaches to express cis-acting antibodies [reviewed in (133)] targeting both CD127 and membrane-bound HIV proteins may increase specificity and facilitate longer-term treatment.

SPECIFIC CONSIDERATIONS/LIMITATIONS

There are number of considerations and limitations to this perspective to HIV cure.

1. Firstly, antagonizing CD127 signaling at ART initiation will not block HIV-1 already integrated into long-lived $CD4^+$ T cells. Both studies by Brodin et al. (3) and Abrahams et al. (2) as well as another smaller study by Jones et al. (134) found that HIV-1 variants from much earlier in infection were genetically similar to a subset of post-ART viruses. Further work is required to understand the mechanism/s by which HIV-infected cells harboring these viruses were able to persist during long periods of untreated infection. One possibility is that while HIV-1 infection impairs $CD4^+$ T cell memory formation, this is incompletely abrogated and a small number of cells infected early in untreated infection may become long-lived memory $CD4^+$ T cells despite profound dysregulation in IL-7 signaling at the population level (**Figure 1**).
2. The observations of Brodin et al. (1) and Abrahams, Joseph et al. (2) were made in peripheral $CD4^+$ T cells. HIV reservoirs are not limited to the blood. Recent studies have identified T follicular helper cells (TFH) (135) that reside in lymph nodes as the major $CD4^+$ T cell subset for HIV infection and replication in PLWH (136, 137) that continue to serve as a persistent HIV reservoir in PLWH on ART (138, 139). Whether replication competent viruses in TFH also cluster with viruses circulating around the time of ART, suggesting TFH could also be targeted by CD127 blocking, requires investigation. Similar studies are needed to examine other tissue reservoirs. Here, animal models of HIV infection and persistence will be particularly useful.
3. While α-CD127 antibody therapy has been well-tolerated in healthy individuals [(120) NCT02293161, NCT02293161], treatment of PLWH must be evaluated for risks associated with delayed recovery of $CD4^+$ T cell homeostasis [immunological non-responders (140)], particularly in individuals with advanced disease and/or low $CD4^+$ nadir (141).

   First-line INSTI ART regimens are associated with a higher incidence of immune reconstitution immunodeficiency syndrome (IRIS) (142, 143). IRIS, that can worsen existing opportunistic infections or unmask previously subclinical infections, very commonly *M.tb*, is associated with redistribution and restoration of functional memory T cells within the first months of ART (144). A single dose of α-CD127 antagonist mAb in NHP produced significant and prolonged decreases of IFN-γ secreting *M.tb*-specific CD4 T cells without $CD4^+$ T cell depletion (111). It is attractive to speculate whether short-term α-CD127 antibody therapy when given in combination with ART-initiation to PLWH, could also afford some protection against IRIS-associated events.
4. T regulatory (Treg) $CD4^+$ T cells function to suppress potentially deleterious activities of other T helper cells particularly TH1 and TH17 cells. Like other CD4 T helper cell subsets, Treg $CD4^+$ T cells express CCR5, are readily infected in untreated HIV infection (145) and in PLWH on ART, can harbor replication competent virus (139); with some reports that Treg $CD4^+$ T cells are enriched for latent HIV relative to conventional T helper subsets [reviewed in (146)]. Treg $CD4^+$ T cells differ from other conventional $CD4^+$ T helper subsets in that they are CD127lo (147). IL-7 can however induce STAT-5 phosphorylation in these cells in a dose-dependent manner (115). Following ART initiation, Treg $CD4^+$ T cells frequencies increase further in the first week of ART initiation then decrease to normal ranges in most individuals (148). The effect of CD127 antagonism on Treg $CD4^+$ T cells appears different in NHP and mice models. In NHP, CD127 blocking did not increase PD-1 expression (111) but increases in PD-1 expression as well and increases Treg $CD4^+$ T cells frequencies were observed in mice (112). Further studies are needed, particularly to investigate if outgrowth viruses from Treg $CD4^+$ T cells cluster with early or late (pre-ART) viruses. Treg $CD4^+$ T cells in PLWH however represent a cell subset that may be refractory to IL-7/IL-7R blocking strategies following ART initiation (112).
5. A CD127 blocking strategy will impact the signaling of all CD127 expressing cells. This includes naïve $CD4^+$ T cells, naïve and memory $CD8^+$ T cells (111), γδ T cells and innate lymphoid cells (ILCs) including NK cells. In humans, clinical administration of an anti-CD127 antagonist antibody induced minimal changes beyond short-term B cell loss (120), consistent with observation in NHP in which anti-CD127 mAbs produced no changes in peripheral T and B cell frequencies, nor changes in T cell subsets including Treg cells (111). In that study, CD127 antagonism did not increase PD-1 levels on $CD8^+$ T cells (111), however, studies in humanized mice using the same antibody clone increased the exhaustion signature particularly Tim-3 and PD-1 on $CD8^+$ T cells (115). Given the disparities in animal studies, detailed functional

studies are needed in humans to examine the impact of CD127 antagonism on lymphocyte subsets, particularly cytolytic, subsets. Non-cytolytic ILCs do not recover on ART (149) and similarly, studies are needed to investigate whether there are any additional, deleterious effects of CD127 antagonism on this cell subset (150).

6. While resting CD4+ T cells constitute the largest HIV-1 reservoir in the body, other CD4+ expressing cells, such as macrophages, can harbor HIV-1 and may contribute to virus rebound (151). Much less is understood about formation of HIV latency in these cell subsets. Macrophages express CD127 and antibody blocking of this pathway increases autophagy (152). How this process would impact the HIV persistence is unclear. γδ T cells have been shown to harbor replication competent HIV in PLWH on ART (153) and also express CD127. Future studies will need to investigate how CD127 blocking modulates HIV persistence in non-CD4+ T cell subsets.

SUMMARY

We propose that restoration of CD4+ memory transition in ART treated participants, which enables the generation of long-lived CD4+ T cells, drives the majority of HIV-1 reservoir formation. A temporary blockade of IL-7/IL-7R signaling at the time of ART initiation, by delaying memory CD4+ T cell restoration until virus has been cleared, could limit the size of the stable HIV reservoir, facilitating HIV-1 cure efforts. Limiting the size of the long-lived reservoir could also be combined with other strategies aimed at minimizing homeostatic proliferation of memory CD4+ T cells harboring HIV by limiting CD4+ T cell proliferation (154) or strategies to further reduce the established HIV reservoir following latency reversal and immune-mediated clearance. It is likely a combination of HIV cure strategies will be required to enable long-term ART interruption without virus rebound.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

NG conceived the manuscript. All authors contributed to the writing of the manuscript.

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Immunotherapy in People With HIV and Cancer

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HIV infection alters the natural history of several cancers, in large part due to its effect on the immune system. Immune function in people living with HIV may vary from normal to highly dysfunctional and is largely dependent on the timing of initiation (and continuation) of effective antiretroviral therapy (ART). An individual's level of immune function in turn affects their cancer risk, management, and outcomes. HIV-associated lymphocytopenia and immune dysregulation permit immune evasion of oncogenic viruses and premalignant lesions and are associated with inferior outcomes in people with established cancers. Various types of immunotherapy, including monoclonal antibodies, interferon, cytokines, immunomodulatory drugs, allogeneic hematopoietic stem cell transplant, and most importantly ART have shown efficacy in HIV-related cancer. Emerging data suggest that checkpoint inhibitors targeting the PD-1/PD-L1 pathway can be safe and effective in people with HIV and cancer. Furthermore, some cancer immunotherapies may also affect HIV persistence by influencing HIV latency and HIV-specific immunity. Studying immunotherapy in people with HIV and cancer will advance clinical care of all people living with HIV and presents a unique opportunity to gain insight into mechanisms for HIV eradication.

Keywords: HIV, immunotherapy, HIV reservoir, cancer, PD-1, Kaposi sarcoma, lymphoma

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INTRODUCTION

People living with HIV (PLWH) have an elevated risk of developing cancer compared to the general population. This increased risk is partially attributable to comorbid conditions and social factors such as smoking or poorer access to preventative services. However, there is strong evidence that immunologic factors such as decreased immunologic surveillance and increased susceptibility to oncogenic viral infection play a significant role (1–5). Historically, cancers developing in the setting of HIV have been classified as AIDS-defining malignancies (ADM; cancers that, when present, confer a diagnosis of AIDS) and non-AIDS defining malignancies (NADM; cancers whose presence does not necessarily indicate AIDS) (6). Many HIV-related cancers have a viral etiology (7). These include Kaposi sarcoma (KS) [Kaposi sarcoma herpes virus (KSHV)]; cervical, anal, penile and vulvar squamous cell cancer and oropharyngeal cancers [human papilloma virus (HPV)]; B cell non-Hodgkin lymphomas (NHL) including diffuse large B-cell lymphoma, Burkitt lymphoma, plasmablastic lymphoma, primary central nervous system lymphoma, primary effusion lymphoma, classic Hodgkin lymphoma, and lymphoproliferative disorders [in some cases, Epstein-Barr virus (EBV) and/or KSHV]; hepatocellular carcinoma [hepatitis B and C viruses (HBV/HCV)], and Merkel cell carcinoma [Merkel cell polyoma virus (MPV)]. In epidemiological studies of

non-Hodgkin lymphoma, Kaposi sarcoma, and anal cancer, uncontrolled HIV viremia is an independent risk factor (4, 5, 8).

The introduction of antiretroviral therapy (ART) after 1996 resulted in a reduction in the incidence of many ADMs by 75–80% (9), largely due to reduced prevalence of profound immunodeficiency. NADMs including lung cancer, Hodgkin lymphoma, anal cancer, and oropharyngeal cancer now comprise an increasing proportion of total cancers in PLWH in North America (10, 11). A similar trend has been documented in Europe, Australia (12) and the Asia-Pacific region (11, 13). This epidemiological switch in prevalence away from ADMs and virally-associated malignancies corresponds with increasing life expectancy of PLWH, increased availability of ART and promotion of viral suppression (14–16).

HIV LEADS TO PARTIALLY REVERSABLE PERTURBATION IN T-CELL FUNCTION

HIV has multiple effects on T-cell immunity that may contribute to cancer risk. Absent effective ART, uncontrolled HIV infection leads to massive depletion of HIV-infected CD4 cells and uninfected bystander CD4s in both blood and tissue (17). In the same setting, CD8 counts often rise, leading to inverted CD4/CD8 ratios that are an independent measure of immune dysfunction. Moreover, HIV and other chronic viral infections lead to increased expression of immune checkpoint proteins (such as PD-1), exhaustion markers, and impaired CD8 T cell function (18–20), causing systemic immune dysfunction and dysregulation (21). Untreated HIV perturbs not only the quantity but also the breadth of T-cell immunity. HIV leads to decreased numbers of naïve T cells, less diversity of the T-cell repertoire in the blood (22, 23), and skewing of the T-cell receptor (TCR) repertoire secondary to CD4 depletion and expansion of oligoclonal CD8 populations (24). HIV viremia is rapidly suppressed with modern ART. Immune reconstitution after initiation of ART leads to CD4 recovery and CD8 decline over time (25). The likelihood of full immune recovery improves with earlier diagnosis and a younger age at ART initiation (26), although immune recovery is often incomplete (27). The heightened pro-inflammatory state associated with both untreated and treated HIV contributes to long-term adverse outcomes (28, 29).

ONCOGENESIS IN THE SETTING OF HIV-INDUCED IMMUNE DYSFUNCTION

Immunodeficiency is an established risk factor for the development of cancer, and the underlying causes are likely many, including uncontrolled proliferation of oncogenic viruses and inadequate immune surveillance. Many oncogenic viruses have been shown to cause cancer in other immunosuppressed states, including inherited immunodeficiencies and solid-organ transplantation (30). CD4 deficiency is strongly linked to malignancy (31), independent of HIV infection (32–35). The presence, number, and functionality of CD4 T cells are important in multiple steps of the oncogenic pathway, including recognition of tumor antigens, development of effective neutralizing antibody, and cellular responses to viral pathogens, and clearance of premalignant lesions. The risk of many HIV-associated malignancies decreases with improved CD4 count on ART (9, 12, 36–39) and cancer-specific mortality correlates inversely with CD4 count (12, 40). The link between reduced CD4 count and elevated cancer risk is profound in KS and NHL (41–43), but also present in other malignancies (37). An individual's risk of cancer (and long-term immune dysfunction) is likely influenced by the CD4 nadir, perhaps indicative of a synergistic relationship between chronic inflammation and impaired immune surveillance (10, 44–49).

CD4 lymphocytopenia, ineffective CD8 response, and associated immune dysregulation lead to a reduction in immunosurveillance, a key mechanism in HIV-associated oncogenesis (21, 50). This is illustrated in the link between HIV, immune status, and cervical cancer (37). PLWH are more likely to acquire high risk HPV (51, 52), less likely to clear HPV, and more likely to progress to higher-grade forms of dysplasia (53). PLWH with lower CD4 counts are also more likely to progress from dysplasia to invasive cancer (54). In an HPV vaccine trial in adolescents with HIV, the induced antibody titer correlated positively with CD4 count (55), supporting the importance of CD4 T cells in the production of high-affinity antibodies (51), the primary correlate of protection of the HPV vaccine (56). Tissue-localizing HPV-specific CD4 and CD8 T cells are also potentially important to tumor regression (57, 58).

Immune exhaustion and T-cell senescence are prominent features of both chronic viral infections and malignancies (59). In PLWH, T-cell dysfunction is most strongly implicated in the development of EBV-related lymphomas and KS (60). In HIV-associated B cell NHL, reduced T-cell polyfunctionality and TCR diversity is associated with poorer prognosis (61). These observations, among others (62), have led to interest in remedying immune dysfunction to treat malignancy in PLWH (63).

ANTIRETROVIRAL THERAPY AND OTHER FORMS OF IMMUNOTHERAPY IN HIV-RELATED CANCER

ART is itself an effective form of immunotherapy for ADM. Improvements in ART in 1996 resulted in a decline in the incidence and severity of KS, as well as changes in its natural history (9, 64–66): the risk of death due to KS decreased at similar HIV RNA levels and CD4 count (66), suggesting that ART resulted not only in improved immune control of KSHV but also decreased immune dysregulation. ART-induced immune reconstitution results in regression of KS lesions in ∼80% of PLWH with early KS (67). However, ART alone is often insufficient in advanced KS.

Several immunotherapies have shown efficacy in KS and other HIV-related cancers (**Table 1**). Interferon alpha (IFN-α), the first true immunotherapy used in HIV-associated KS, generated a 20–40% response rate (98–100). IL-12, which enhances Th-1 type immune responses (91), has been shown to have anti-KS

TABLE 1 | Select immunotherapeutic agents used in cancers that occur at increased frequency in people with HIV and their demonstrated or hypothesized effect on measurements of the HIV reservoir.

Agent	Mechanism	Indication in cancer that is associated with HIV	Adverse events	Potential effect on HIV reservoir	References
Checkpoint inhibitors (ipilimumab nivolumab, pembrolizumab, durvalumab, etc.)	Block inhibitory T cell receptors including CTLA4, PD-1, or PD-L1, allowing T cell activation and promoting cytotoxic killing of target cells	Lung cancer, classical Hodgkin lymphoma, head and neck cancer, liver cancer	Fatigue, rash, arthralgia, pruritis, GI toxicity, asthenia, pulmonary toxicity, pyrexia, autoimmune phenomena, headache	Transient increases in unspliced HIV RNA and decreases in HIV DNA in blood, variable effects on plasma HIV RNA	(68–72)
Pomalidomide	Modulates substrate specificity of cereblon E3 Ubiquitin ligase, altering protein expression. Induces cell cycle arrest and apoptosis in plasma cell malignancies. Enhances T cell- and natural killer (NK) cell-mediated cytotoxicity, inhibits angiogenesis, modulates cytokines, and cell microenvironment	Under evaluation for KS	Thromboembolic events, teratogenicity, fatigue and asthenia, cytopenias, GI toxicity, dyspnea, back pain, pyrexia	Immune stimulation, increased killing of reservoir cells	(73–75)
Brentuximab vedotin	Monoclonal antibody drug conjugate with anti-CD30 antibody (expressed on Hodgkin Reed-Sternberg Cells) and MME (microtubule disruptor) payload	Classical Hodgkin lymphoma	Cytopenias, peripheral sensory neuropathy, fatigue, GI toxicity, pyrexia, rash, cough	Transient loss of detectable CD4 T-cell HIV RNA and reduction in plasma HIV viremia	(76, 77)
Alemtuzumab	Monoclonal antibody to CD52 (expressed on lymphocytes, monocytes, macrophages, NK cells, and some granulocytes)	Hematopoietic stem cell transplant conditioning	Infusion reaction, serious infections, cytopenias, secondary autoimmune disorders	*Ex vivo* elimination of latently-infected CD4 T cells. Evidence of decreased frequency of HIV-infected CD4 T cells *in vivo*.	(78–81)
IL-7	Modulates T cell development and maturation in the thymus. Modulates T cell homeostasis and proliferation and memory differentiation. Inhibits T cell apoptosis and promotes proliferation.	Under evaluation in combination with CD19 CAR T-cells in relapsed B-cell lymphoma	Infusion reaction, hypersensitivity	Transient increases in HIV viral load without observed clinical sequelae, as well as enhanced anti-HIV CD8 activity	(82–90)
IL-12	Promotes activation and differentiation of T lymphocytes and NK cells	Under evaluation in therapeutic vaccines for HPV associated cancers, phase 1 studies in solid tumors.	Immune activation	Latency reversal *ex vivo*	(91–93)
IL-15	Stimulates the proliferation of memory T cells and regulates their turnover. Promotes the survival of naive T cells.	Under evaluation in refractory B-cell lymphomas and solid tumors	Infusion reaction, hypersensitivity	*Ex vivo* killing of latently-infected CD4 T cells by cytotoxic CD8 T cells	(94–97)

activity in patients who are progressing despite ART (92) and is currently being developed as a tumor-targeted immunocytokine, NHS-IL12 (101). A recent trial of the immunomodulatory drug pomalidomide in 22 participants with heavily pretreated KS who were virally suppressed on ART noted an overall response rate of 60% among HIV-infected participants, which is comparable to traditional cytotoxic chemotherapy for KS. The investigators observed expansion of central memory cells and decreases in CD57+ immunosenescent T-cells (73, 74).

Despite immune dysfunction due to HIV, cancer in PLWH is often responsive to immunotherapy. Thus far, the best-studied agents are tumor-targeting monoclonal antibodies in the management of HIV-associated lymphomas. Rituximab, a monoclonal antibody to the B-cell antigen CD20 that works in part through antibody-dependent cell-mediated cytotoxicity, is associated with improved overall survival in NHL when compared to chemotherapy alone (102–104). In people with HIV-associated lymphoma, a pooled analysis of over 1,500 patients noted that rituximab improved overall survival in those with a CD4 count >50 cells/μL (105). Brentuximab vedotin, an antibody-drug conjugate directed at CD30 on Reed-Sternberg cells, has been shown to have activity in HIV-associated Hodgkin lymphoma: in a study of 6 patients with HIV and classical Hodgkin lymphoma, all achieved a complete response with minimal hematologic toxicity or infectious complications (106).

More recently, immune checkpoint inhibitors (CPIs), monoclonal antibodies to cytotoxic lymphocyte associated protein 4 (CTLA-4) or programmed cell death 1 or its ligand (PD-1 and PD-L1), have gained widespread use due to their demonstrated activity and favorable toxicity profile in many malignancies. CPIs, which function by blocking T-cell inhibitory signaling, have performed well in clinical trials of many malignancies that are common in the setting of HIV, including lymphoma, lung cancer, cervical cancer, liver cancer, and

head and neck cancers (107, 108). While nearly all these trials excluded PLWH (109), case reports and retrospective cohort studies from US and European collaborative groups have described an acceptable safety profile with the use of nivolumab, pembrolizumab, and ipilimumab in PLWH, with reported tumor responses in classical Hodgkin lymphoma, melanoma and lung cancer (68, 69, 110–116). A systematic review of CPIs in PLWH noted overall response and adverse event rates that were similar to the general population. In the subset of patients in whom viral load was measured, HIV remained suppressed in 93% of participants, and CD4 counts increased modestly. Notably, CPI use in KS was associated with an overall response rate of 63% (117). A prospective cohort study of 10 PLWH with NSCLC treated with nivolumab noted similar response rates to HIV-uninfected patients: 2 patients had a partial response, 4 had stable disease, and 4 progressed. All patients tolerated nivolumab well with no serious adverse events (70). A prospective phase 1 study of pembrolizumab in PLWH with a CD4 count >100 cells/μl and advanced cancer demonstrated evidence of safety and activity in KS, NHL, lung cancer, and liver cancer (118). A study of durvalumab in 20 aviremic PLWH with advanced solid tumors likewise reported no serious adverse events, nor evidence of HIV reactivation during durvalumab therapy (119). Ongoing studies evaluating CPIs in HIV-associated cancers include a phase 1 study of nivolumab (anti-PD-1) combined with ipilimumab (anti-CTLA-4) in relapsed classical Hodgkin lymphoma or solid tumors (NCT02408861), a phase 2 study of nivolumab in advanced non-small cell lung cancer (NCT03304093), a phase 2 study of durvalumab in advanced cancer (NCT03094286), a study of pembrolizumab as first systemic therapy in KS (NCT02595866), and intralesional nivolumab for limited cutaneous KS (NCT03316274).

CANCER IMMUNOTHERAPY AND HIV PERSISTANCE

Although HIV-infected individuals on ART may have undetectable plasma HIV RNA by standard clinical assays, a reservoir of latently HIV-infected cells (120, 121) persists from which the virus will resurface after discontinuation of ART (122). Persistence of the HIV reservoir is partly due to the inherent longevity of resting memory CD4 T cells; growing evidence suggests that its persistence is maintained by clonal expansion (123, 124). In whole genome-based studies, HIV integration favors sites of active gene transcription (125) which benefits HIV replication and establishment of latency (126, 127) and promotes pathways associated with oncogenesis (124). The HIV reservoir has been a major subject of research into a functional cure for HIV. One theory called "kick and kill" (**Figure 1**) (128, 129) proposes that HIV latency reversal in the setting of ART (meaning activation of HIV replication within a latently infected cell), can lead to increased immunogenicity of HIV infected cells, enhancement of anti-HIV immunity, and increased cell death of HIV reservoir cells.

Several immunotherapeutic agents used in the treatment of cancer may have cause HIV latency reversal and/or have a targeted effect on HIV persistence. CPIs have been proposed to have latency reversal activity. Anti-PD-1 therapy is associated with changes in CD4 count and HIV RNA (130–132), perhaps due to direct targeting of the HIV reservoir. PD-1 and CTLA-4 expression are increased in the setting of chronic HIV infection, and HIV DNA and unspliced RNA are enriched in PD-1+ cells in blood and lymph nodes of individuals with HIV on ART (133–136). Multiple case reports and prospective studies have documented transient increases in HIV transcription in CD4 cells in people with HIV-associated malignancies on ART who are treated with anti-PD-(L)1 drugs, although many of these participants later experienced decreases in plasma HIV RNA (117, 128, 129, 132, 137). In one study, 2 of 28 patients who had undetectable HIV RNA prior to CPI therapy developed detectable HIV RNA, whereas 5 of 6 patients who had detectable viremia experienced a decrease in their viral load (117). A prospective study of the effect of ipilimumab in 24 PLWH with detectable viremia and without cancer, of whom 17 were on ART, also demonstrated a range of responses: 2 participants had slight decreases in HIV RNA but 14 had slight increases. None experienced significant change in CD4 or CD8 T cell

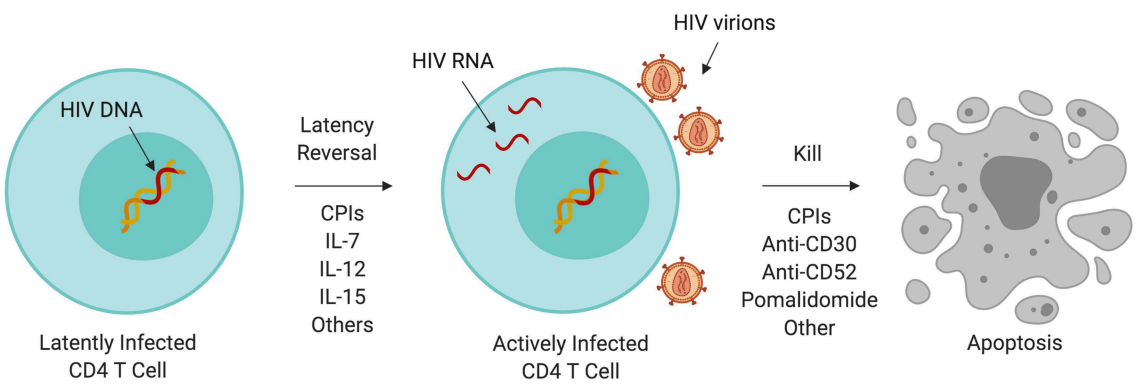

FIGURE 1 | Immunotherapy and the HIV reservoir. A variety of immunotherapeutic agents used to treat cancer may perturb the HIV reservoir through induction of latency reversal or increased cell killing. Some of these agents are being evaluated in clinical trials targeting HIV persistence. CPI, immune checkpoint inhibitor; IL, interleukin.

count (138). These observations support the activity of CPIs to produce latency reversal. Additional studies are being performed to evaluate the effects of CPIs on anti-HIV T-cell function.

The effects of anti-CD30 monoclonal antibodies on HIV latency have also been investigated. Early work in HIV demonstrated that cross-linking of CD30 on latently-infected CD4 T cells induced HIV transcription (139). More recently, brentuximab vedotin has been associated with transient loss of detectable CD4 T-cell HIV RNA and reduction in plasma HIV viremia (76). CD30 is therefore speculated to be a marker of latent, but transcriptionally-active, HIV-infected cells and a potential therapeutic target for HIV eradication (140).

Alemtuzumab is a monoclonal antibody targeting CD52, which is expressed by T cells including HIV-infected T cells, regardless of CD4 count or plasma viremia. Latently-infected CD4 T cells have been eliminated *in vitro* with alemtuzumab (78). *In vivo*, a case report of alemtuzumab in an individual with HIV and Sezary syndrome described decreased frequency but not elimination of HIV-infected CD4 T cells (79). Alemtuzumab was also part of the conditioning regimen of one of the patients with sustained HIV aviremia after HSCT (141).

T-cell growth factors, many of which are being investigated for cancer indications, have also been shown to affect the HIV reservoir. Interleukin 7 (IL-7) is a homeostatic cytokine that increases T-cell repertoire diversity through expansion of naive T cells (82) and is being investigated in several malignancies. IL-7 levels increase in HIV-associated CD4 lymphocytopenia and decrease with immune reconstitution (142). Exogenous administration of IL-7 is associated with dose-dependent increases in CD4 and CD8 T cells in PLWH on ART (143), including HIV-specific CD8 T cells (83). In patients with suppressed HIV, administration of IL-7 led to transient increases in HIV viral load without observed clinical sequelae (84), as well as enhanced anti-HIV CD8 activity. Another T-cell growth factor, IL-15, induces antigen-specific T-cell proliferation, most pronounced in the CD8 compartment (94, 95, 144, 145). IL-15 is produced during acute HIV infection (95). Stimulating NK cells with IL-15 *ex vivo* from participants with suppressed HIV on ART led to *ex vivo* killing of latently-infected CD4 T cells by cytotoxic CD8 T cells (96). Early phase studies of IL-7 and IL-15 in several malignancies are underway.

HEMATOPOIETIC STEM CELL TRANSPLANTATION IN HIV

In 2007, an individual with HIV infection and leukemia underwent hematopoietic stem cell transplant (HSCT) in Berlin, using cells from a donor who was homozygous for CCR5-delta32, a mutation that renders CD4 cells resistant to CCR5-tropic HIV. After transplant, HIV was undetectable in blood and biopsy specimens, despite discontinuation of ART (146, 147). Recently, a second patient who underwent allogeneic HSCT for Hodgkin lymphoma using cells from a homozygous CCR5-delta32 donor and whose HIV remained undetectable 18 months after stopping ART (141) was described. Allogeneic stem cell transplant itself appears to substantially decrease the HIV reservoir. In the European IciStem cohort of PLWH on ART who underwent HSCT for hematologic malignancies from CCR5 wild-type donors with full donor engraftment and who remained on ART, 5 of 6 were found to have no detectable HIV DNA in CD4 cells from blood and tissues and no evidence of HIV in a humanized mouse viral outgrowth assay (148). However, ART interruption is required to demonstrate functional cure, and in cases of allotransplants from CCR5 wild-type donors, HSCT has failed to produce long-lasting viral suppression in the absence of ART. In an ART interruption study of 2 PLWH who underwent HSCT for hematologic malignancies from CCR5 wild-type donors and had undetectable HIV RNA for years post-transplant while on ART, both participants developed detectable viremia after ART interruption: patient A at day 84 and patient B at day 225 (149).

Given the success of allotransplants from homozygous CCR5-delta32 donors, CCR5-mutant cell products have been developed via gene editing and have been shown to be safe when infused into participants with chronic aviremic HIV. When ART was interrupted, the edited CD4 cells declined at a slower rate than endogenous CD4 cells. While these results are promising, additional work is required to develop a scalable approach to address HIV persistence on ART (150–153).

IMPROVING OUR UNDERSTANDING OF HIV-RELATED CANCER

As PLWH are living longer, cancer has become a major cause of morbidity and mortality, well above the burden faced by the general population. Although the incidence of AIDS-defining malignancies has decreased, mortality associated with NADMs is rising. Given the persistent immune abnormalities despite ART and the implications for cancer risk, immunotherapy is uniquely poised to improve outcomes in HIV-associated cancers. In order to advance our understanding, PLWH must be included in immuno-oncology studies. Recent recommendations from ASCO and the FDA provide guidance for appropriate inclusion of PLWH and cancer in clinical trials (109, 154). Furthermore, studying cancer immunotherapy in this population represents an opportunity to gain a better understanding of HIV itself. Investigation of the immunologic and viral responses to cancer immunotherapy in PLWH will lead to novel insights into HIV elimination and, above all, improve the outcomes of people with HIV and cancer.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Toward T Cell-Mediated Control or Elimination of HIV Reservoirs: Lessons From Cancer Immunology

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As the AIDS epidemic unfolded, the appearance of opportunistic infections in at-risk persons provided clues to the underlying problem: a dramatic defect in cell-mediated immunity associated with infection and depletion of $CD4^+$ T lymphocytes. Moreover, the emergence of HIV-associated malignancies in these same individuals was a clear indication of the significant role effective cellular immunity plays in combating cancers. As research in the HIV field progressed, advances included the first demonstration of the role of PD-1 in human T cell exhaustion, and the development of gene-modified T cell therapies, including chimeric antigen receptor (CAR) T cells. In the intervening years, the oncology field has capitalized on these advances, effectively mobilizing the cellular immune response to achieve immune-mediated remission or cure of previously intractable cancers. Although similar therapeutic advances have not yet been achieved in the HIV field, spontaneous $CD8^+$ T cell mediated remission or functional cure of HIV infection does occur in very small subset of individuals in the absence of anti-retroviral therapy (ART). This has many similarities to the $CD8^+$ T cell mediated functional control or elimination of cancers, and indicates that immunotherapy for HIV is a rational goal. In HIV infection, one major barrier to successful immunotherapy is the small, persistent population of infected $CD4^+$ T cells, the viral reservoir, which evades pharmacological and immune-mediated clearance, and is largely maintained in secondary lymphoid tissues at sites where $CD8^+$ T cells have limited access and/or function. The reservoir-enriched lymphoid microenvironment bears a striking resemblance to the tumor microenvironment of many solid tumors–namely high levels of anti-inflammatory cytokines, expression of co-inhibitory receptors, and physical exclusion of immune effector cells. Here, we review the parallels between $CD8^+$ T cell-mediated immune control of HIV and cancer, and how advances in cancer immunotherapy may provide insights to direct the development of effective HIV cure strategies. Specifically, understanding the impact of the tissue microenvironment on T cell function and development of CAR T cells and therapeutic vaccines deserve robust attention on the path toward a $CD8^+$ T cell mediated cure of HIV infection.

Keywords: HIV, cancer, remission, CTL (cytotoxic T lymphocyte), immunothearpy

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INTRODUCTION

Human immunodeficiency virus (HIV) remains one of the most pervasive global health challenges of our time. Currently there are an estimated 37 million persons infected with HIV worldwide with more than 35 million AIDS-related deaths to date (1). The development of combination anti-retroviral therapy (ART) has mitigated the severity of this disease, significantly improving survival rates and life expectancy for persons infected with HIV.

Despite these encouraging developments, the number of new HIV infections has remained largely static and co-morbidities including cancers continue to develop in HIV treated individuals. Furthermore, individuals must remain on life-long therapy due to the persistence of latently-infected $CD4^+$ T cells, intractable to ART and immune detection due to proviral integration into the host chromosome and being transcriptionally silent, and due to sequestration in anatomical sites largely devoid of HIV specific $CD8^+$ T cells [reviewed in (2)]. In particular, secondary lymphoid sites, such as the gut-associated lymphoid tissue (GALT) and lymph nodes (LN), bear the largest fraction of the HIV burden in ART suppressed individuals (3). Unique microenvironments and distinct compartmentalization of immune subsets within these anatomical sites provide an ideal niche for ongoing viral persistence and limited immune pressure. Although T cell exhaustion and immune escape further hinder the impact of adaptive HIV-specific $CD8^+$ T cell responses, there are clear examples of persons who spontaneously control HIV for decades without medications (4), indicating that effective HIV immune containment, if not eradication, can be achieved despite these barriers.

As the HIV field has attempted and largely failed thus far to mobilize the immune system to better prevent, treat, and cure infection, the cancer field has experienced dramatic advances through application of immunotherapeutic interventions that either genetically modify and re-direct T cells or liberate endogenous T cell responses to tumor neoantigens. Remarkable examples of immune-mediated disease-free remissions have been achieved for some previously intractable malignancies, such as melanoma (5–7), non-small cell lung cancer (8, 9), and chemotherapy-refractory leukemia and lymphoma (10, 11). Indeed, key barriers to cancer eradication bear multiple similarities to hurdles experienced in immune control of HIV, such as lack of accessible antigens, chronic immune dysfunction, and tissue microenvironments that impede effective clearance of cancerous cells. The dramatic advances in therapeutic interventions to augment effective $CD8^+$ T cell immunity in cancer provide important insights for therapeutic interventions in HIV infection. Here, we discuss the role of $CD8^+$ T cell mediated immunity in HIV and cancer, and lessons learned from the advances in cancer treatment that may aid in the development of HIV cure strategies.

EVIDENCE FOR CTL-MEDIATED CONTROL OF HIV AND CANCER

Among the most striking data implicating $CD8^+$ CTLs in control of AIDS virus infections come from rapid rebound of viremia following $CD8^+$ T cell depletion in the non-human primate (NHP) model of SIV infection (12). These data are supported by human data demonstrating rapid emergence of HIV specific $CD8^+$ T cells mediating strong selection pressure concomitant with post peak viral decline (13–15) the observed inverse relationship of HIV-specific CTLs with both viral set-point and rate of $CD4^+$ T cell loss (16, 17) and the profound viral control exhibited by a select group of elite controllers who, in the absence of ART, maintain potent HIV-specific T cell responses and do not progress immunologically [reviewed in (4)]. These untreated elite controllers represent <1% of HIV-infected persons, some of whom have been infected for more that three decades and maintain prolonged control of plasma viremia (HIV RNA <50 copies/mL of plasma) (18, 19).

The role of $CD8^+$ T cells in this remarkable control of HIV is consistently seen in the context of expression of certain "protective" HLA class I alleles such as B*27 and B*57, and specific amino acids lining the class I peptide binding groove that present viral peptides for $CD8^+$ T cell recognition (20, 21). Containment of viremia in elite controllers has been linked to more polyfunctional $CD8^+$ T cells than in persons with progressive disease (22), perhaps in part due to maintenance of virus-specific $CD4^+$ T cells (23), as well as enhanced recognition of epitope variants (24).

Complementary evidence of $CD8^+$ T cell mediated immune control of HIV also derives from studies of the virus itself. Transmission of amino acid "escape" mutations within the 8–10 amino acid epitopes targeted by CTL is associated with worse outcomes due to replication of pre-adapted viruses (25, 26). Other studies have shown impaired viral fitness due to viral mutations associated with $CD8^+$ T cell selection pressure (27, 28). More recent studies indicate that persons who spontaneously control HIV without the need for medication do so at least in part by targeting epitopes containing highly networked amino acids that are critical to structure and function of the virus (29, 30). These sites are highly mutationally intolerant, such that immune driven mutations are likely to impair viral fitness and be less resolvable by compensatory mutations at secondary sites. In addition, HIV infection and depletion of $CD4^+$ T cells, with preferential infection of HIV-specific $CD4^+$ T cells (31), exacerbates immune impairment by providing insufficient help for HIV-specific $CD8^+$ T cells. Indeed, immediate treatment of acute infection leads to preservation of $CD4^+$ T cell responses and induction of $CD8^+$ T cells with greater functionality (32).

Despite a long history of debate as to whether the immune system plays a role in controlling cancers, particularly of non-viral origin, it is now clear $CD8^+$ T cell-mediated immunity is also a major host defense against tumors. In 1909, it was first hypothesized that immune surveillance suppressed the outgrowth of cancers (33), but it took decades to identify cancer neoantigens, giving credence to the idea that tumors could be recognized as foreign (34). Early, *in vitro* studies demonstrated that melanoma-specific $CD8^+$ T cells could lyse tumor targets (35). Further evidence included the identification of tumor associated antigen (TAA) expressed on tumor cells but not on normal cells, and the observation that a high frequency of TAA-specific $CD8^+$ T cells localized within tumors that spontaneously regressed (36). Density of tumor infiltrating

$CD8^+$ T cells (TILs) has been shown to negatively correlate with progression of colorectal metastasis (37) and oligoclonal expansions of tumor-infiltrating T cells have been associated with tumor regression (38). Furthermore, the development of checkpoint inhibitors that target and effectively block the PD-1 and CTLA-4 axes have convincingly underscored the importance of endogenous $CD8^+$ T cells in the recognition and elimination of tumor cells, but most importantly that the cancer-specific immune response can be manipulated for therapeutic benefit. Of note, this checkpoint blockade-mediated liberation of anti-tumor T cell responses is most effective in tumors that have a high mutational burden (39, 40) [i.e., that result in greater presentation of neo-antigens, especially those with mismatch-repair defects (41, 42)], and in those that upregulate the checkpoint ligands such as PD-L1 (43, 44). In addition, engineered autologous T cells transduced to express synthetic, chimeric antigen receptors, or CAR T cells, have demonstrated that T cells can be engineered to recognize surface antigens present on tumor cells and successfully eliminate the cancer, particularly lymphoid malignancies like B-cell leukemia (45), lymphoma (46, 47), and multiple myeloma (48).

MECHANISMS OF $CD8^+$ T CELL IMMUNE FAILURE IN HIV AND CANCER

Immune failure is a hallmark of cancer and persistent viral infections such as lymphocytic choriomeningitis infection (LCMV), simian immunodeficiency virus (SIV) and HIV. Understanding the mechanisms driving immune dysfunction is critical to the rational development of immunotherapies for the treatment of both HIV and cancer. There are three areas that are particularly relevant to both HIV and cancer, namely immune exhaustion, immune escape, and immunoregulatory factors in the lymphoid tissue (HIV) and tumor microenvironment (cancer).

Immune Exhaustion

One of the major obstacles to immune control of both HIV and cancers is progressive T cell exhaustion in the face of ongoing pathogen burden. The original demonstration of this phenomenon came from the lymphocytic choriomeningitis virus (LCMV) model (49). Armstrong and Clone 13 LCMV variants result in vastly different immunological outcomes, associated with differences in antigen load and persistence (50). Clone 13 has two nucleotides that differ from LCMV Armstrong, resulting in ineffective clearance by $CD8^+$ T cells, chronic viremia, and progressive dysfunction of LCMV-specific $CD8^+$ T cells. This includes impaired proliferative capacity and decreased polyfunctionality. Gene expression analysis of virus-specific $CD8^+$ T cells revealed upregulation of the negative immunoregulatory molecule PD-1 on these cells in the context of Clone 13 infection compared to Armstrong (49), indicative of immune dysfunction with ongoing antigen persistence. Importantly, the immune exhaustion was shown to be reversible through blockade of the interaction of PD-1 with its ligand PD-L1 or PD-L2.

These features of T cell exhaustion are strikingly similar to what is observed in untreated HIV infection and cancer. Chronic viral infection and cancer are both disease states with inadequate antigen clearance. Memory T cell (T_{mem}) development is impaired, and effector T cell (T_{eff}) become functionally exhausted with elevated and sustained expression of the check-point receptors like PD-1. The first evidence that reversible T cell exhaustion occurs in humans came from studies of HIV infected persons, and like in the LCMV model, blockade of the interaction with PD-L1 or PD-L2 could at least partially reverse cellular dysfunction (51). In cancer, *in vitro* studies showed that tumor-specific T cells in human melanoma metastases share many features of the exhaustion signature that was characterized in LCMV infection (52). Exhaustion was found to be associated with altered epigenetic and transcriptional profiles, a distinct metabolic signature (53–55) and impaired responses to homeostatic cytokines (56). In HIV infection, PD-1 levels are significantly increased on $CD8^+$ T cells during chronic HIV infection, directly correlating with plasma viremia and inversely with $CD4^+$ T cell counts (51, 57). It was also found that T cells residing within the LN compartment exhibited even greater levels of inhibitory receptors when compared to the peripheral blood (57) demonstrating anatomical differences in parameters of immune exhaustion, posing the question of how distinct microenvironments shape T cell function. Indeed upregulation of these immunoregulatory ligands on tumor cells is an important mechanism of immune dysregulation (57). Beyond inhibitory receptor expression, the transcriptional and epigenetic profiling of virus-specific and tumor-specific CD8 T cells has revealed key similarities and differences between $CD8^+$ T cell responses in the two disease settings. Multiple transcriptional regulators have been associated with $CD8^+$ T cell exhaustion, including NFAT, Eomes, BLIMP-1, BATF, FOXO1, FOXP3, IRF4, VHL, c-Maf, implicating various metabolic, and signaling pathways as important contributors to various states of CTL exhaustion [(58–62); reviewed in (50)].

Another consideration in loss of T cell effector functions in HIV and cancer is depletion or diminished activity of antigen-specific $CD4^+$ T cells [reviewed in (63, 64)]. These cells enhance CTL expansion, activity, migration, tissue invasion, and memory differentiation. HIV preferentially infects HIV-specific $CD4^+$ T cells (31), and loss of these cells is associated with a reversible defect in $CD8^+$ T cell *in vitro* proliferation (65). CD27 agonism was shown to recapitulate $CD4^+$ T cell help by improving induction of effector $CD8^+$ T cells, antigen-specific cell killing, and overall survival in a murine cancer vaccine model (63). Loss of $CD4^+$ T cells by HIV infection, or diminished antigen-specific $CD4^+$ T cell activity by tumor or virus-induced downregulation of MHC class II impairs induction, expansion, and efficacy of CTL responses capable of viral or tumor clearance, and means to rectify this are needed for both HIV and cancer.

Immune Escape

Effective primary $CD8^+$ T cell responses may drive viral or tumoral evolution, particularly in the context of rapidly mutating pathogens, allowing the outgrowth of variants that are no longer recognized by the host $CD8^+$ T cell response. This has been

termed $CD8^+$ T cell escape in the HIV context [reviewed in (66)], and tumor immunoediting in the cancer field [reviewed in (67)], rendering initial $CD8^+$ T cell responses ineffective. Following immune escape, induction of effective *de novo* $CD8^+$ T cell responses targeting the mutated epitope or a different epitope is necessary to restore antigen-specific immune control.

In HIV infection, where more than 300 viral epitopes and their restricting class I alleles have been defined, immune escape occurs during the initial period of peak viremia [reviewed in (68)]; moreover, transmission of $CD8^+$ T cell immune escape variants is already shaping global viral evolution (69). Of particular relevance to any immunotherapeutic approaches is the finding that the majority of immunodominant CTL epitopes in persons with chronic infection may harbor escape mutations (70), such that simple reversal of CTL dysfunction may be insufficient to augment an antiviral effect.

T cell responses against tumor-associated antigens (TAAs) may be rendered ineffective by immune escape in tumors with high mutation burden. The concept of tumor immunoediting encompasses three phases of the interaction between the protective aspects of adaptive immunity against cancers as well as the "tumor sculpting" functions of the immune response: elimination, equilibrium, and escape (71). TAAs arise from non-synonymous somatic mutations (NSSMs) in protein-coding genes, aberrant expression of an embryonic, placental, testes or other tissue-specific differentiation genes, aberrant overexpression of a wild type gene, and viral proteins expressed by cancer cells. In contrast, the high mutability of HIV is due to the infidelity of the viral reverse transcriptase, which induces errors during the process of converting incoming viral RNA into proviral DNA. On the positive side, mutations that escape adaptive immune surveillance may also inflict a fitness cost to the virus or cancer cell and thus serve to the advantage of the host. And in the HIV context, predictable mutations that arise under immune selection pressure can be incorporated into vaccine immunogens, such as is currently being tested in an efficacy trial of a mosaic vaccine (72).

Tumor and Lymph Node Microenvironments

One of the shared challenges for $CD8^+$ T cell mediated clearance of HIV or cancer is the need for migration into and induction of effector function within immunosuppressive tissue environments. In HIV infection, this involves the lymph node microenvironment (LNME), whereas in cancer the tumor microenvironment (TME) is the major site of immune engagement (**Figure 1**).

Lymph nodes (LN) are not only the inductive site for adaptive immune responses, but are a major site of HIV infection [reviewed in (79, 80)]. They are characterized by interaction of lymphocytes and antigen-bearing dendritic cells (DC) within a fibroblastic reticular network (FRC) (81). Localization of DC subsets, stromal cells, and immune cells within the LN in combination with various cytokines, costimulatory signals, secondary metabolites and the amount and nature of foreign antigen (82–84) impact T cell differentiation by establishing distinct microenvironmental niches (85). Moreover, the LNME is largely immunosuppressive, regulating both naïve and pre-activated T cells through the production of indolamine 2–3 dioxygenase (IDO), Prostaglandin E2 (PGE_2), adenosine 2A receptor (A2AR) agonists and tumor growth factor β (TGFβ) (78, 86).

As the major site of HIV replication is in $CD4^+$ T cells, LNs and the gut associated lymphoid tissue are the initial and persistent targets of infection. Importantly, germinal centers (GC) within LN are important anatomic sites for HIV persistence [reviewed in (87)]. Peripheral blood $CD4^+$ T cells constitute ∼0.2% of the HIV reservoir, whereas lymphoid resident $CD4^+$ T cells represent >50% of the overall HIV burden (3). T follicular helper cells (Tfh, defined as $CXCR5^{hi}$ $PD\text{-}1^{hi}$ $CD4^+$ T cells) accumulate during chronic HIV/SIV infection, and are highly susceptible to HIV infection (88–91), contributing to both viral production and persistence during chronic untreated and treated HIV infection. Importantly, germinal centers largely exclude HIV-specific CTLs (92).

Progressive dismantling of the FRC networks within lymphoid tissue during HIV infection (93), a consequence of profound $CD4^+$ T cell loss, results in increased collagen deposition and significant fibrosis (93–95). These alterations restrict access to IL-7 and limit the life-span of naïve $CD4^+$ and $CD8^+$ T cells within the LN and the overall generation of T cell immunity within the lymphoid tissue. Excessive accumulation of collagen and other extracellular matrix (ECM) components that occur during HIV/SIV infection has been linked to an early induction of an immunoregulatory response within secondary LT such as increased levels of TGFβ (96–98).

In contrast to the limited understanding of the LNME during chronic HIV/SIV, the TME and its corresponding impact on immune function has been well-characterized. The physical and chemical content within the TME such as the extracellular matrix (ECM), fibroblasts, stromal cells, myeloid cells, and immune cells as well as secreted chemokines and cytokines, collectively impact tumor progression and impair immune function either directly or in *trans* (99). The induction and localization of immune subsets such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor infiltrating DCs (TIDCs), and tumor-associated macrophages (TAMs) can hinder effector function and $CD8^+$ T cell infiltration and actively contribute to the maintenance of $CD8^+$ T cell exhaustion. Tumor cell oncogenic pathways, including oncogenic Wnt/ß-Catenin signaling and gain of function MYC have also been shown to impart immunosuppressive signals within the TME that limit T cell recruitment, activation, and infiltration [reviewed in (100)]. Transcriptional regulation through Signal Transducer and Activator of Transcription 3 (STAT3) within $CD8^+$ T cells has also been implicated in limiting $CD8^+$ T cell recruitment to (101) and cytotoxic function within (102) tumors. Significant metabolic challenges also occur within the TME which impact T cell function and tumor regression including hypoxia, decreased pH, increased levels of extracellular adenosine, high interstitial fluid pressure, and increased extracellular matrix (ECM) stiffness, akin to what is observed during LN fibrosis in chronic HIV infection.

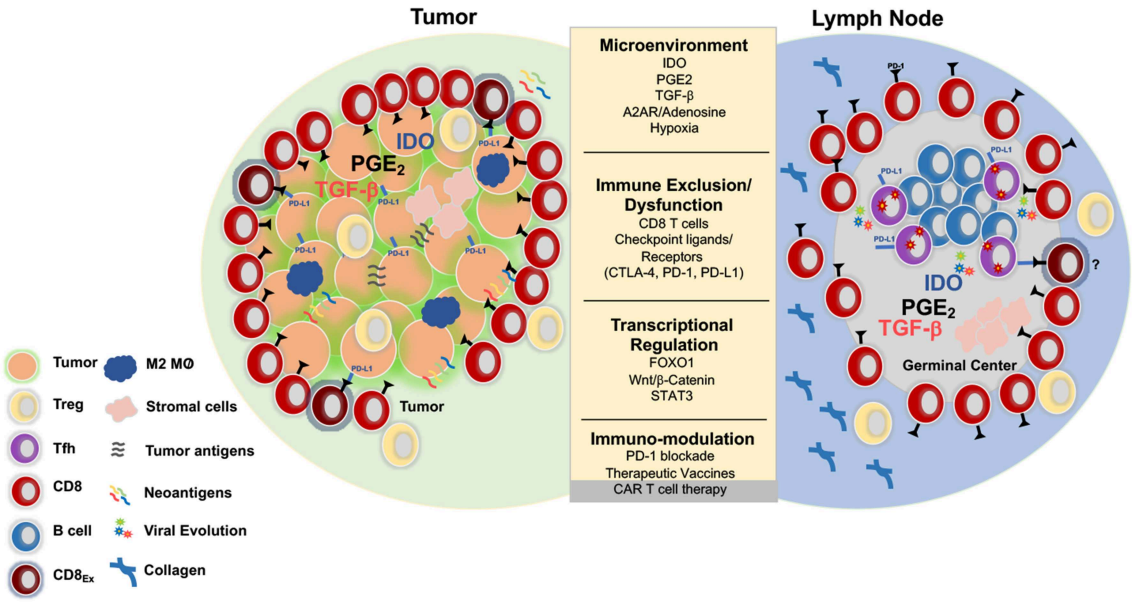

FIGURE 1 | Parallels between immunoregulation in solid tumors and lymph nodes. Tumors and LNs are composed of stromal and immune cells that secrete cytokines and growth factors such as transforming growth factor b (TGFβ), prostaglandin E2 (PGE2), indolamine 2-3-dioxygenase (IDO), and adenosine that shape the tumor and LN microenvironment and collectively contribute to suppression of the T cell response. Adenosine signals through the adenosine 2A receptor (A2AR) and promotes production of cyclic AMP, which impairs T cell trafficking, proliferation, and cytotoxicity (73). Immunosuppression is also induced by regulatory T cells (Treg) that express higher levels of CTLA-4, an inhibitory receptor that outcompetes CD80/CD86 on the surface of effector cells and promotes the production of IDO, an enzyme that degrades tryptophan and leads to impaired proliferation and Treg differentiation. Additionally, Tregs express high levels of CD73/CD39, enzymes that convert ATP to adenosine, which inhibit immune function (74, 75). Tumors upregulate inhibitory ligands such as PD-L1 that bind to inhibitory receptors resulting in suppression of adaptive immune responses (75). Several strategies have been developed in the immune-oncology field to overcome these barriers such as checkpoint blockade, small molecule inhibitors, therapeutic vaccines, and CAR T cell therapy. These immune based therapies can all be extended to the HIV cure field. Several mechanisms that result in resistance to checkpoint blockade and CD8 T cell exclusion include activation of the WNT/β -catenin pathway (76), localization of M2 macrophages within the tumor (77), and the secretion of TGFβ (78).

Tumor associated hypoxia commonly occurs during the later stages of cancer, but hypoxia inducible factors (HIFs) can be upregulated due to acidification and glycolytic metabolites within the TME. The concerted effort to understand the TME has led to the development of immune based therapies, currently in clinical trials for the treatment of solid tumors [reviewed in (103)].

Limitations to $CD8^+$ T cell trafficking act to impede immune clearance in both HIV infection and solid tumors. Through a variety of mechanisms, $CD8^+$ T cells appear to be excluded from both solid tumor masses and LN germinal centers. In both HIV and numerous tumors the relative frequency of tumor infiltrating or GC infiltrating $CD8^+$ T cells is inversely correlated with disease outcome in numerous cancers and HIV infection, respectively (104–108). Studies have demonstrated that intra-follicular localization of HIV specific $CD8^+$ T cells is correlated with lower plasma viremia (106); however, whether the cytolytic function of these $CD8^+$ T cells mediates control remains unknown. Studies from patients with follicular lymphomas (FL, tumors situated in LN) indicate that the presence of functional granzyme B^+ CTLs at the follicular border within the LN correlated with prolonged progression free-survival (109), whereas higher levels of the inhibitory receptor TIM3 on FL CTLs correlated with shorter relapse-free survival (110).

Understanding the immune suppressive elements of the LNME and TME are likely to lead to additional avenues to immunotherapy. For example, one potential mechanism of immunoregulation shared between the LNME and TME is the pleiotropic cytokine TGFβ. TGFβ has been shown to promote immune exclusion, impair immune function, and limit responsiveness to check-point blockade in metastatic urothelial cancer and other tumors (78, 111). Administration of a TGFβ blocking antibody in combination with anti-PD-L1 has been shown to promote T cell localization within tumors and enhance anti-tumor immunity, leading to increased regression (78, 112). Higher levels of TGFβ have also been observed in the LN during progressive HIV infection. These shared observations suggest that immunoregulation via TGFβ might be playing a similar role in restraining $CD8^+$ T cell effector function in the LNME and TME. These data demonstrate the potential for $CD8^+$ T cells within LN sites to exhibit cytotoxicity. However, further investigation is required to elucidate the conditions under which $CD8^+$ T cell cytotoxicity can occur within the LN, which will have direct implications for the development of HIV cure therapeutics.

IMPLICATIONS FOR IMMUNOTHERAPEUTIC INTERVENTIONS

Therapeutic vaccines and immune based therapies aimed at achieving durable remission or cure of HIV have garnered significant interest within the HIV cure field with the identification of the first functionally cured individual known as the "Berlin patient" (113, 114) and hopeful second case reported in London earlier this year (115). Both underwent allogeneic hematopoietic stem cell transplant (HSCT) from HIV-resistant *CCR5Δ32* homozygous donors, resulting in reduced expression of the CCR5 co-receptor required for HIV entry. Both patients exhibited virological and immunological features of remission and have been considered cured. However, there is limited feasibility in applying HSCT as a standard of care approach to curing HIV due to toxicity, cost, availability of CCR5Δ32 HSCs and continued susceptibility to infection with CXCR4-utilizing strains (116, 117). The profound outcomes observed in these two cases have nevertheless energized efforts to develop safe and effective HIV cure strategies. Since robust immunological remissions occur in the 1 in 300 HIV infected persons (elite controllers), immune based approaches toward a functional cure are in our view the most rational approach. Given that immunotherapeutic interventions have transformed the cancer field, review of those therapeutic successes is likely to provide critical information for advancing HIV immunotherapy efforts.

Biological Inhibition of Immuno-Regulatory Pathways

Immune check-point inhibitor (ICI) therapy targeting the CTLA-4 and PD-1 pathways has profoundly altered the management of several cancers, significantly enhancing anti-tumor responses and prolonging progression-free survival. CTLA-4 competes with the co-stimulatory molecule CD28 for binding to CD80/86 on antigen presenting cells, resulting in attenuation of T cell signaling. Ipilimumab, a monoclonal antibody to CTLA-4, blocks this interaction and prevents the inhibitory signal, allowing CTL to kill cancerous or virus infected cells. Pembrolizumab and nivolumab, monoclonal antibodies targeting the PD-1 pathway, engage the PD-1 ligand on target cells, resulting in dephosphorylation of TCR proximal signaling and decreased polyfunctionality, cell cycle progression, survival, and effector function (118, 119). Ipilimumab was the first FDA approved ICI, based on studies in advanced melanoma showing a modest improvement in the overall survival of patients previously treated for metastatic melanoma (120). At present, overall response rate of single ICI therapy is only about 30% in most tumor types for which activity has been shown, such as non-small cell lung cancer (NSCLC), renal cell carcinoma, and metastatic melanoma (7, 121, 122). Biomarkers of ICI responsiveness include an immune inflamed tumor phenotype, described as a gene signature of immune related genes (123), pre-existing anti-tumor $CD8^+$ T cells (124), low levels of circulating immunoregulatory cells and cytokines such as IL-10 and TGFβ (125), and a high tumor mutational burden which leads to high levels of tumor associated neoantigens, presumably associated with neoantigen-specific T cells (126, 127).

Efforts to better predict treatment outcomes are advancing effective implementation of ICI therapy for cancer. Parameters including displayed increased localization of $CD8^+$ T cells to the tumor core, and increased expression of check-point regulators such as PD-L1 expression on tumor stroma have been shown to correlate with positive disease response to ICI (126, 128). Interestingly, these findings parallel the association of follicular infiltrating $CD8^+$ T cells within germinal centers during HIV/SIV infection. Higher frequencies within the GC are associated with reduced plasma viral loads during chronic infection, and these cells retain higher levels of inhibitory receptors (105, 106, 108, 129) and are more responsive to anti-PD-1 therapy (130).

Despite the fact that the initial demonstration of ICI leading to augmentation of CTL function came from studies of HIV, the effective use of ICI in HIV infected individuals is yet to be realized. Indeed, most *in vivo* data assessing ICI for the treatment of AIDS virus infection have been generated in the SIV macaque model. *In vivo* PD-1 blockade of progressive SIV infection resulted in an increase in magnitude and quality of SIV specific $CD8^+$ T cells (131, 132), anti-viral B cells (131) and a transient decline in plasma viremia—a clear signal but far short of the best outcomes in cancer. A separate study observed a decrease in hyperimmune activation and microbial translocation in macaques treated with anti-PD-1 (133). Several limited case reports demonstrated that PD-1 blockade promoted increased anti-viral immunity in HIV infected patients and was tolerated (134, 135), but toxicity concerns remain.

PD-1 expression on $CD4^+$ T cells has also been explored as a potential cellular biomarker of immune cells enriched in active and latent SIV/HIV (89–91, 136–138). *In vitro* studies have described variable effects of PD-1 blockade on disrupting the latent viral reservoir (139), and substantial reactivation of the latent HIV reservoir with anti-PD-1 alone (140) and in combination with the latency reversal agent (LRA) bryostatin (141). In a macaque study, anti-PD-1 administration during suppressive ART led to transient increase in plasma viremia and a reduction in replication competent virus (142). These data suggest that PD-1 signaling may play a role in maintaining viral latency and blockade may allow for disruption and reactivation of the latent viral reservoir. $CTLA\text{-}4^+$ $PD\text{-}1^-$ $CD4^+$ T cells have also been implicated as a subset of T cells enriched in viral DNA during suppressive ART (143). A recent open-label study found that ascending doses of anti-CTLA-4 were well-tolerated and showed variable changes in detectable plasma viral RNA (144). Check-point blockade monotherapies have elicited modest immunological responses and reactivation of the viral reservoir, suggesting that combination therapeutic approaches may be required for significant destabilization of the HIV reservoir.

We believe that check-point blockade should be considered cautiously as a treatment modality for HIV, as ICIs carry significant toxicity profiles, setting a higher bar when alternative HIV treatments are available. Following ICI therapy for cancer, immune-related adverse events (irAEs), and increased immune cell infiltration into healthy tissues have caused autoimmune-like

toxicities. Severe irAEs are more common with ipilimumab (15–43% of patients) than nivolumab or pembrolizumab. However, 10–23% of patients given anti-PD-1 therapy still develop potentially life-threatening toxicities, that increase with co-administration of anti-CTLA-4 (145). A comprehensive meta-analysis conducted to assess irAE's resulting from ICI found higher risk of all-grade rash and colitis with anti-CTLA-4 treatment (146) and a case study of a patient with widespread uveal melanoma had an exceptional response to ipilimumab and nivolumab but suffered severe immune-related sequelae, with identical T cell clones found in the tissues affected (147). Moreover, a recent report assessing patients treated with a single-agent nivolumab or pembrolizumab for advanced cancer found an overall response rate of 82.5% in patients experiencing irAE (148), highlighting autoimmunity as an emerging biomarker for responsiveness to ICI. Thus, there is an ongoing medical need to not only define biomarkers of ICI resistance, but identify mechanisms underlying cross-reactivity and toxicity as well, in an effort to develop therapies that promote remission while limiting immune toxicities.

Adoptive T Cell Therapy

Chimeric antigen receptor (CAR) T cell immunotherapy has emerged as an important adoptive T cell therapy for the treatment of cancer with the recent FDA approval of the CD19-targeted CAR T cell "living drug," tisagenlecleucel (Kymriah) for the treatment of adult and pediatric B cell malignancies (45). CARs are synthetic receptors comprised of a single-chain variable fragment (scFV) of an antibody fused to a transmembrane domain and intra-cellular signaling complex [reviewed in (149)]. CAR T cells can re-direct specificity, functionality, and localization of T cells. Clinical trials have shown dramatic outcomes in patients with relapsed, refractory B cell cancers. A phase II clinical trial utilizing the CD19-targeting CAR for the treatment of B cell acute lymphoblastic leukemia (ALL) observed an 81% complete response (CR) rate at 28 days of follow-up, and a relapse-free survival of 59% with a short median 12-month follow-up. Despite initial high rates of remission, a significant fraction of patients will relapse with $CD19^+$ or $CD19^-$ tumors due to decreased persistence/function of the CAR T cells, antigen loss, and impairment due to the immunosuppressive tumor microenvironment (150). Increased persistence of circulating CAR T cells correlated with durable responses and improved clinical outcomes, indicating that these therapies can be further improved (151). This is especially true for CAR T cells that contain the 4-1BB costimulatory domain, which allows the CAR T cells to primarily utilize oxidative metabolism vs. glycolysis which CD28 costimulatory CARs rely on, allowing for enhanced persistence (152).

Despite persistence of CAR T cells, relapses can occur due to antigen loss post CAR infusion, which accounts for 40% of relapses (153). Moreover, the immunosuppressive tumor microenvironment significantly contributes to poor clinical outcomes by inducing early dysfunction, decreased expansion of CAR T cells, and limited persistence *in vivo* (154). A new generation of CAR T cells is being constructed to overcome these immune barriers. Alternative strategies include the development of CAR constructs targeting antigens other than CD19, the generation of bi-specific CARs that target more than one antigen, cytokine secreting CARs that produce IL-12 (155) and IL-18 (156), or anti-PD-1 (157). Additionally, CAR T cells may be engineered to express chemokine receptors and cytokines to improve their homing and tumor infiltration, but the efficacy of these approaches has not yet been confirmed in clinical trials. One example of this approach is engineering CAR T cells to express IL-7 and CCL19 (158) to enhance survival and T cell trafficking to secondary lymphoid sites, respectively.

Chimeric antigen receptor (CAR) T cell therapy for HIV actually predates its use in cancer, with the first studies completed in the mid 1990's, when a CD4-based CAR, shown to be effective *in vitro* and safe and well-tolerated *in vivo*, provided no clear clinical benefit and no reduction in the peripheral viral reservoir (159, 160). Follow-up studies attributed lack of efficacy to limited CAR T cell persistence, likely due to the high IL-2 dose used in manufacturing. The CAR contained CD4 extracellular and transmembrane domain, which might have increased CAR T cell susceptibility to infection, but lacked costimulatory domains, which could limit cellular functionality (161). Inclusion of costimulatory domains has been shown to be critical for CAR T cell efficacy in cancer. Despite limited function, there were no associated malignancies found with the transduced infused HIV CD4 CARs, which was promising for virally transduced adoptive T cell therapy. In the last several years, a growing number of high affinity broadly neutralizing antibodies (bNAbs) has been identified against HIV passive antibody infusion trials assessing the efficacy of HIV bNAbs have produced modestly decreased viral loads in viremic patients (162), increased clearance of infected cells (163), a delay in viral rebound (164, 165) and viral suppression post treatment interruption in 30% of patients until bNAb titers waned. Moreover, VRC01 and PGT121, bNAbs targeting the CD4 binding site and the V3 glycan of *env,* respectively, blocked HIV-1 replication from reactivated latently infected cells *in vitro* (166).

The growing repertoire of HIV bNAbs and enhanced function and persistence of second and third generation CAR T cell vectors have propelled efforts to design bNAb CARs for HIV remission or cure. Several groups have reported the development of bNAb and CD4 expressing CAR T cells (CD4 CAR) that can effectively limit HIV replication *in vitro* (159, 167–169). Moreover, the recent identification of follicular $CXCR5^+$ $CD8^+$ T cells and their potential contribution to control of viral replication within GC "hotspots" of active and latent HIV, has led to the development of HIV CAR T cells that over-express the chemokine receptor CXCR5, to promote trafficking into B cell follicles. A proof-of-concept study in macaques showed that infusion of $CD8^+$ T cells overexpressing rhesus CXCR5 increased localization within the GC (170). A separate group developed CXCR5+ CAR T cells expressing the CD4 co-receptor for HIV *env* specificity and found the cells functionally capable of limiting SIV infection *in vitro* and chemotaxing in response to CXCL13 in transwell and LN organoid cultures (171). These studies highlight the potential feasibility of developing virus-specific CAR T cells with an increased ability to traffic to specific anatomical sites such as GC that harbor a large fraction of the HIV reservoir.

Several considerations should be taken in developing adoptive cell therapies that enhance $CD8^+$ T cell trafficking to and detection of infected $CD4^+$ T cells in GC of lymphoid tissue. In contrast to tumor masses, GC/B cell follicles are critical anatomical sites for the induction of systemic immunity. Tfh cells localized within GC not only harbor a significant fraction of the HIV reservoir but are key mediators in the development of humoral immunity. Enhanced infiltration of $CD8^+$ T cells into the GC of LN may impact antibody development and pathogen specific immunity. Of note, recent trials assessing the efficacy of re-programmed autologous CAR T cells for the treatment of follicular lymphoma (FL) demonstrated successful restoration of immune function in patients with relapsed or refractory disease (172); however cytokine release syndrome (CRS) and neurotoxicities were experienced in line with what has been observed in other CAR T cell trials. Additional studies to assess the efficacy of ICI in the context of FL found varying objective response rates with some measured as high as 67% (172). However, with limited power in these studies, it is unknown whether ICI treatment of FL comes with a similar toxicity profile to what has been observed in other ICI responsive cancers. These findings suggest that enhanced accumulation of CD8+ T cells within lymphoid sites may be mechanistically supported and immunologically tolerated, but further studies need to be conducted to understand $CD8^+$ T cell targeting of LNME and the associated toxicities of targeting cells at LN sites.

Additionally, although the LNME has not been fully characterized in the context of HIV infection, collective data suggest an immunosuppressive environment may hamper the local functionality of CAR T cells. Immuno-oncology strategies to enhance intra-tumoral CAR T cell efficacy may serve to overcome similar constraints of the LNME. Strategies include combining CAR T cells with ICI therapy and development of CAR T cells with endogenous PD-1 knockout or encoding secretable check-point inhibitors and effector cytokines such as IL-18 (156, 173), IL-12 (174), or a tethered IL-15 (175), as well as engineering T cells to express a domain-negative form of the TGFβ receptor (176, 177). A recent *in vivo* trial in macaques using the human IL-15 superagonist ALT-803 demonstrated enhanced trafficking of SIV specific $CD8^+$ T cells to B cell follicles. HIV specific CARs with the ability to secrete IL-15 *in situ* may direct localization of these cells within B cell "sanctuary sites." Additionally, there is potential for viral escape following treatment with HIV-specific CAR T cells that target a single HIV *env* epitope, similar to what was observed in phase 1 studies assessing single infusions of bNAbs targeting distinct HIV-1 envelope epitopes (164, 178). However, more recent studies have found that despite selection of escape variants, rebound viruses did not show further resistance to other antibodies that targeted different envelope epitopes (164, 165, 178) and combination approaches with multiple bNAb scFVs can maintain viral suppression (179) and limit viral resistant variants. Thus, combination multi-specific CAR T cell approaches could be taken to promote durable viral control. Further studies need to be conducted to assess the *in vivo* potential of the single and multi-specific bNAb CAR T cells to reduce the HIV reservoir and mediate post-treatment viral control. What is very clear is that advances in CAR T cell therapy for cancer and HIV will benefit both.

Therapeutic Vaccines

The rationale for therapeutic vaccines is similar for cancer and for HIV: for both tumors and viruses, genomic heterogeneity limits the efficacy of naturally induced immune responses, and in both diseases, there is compelling evidence revealed by next generation sequencing and advances in bioinformatics to suggest that targeting the $CD8^+$ T cell response to specific epitopes may be beneficial. Moreover, in both diseases there is evidence for dysfunctional natural immune responses that might be countered by therapeutic immunization. For cancer, there are now multiple vaccines that have been licensed by the FDA, and exciting new advances from early phase human clinical trials targeting cancer neoantigens (180), but for HIV, despite some promising results in monkeys infected with SIV, human studies have mostly been disappointing. This is thus an overlap area that deserves considerable attention.

The first attempt at therapeutic vaccination for cancer came over a century ago with the administration of bacterial toxin directly into tumors, which led to tumor regression in a person with an advanced sarcoma (181). This was the first evidence that a tumor-specific immune response could be augmented by immunization. In the cancer field today, there are multiple licensed therapeutic vaccines, starting in 2010 with FDA approval of Sipuleucel-T (Provenge; Dendreon), an autologous dendritic cell vaccine for prostate cancer (182). Autologous and allogeneic tumor vaccines have been tested in different cancer modalities, enhancing anti-tumor responses and prolonging survival (183, 184). GVAX, the most extensively studied whole cell vaccine which is comprised of irradiated, allogeneic, or autologous pancreatic tumor cells genetically engineered to secrete granulocyte colony stimulating factor (GM-CSF), has been used in pre-clinical and clinical studies in an attempt to stimulate dendritic cell activation and T cell priming (185). Despite an observed increase in anti-tumor immunity (186), a phase II trial with GVAX in combination with cyclophosphamide for the treatment of pancreatic cancer failed to show an increase in overall survival (187). More recent advances have been associated with individualized vaccines using tumor whole exome sequencing to identify autologous neoantigens, which have been shown to be immunogenic for intratumoral $CD4^+$ and $CD8^+$ T cell responses in early phase clinical trials in glioblastoma (180). Thus, far the impact on disease course has been modest, and none of these approaches has effected a cure or sustained remission.

In the setting of HIV infection, there have been multiple attempts at therapeutic vaccination to augment HIV-specific $CD8^+$ T cell responses, but thus far there have been no clear successes in humans. Studies of DC based immunotherapy clinical trials conducted for the treatment of HIV have shown modest immunogenicity and modest impact on viral load (188–191), which has often been difficult to interpret due to lack of appropriate controls. More promising results come from recent studies in NHPs suggest that immune modulation

may be possible. Several studies conducted in rhesus macaque models have underscored the importance of generating a robust anti-viral CTL response for therapeutic SIV/HIV control. An epidermally administered DNA vaccine expressing highly conserved elements (CE) of the SIV capsid protein p27 in SHIV infected macaques experiencing chronic but controlled SHIV infection. Macaques experiencing a stronger induction of CE specific responses exhibited lower plasma viral loads (192). In a separate study, Ad26/MVA (recombinant adenovirus 26 serotype (Ad26) prime/modified vaccinia Ankara (MVA) boost) with a TLR-7 (Toll-like receptor 7) adjuvant demonstrated a delay in viral rebound and a 2-log reduction in plasma viral loads post treatment interruption (193), where the breadth of the immune response directly correlated with time to rebound and inversely with plasma viral loads. However, a recent randomized controlled trial utilizing a therapeutic vaccine regimen in HIV infected patients who began cART early during the course of infection, showed a limited induction of anti-viral $CD8^+$ T cells, no significant effects on the kinetics of viral rebound, and no reduction in the viral reservoir post discontinuation of ART (194). This was despite the addition of human interleukin (IL)-12p35 and p40 proteins via *in vivo* electroporation to maximize immunogenicity, previously shown in non-human primates to enhance the potency of the HIV DNA based vaccine (195, 196). Moreover, the subjects enrolled in this study were within the acute phase of infection with early cART treatment and potentially better preserved immune function, but still failed to show any effect on post-treatment control.

For both HIV and cancer, epitope mutation resulting in immune escape appears to play a major role in the lack of efficacy of host T cell responses, but recent studies suggest that this property may be exploited to therapeutic benefit. In cancers, neoantigens which can be bioinformatically identified have been used as immunogens and shown promise in early phase human trials (180, 197). In HIV infection, application of network theory to HIV structure has revealed that mutation of epitopes at important network positions disproportionately impairs viral replication capacity and that $CD8^+$ T cell targeting of highly networked epitopes distinguishes persons who naturally control HIV, even in the absence of protective HLA alleles (30). These data suggest that targeting mutationally constrained epitopes is a promising approach for vaccine design. Support for induction of immune responses to neoantigen epitopes or highly networked epitopes comes from recent studies showing that a synthetic DNA, multi-neoantigen cancer vaccine in a mouse model drives robust MHC class I $CD8^+$ T-cell responses which are able to impact tumor growth (198). It is still unknown whether therapeutic vaccinations alone can increase the magnitude of the HIV specific response to a level that can both detect very low antigen levels in ART treated patients as well as induce durable viral suppression upon ART cessation, but future studies incorporating check-point blockade, cell based therapies, and tissue specific/LNME agonists in combination with therapeutic vaccines to develop a functional, highly potent CTL response may be key to containing or eradicating the latent HIV reservoir.

CONCLUSIONS

HIV remains a significant global health burden and despite the profound efficacy of ART in preventing viral transmission (199), the number of individuals living with HIV and on treatment continues to rise each year and non-AIDS related morbidities are increasing with the duration of HIV and time on ART (200, 201). Given the limited impact of ART on viral "sanctuaries," there is a critical need to identify immune mechanisms within tissue sites that harbor the HIV reservoir and hinder anti-viral immunity, similar to the need for immune based therapies in cancer to access malignant cells in tissue sites and overcome tumor immunosuppressive environments. In the same way that advances in cancer immunotherapy have resulted in durable remission in patients with seemingly incurable malignancies, there is strong rationale for immune control if not eradication of HIV, given that some persons are able to achieve a state of immune-mediated functional cure of HIV infection without the need for ART. A deeper understanding of common mechanisms of immune dysfunction and exclusion as well as mechanisms of tumor response leading to durable remission, will be critical to attaining a functional state of viral remission or cure in HIV infected patients. These include enhancing T cell trafficking into tumors and lymph node HIV sanctuaries, overcoming immune exhaustion, and escape, reversing tumor and lymph node immunosuppressive environments, and eliciting robust CTL responses against neo-epitopes and highly networked epitopes. Caution, however, must be taken when exploring immunotherapeutic interventions to avoid emergence of autoimmunity and other adverse events. A greater understanding of the immune mechanisms regulating the LN microenvironment and the impact of check-point blockade on CTL function, localization, and viral clearance within the LNME will be crucial to the development of HIV cure strategies. Thus, as the field of cancer immunotherapy progresses, the HIV cure field must take heed in determining what therapeutic interventions will prove safe, effective, and clinically justifiable to explore in HIV infected individuals currently durably suppressed with ART.

AUTHOR CONTRIBUTIONS

GM and AY carried out the primary research and equally wrote the manuscript. MM edited the manuscript. BW provided oversight in preparation and editing.

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REVIEW
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Seeing Is Believing: Nuclear Imaging of HIV Persistence

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A major obstacle to HIV eradication is the presence of infected cells that persist despite suppressive antiretroviral therapy (ART). HIV largely resides outside of the peripheral circulation, and thus, numerous anatomical and lymphoid compartments that have the capacity to harbor HIV are inaccessible to routine sampling. As a result, there is a limited understanding of the tissue burden of HIV infection or anatomical distribution of HIV transcriptional and translational activity. Novel, non-invasive, *in vivo* methods are urgently needed to address this fundamental gap in knowledge. In this review, we discuss past and current nuclear imaging approaches that have been applied to HIV infection with an emphasis on current strategies to implement positron emission tomography (PET)-based imaging to directly visualize and characterize whole-body HIV burden. These imaging approaches have various limitations, such as the potential for limited PET sensitivity and specificity in the setting of ART suppression or low viral burden. However, recent advances in high-sensitivity, total-body PET imaging platforms and development of new radiotracer technologies that may enhance anatomical penetration of target-specific tracer molecules are discussed. Potential strategies to image non-viral markers of HIV tissue burden or focal immune perturbation are also addressed. Overall, emerging nuclear imaging techniques and platforms may play an important role in the development of novel therapeutic and HIV reservoir eradication strategies.

Keywords: human immunodeficiency virus, positron emission tomography imaging, simian immunodeficiency virus, nuclear medicine, molecular imaging

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INTRODUCTION

Despite the overwhelming success of antiretroviral therapy (ART) to achieve complete or near-complete HIV suppression, residual virus that integrates into host cell genomes prior to ART initiation persists indefinitely. Blood-derived resting CD4+ T cells comprise one of the most characterized reservoirs of latent HIV, and integrated viral DNA can exist at frequencies below one copy per million resting CD4+ T cells (1–6). However, HIV largely resides in organized lymphoid or other tissues outside of the peripheral circulation, and many anatomical regions are inaccessible to routine sampling (7–16). Only a small amount of tissue from a small number of sites can be realistically obtained from living human participants, and one of the major barriers to the successful design and implementation of HIV eradication or immune-based therapeutic strategies is the limited ability to characterize the tissue-wide burden of HIV in the setting of ART.

HIV-1 infection leads to immune activation and inflammation throughout all stages of disease. Markers of T-cell activation remain elevated in blood and lymphoid tissues in HIV-infected individuals, even in the setting of elite control or after years of suppressive ART. Certain immune privileged environments may be especially important foci of HIV persistence and viral transcriptional activity. For example, CD4+ T-follicular cells (T_{FH}) within lymph node B cell follicles have been shown to be highly enriched in HIV-1 DNA, are very permissible to HIV infection, and are able to produce high levels of replication competent virus upon *ex vivo* stimulation (12, 17–19). T_{FH} cells may be protected from various host immune responses by their location in the unique histological makeup (12, 17–19). Even outside of infected tissues, persistent HIV has lasting and often profound effects on tissues such as vascular endothelium, gut, and brain, and leads to sustained, systemic inflammatory responses. Markers of inflammation, coagulation, and immune activation remain elevated in effectively treated HIV infection and are strong predictors of mortality and non-AIDS events, which has been demonstrated in a variety of cohorts (20–23). As a result, there are direct and indirect consequences of HIV infection that are clinically relevant, even in the setting of treated and suppressed HIV. For example, HIV has been associated with increased cardiovascular disease, neurological disorders, and various hematological and solid-tumor malignancies (24).

The direct and indirect impact of persistent HIV on immune activation, systemic inflammation, and increased clinical comorbidities has led to interest in positron emission tomography (PET) and other molecular imaging techniques as tools to better understand the whole-body burden and consequences of HIV infection. Molecular imaging has been critical for the diagnosis, treatment, and management of various malignancies and other diseases. Similar modalities have the potential to provide insights into the design, implementation, and analysis of immunotherapies and other interventions to reduce HIV reservoir burden, lower inflammation, and thus reduce HIV-related morbidity.

NUCLEAR IMAGING APPROACHES TO HIV PERSISTENCE AND HIV-RELATED MORBIDITY

The Molecular Imaging Toolbox

Innovative strategies to perform molecular imaging, from microscopic visualization and characterization techniques on the tissue level, to whole-body *in vivo* anatomical and functional imaging incorporating techniques such as SPECT and PET, are rapidly being developed for a wide range of diseases, including HIV and other chronic infections (see **Table 1**).

Ex vivo molecular imaging on the cellular and tissue level has already provided many important insights into HIV pathogenesis such as identifying foci of residual infected cells in the setting of ART and characterizing the immunological microenvironments of such foci (58–65). These studies have focused largely on gut, lymphoid, and central nervous system tissues but may involve a wide variety of other scenarios such as tumor microenvironments and quantifying vascular inflammation. However, the focus of this review covers *in vivo* nuclear medicine approaches with an emphasis on novel PET imaging approaches of HIV persistence.

TABLE 1 | Historical and current PET radiotracers used in the context of HIV infection.

Early SPECT radiotracer	Target or response in disease
^{99m}Tc-HMPAO	Cerebral blood flow (25–34)
^{123}I-Iodoamphetamine	Cerebral blood flow (35–38)
^{123}I-FP-CIT	Cocaine analog, dopaminergic neurotransmission (39)
^{123}I-iodobenzamide	Dopaminergic neurotransmission
^{201}Thallium	Differentiation of CNS lymphoma from toxoplasmosis (40–43)
Current (dates) PET radiotracers	**Target or response in disease**
^{18}F-Fluorodeoxyglucose FDG	Glucose metabolism
TSPO imaging (^{11}C-PBR28, ^{18}F-DPA-714, ^{11}C-DPA-713, ^{11}C-PK11195)	Neuroinflammation (44–50)
Fluoromisonidazole	Reduced hypoxia associated with Nelfinavir (51)
^{82}Rb	Myocardial perfusion (52, 53)
^{11}C-DASB	Dysregulated serotonergic transmission (54, 55)
^{11}C-PiB	Alzheimer disease (AD) plaque tracer—no increased AD risk (56, 57)

Nuclear Imaging Approaches to HIV Infection

Common nuclear imaging approaches that have been applied to HIV infection for over 20 years include SPECT/CT and PET/CT imaging (44). These modalities involve the detection, anatomical location, and kinetics of radioactive tracer uptake, with SPECT involving the detection of single photon gamma emission and PET measuring positron emission. Clinically, these nuclear imaging modalities are commonly used to diagnose various malignancies and provide information on potential tumor burden or sites of metastases, disease staging, and response to various treatment strategies. They are also used to differentiate benign, metabolically quiescent tissues from metabolically active foci, which may be manifested by active infections, reactive lymphoid tissues, vascular inflammation, and more. As a result, nuclear imaging has been applied in the setting of HIV infection and HIV-related comorbidities. HIV imaging studies are diverse and have involved numerous tracers and measured outcomes. As summarized in **Table 1** and below, PET imaging has been used to (1) measure cellular metabolic activity in a variety of different clinical scenarios (e.g., 18F-FDG); (2) carry out anatomical and functional neuroimaging involving various metabolic measures, cerebral fluid, dopamine transport, and cellular activation in the setting of HIV-associated neurological disease (HAND), central nervous system malignancies, and opportunistic infections; (3) determine ART-related toxicities; (4) quantify changes in various immune cell types, such as CD4+ T-cell distribution in the

setting of immunomodulatory therapies in animal studies; and (5) characterize the effects of HIV on cardiovascular disease. A recent PubMed search using HIV or AIDS and PET yielded 537 references, averaging about 10 articles per year.

Over the past several years, there has been increased interest in the development of HIV-specific tracers to provide direct anatomical localization and burden of infection. *In vivo* studies are currently taking place using techniques such as radiolabeling monoclonal antibodies (mAbs) specific for HIV or SIV envelope proteins (66, 67). In addition, traditional nuclear medicine approaches, such as FDG-PET, have been applied to look at HIV persistence in the setting of active infection, HIV controllers (i.e., those who are able to suppress virus without ART), and ART-suppressed individuals (see discussion below). These immunoimaging approaches have the potential to significantly improve our understanding of where and how residual viral replication and HIV-related inflammation resides in the setting of suppressive therapy. More specifically, the diverse nuclear imaging toolbox may prove to be useful in people living with HIV to:

- Understand the temporal changes that occur within the whole body as a function of disease status, ART use, viral recrudescence following cessation of therapy, or foci of HIV reactivation during a "shock and kill" approach to HIV remission.
- Distinguish opportunistic infections and malignancies from direct or indirect impact of active or suppressed HIV infection.
- Assist in the development of new drugs and therapeutic paradigms.
- Aid in participant selection for various therapeutic strategies.
- Monitor individualized responses to various therapeutic interventions (including ART, immunotherapies, etc.).

Radiopharmaceutical, Pharmacokinetic, and Nuclear Imaging Considerations

The utility of a specific nuclear imaging strategy is tightly linked with the various properties of the applied radiopharmaceutical tracer. These properties include radiologic dose, exposure, decay rates and tissue uptake, drug metabolism, and excretion. PET tracers involve a radiolabeled molecule as a source of positrons. These isotopes have a wide range of radiological half-lives ($t_{1/2}$). Decay rates range widely from minutes to many days as summarized in **Table 2**, and ideally are in synergy with the pharmacokinetics of the radiolabeled tracer. For example, mAbs may take several days to reach target tissues and bind to specific targets, therefore requiring longer-acting isotopes such as zirconium-89 ($t_{1/2} = 78$ h), whereas FDG uptake (fluorine-18 $t_{1/2} = 110$ min) is rapid and glucose is internalized relatively quickly by metabolically active cells. Special care in matching the appropriate radioactive molecule with the target drug will be critical in the rational design and implementation of HIV-specific imaging agents. In addition, human studies are limited by the total radiation exposure to a participant, leading to challenges with administration of high enough doses for clinically meaningful target-to-background contrast, restricting the frequency of tracer administration and may limit longitudinal imaging studies. In addition, target densities may be quite low in various clinical scenarios such as ART-suppressed HIV infection, where viral proteins may be expressed in very low amounts or frequencies on cells or in tissue, if at all. As a result, there are expected to be significant challenges to increase signal-to-noise ratios in these participants, and this highlights the continued need for non-viral-specific tracers to provide information on location, burden, and immunological impact of persistent HIV infection.

TABLE 2 | Common radioisotopes used in HIV nuclear imaging.

Radioisotope	Half-lives	Pros and cons
^{11}C	20 min	Short half-life good for repeat studies, carbon-11 for carbon-12 exchange in small molecules/drugs produce the same labeled molecule/drug, half-life may be too short to achieve adequate signal-to-noise ratio, may not be transported to distant scanners
^{18}F	110 min	Ideal positron emission characteristics for high-resolution PET imaging may incorporate into small-molecules/drugs. Half-life suitable for longer imaging and delivery to remote scanner sites. May not be long enough for larger biologic molecules. Free ^{18}F-Fluoride ion accumulates in bone
^{64}Cu	12.7 h	Half-life compatible with imaging larger molecules like mAbs. However, half-life may limit utility when using HIV gp120-specific or other mAb, which take time to penetrate certain target tissues
^{89}Zr	78 h	Half-life compatible with imaging larger molecules like mAbs. Radiation dose to patient is higher so lower administered dose is necessary. Takes a long time to clear from body so repeat studies limited but allows for serial imaging over days with a single radioisotype injection. May be beneficial when using HIV gp120-specific mAb, which takes time to penetrate certain target tissues. Ideal for transport to distant scanners. $^{89}ZrCl_3$ may accumulate in active bone

PET Imaging in HIV Infection—Cellular Metabolic Activity, Immune Activation, and HIV Persistence

In the research setting, PET/CT has commonly been used in conjunction with FDG, which provides a measurement of glucose metabolism as a surrogate for inflammation, which is taken up substantially higher by inflammatory cells and macrophages in the tissue (68, 69). FDG-PET imaging has been reported for HIV in the mid to late 1980s, with monitoring of HIV pre- and post-AZT monotherapy (combination ART was not widely available until the mid-1990s), and workup of HIV-associated neurological disorders along with staging of malignancies (44, 70, 71). In addition, FDG-PET studies have involved anatomical localization of HIV-associated immune activation, correlating lymph node inflammation with disease stage, and associating high areas of FDG uptake in non-human primates with

productive SIV infection (72–77). Since this time, studies in the general population have demonstrated that arterial inflammation assessed using FDG-PET/CT can predict future cardiovascular (CV) events (78). Furthermore, lipid lowering using statin therapy along with thiazolidinedione therapy has reduced arterial FDG-PET uptake in several clinical trials (79–83). Our group also has recently reported that using a mAb to IL-1β significantly reduced inflammatory markers along with arterial and bone marrow metabolic activity assessed using FDG-PET/CT in the setting of treated HIV (84). Studies involving animal models and humans showed that both relative and absolute FDG uptake within inflamed tissues (e.g., atherosclerotic plaques) correlate with the degree of immune cell infiltration (12, 17–19, 85–89). More recently, FDG-PET has been applied to assess altered glucose metabolism in HIV-associated inflammation and has demonstrated that HIV patients have higher arterial inflammation that is associated with sCD163 (87). Initiation of ART reduced bone marrow activity but did not affect arterial inflammation; furthermore, metabolic activity on FDG-PET/CT prior to ART was predictive of immune reconstitution inflammatory syndrome development (90).

Subsequently, our group showed that HIV-infected individuals on ART have higher metabolic activity as measured by FDG-PET/CT in the arterial vasculature and lymph nodes than matched uninfected controls and that these markers correlated with measures of HIV persistence in peripheral blood (91). Importantly, individuals on ART had higher FDG uptake in lymph nodes and arterial vasculature than matched uninfected controls. Overall, lymph node FDG activity was significantly associated with levels of integrated HIV DNA measured in peripheral blood mononuclear cells (91). This study suggests that PET-based imaging of inflammation or immune activation has the potential to provide information regarding regional areas of HIV persistence. However, FDG is likely taken up by immune activation/inflammation even when not in tissue with HIV-persistent foci (e.g., arterial wall, which may be influenced by monocyte activation); therefore, more specific markers of T-cell trafficking and targeting of infected tissues are needed.

Recently, advances in molecular imaging of immune activation by PET have made it possible to use non-invasive strategies to monitor immune activation with increased T-cell specificity than FDG. Increased activity of nucleoside salvage pathways has been associated with the proliferation of adaptive immune cells (92). In preclinical models, the PET probe [^{18}F]-2-fluoro-d-(arabinofuranosyl)cytosine ([^{18}F]-FAC), which targets the deoxycytidine salvage pathway, was shown to localize to focal sites of immune activation (93) and is predominantly accumulated in proliferative T cells (94). Recently, a radiofluorinated imaging agent [^{18}F]F-AraG (95) was synthesized with a goal of development for human use. F-AraG is a fluorinated purine derivative with selective T-cell uptake. A water-soluble AraG prodrug, Nelarabine, is FDA-approved for the treatment of relapsed T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphomas (96, 97). [^{18}F]F-AraG is a high-affinity substrate for deoxyguanosine kinase (dGK) and a low-affinity substrate for deoxycytidine kinase (dCK). Both dGK and dCK are over-expressed in activated T cells. Blocking the expression of either dGK or dCK causes reduction in [^{18}F]F-AraG uptake, while over-expression of either dGK or dCK leads to increased accumulation of [^{18}F]F-AraG. T-cell-specific tracers such as these may play an important role in imaging HIV persistence, with the potential to be more specific to regional areas of immune perturbance as a result of HIV replication or residual viral transcriptional activity.

Neuroimaging Microglia Activation in HIV Infection and Related Neurologic Disorders

PET imaging using tracers specific for activated microglial cells is another example of how non-specific markers of increased immune activation has been successfully applied to study HIV-related comorbidities in the central nervous system. More specifically, molecules have been developed that target the 18-kDa mitochondrial translocator protein (TSPO) that shuttles cholesterol into mitochondria for steroid biosynthesis (45–50). TSPO is upregulated in activated microglia, and, as a result, has been used in neuroimaging to determine differences between HIV-infected and uninfected individuals and to characterize differences between various HIV clinical disease manifestations, including HAND (50). PET imaging with TSPO-specific tracers appear to be more specific to innate immune activation than FDG (45) and have led to some important insights into central nervous system persistence of HIV. For example, ART-suppressed individuals without cognitive impairment have been observed to have chronically elevated microglial activation (48), whereas other studies showed that TSPO levels correlated with worse executive brain performance and other HIV-associated cognitive vulnerabilities (46, 49). Despite varying results complicated by various experimental designs and definitions of cognitive impairment (50), there is continued interest in using PET-based immune activation approaches to study the direct impact of residual HIV infection in the setting of suppressive ART.

Antiretroviral Drug Labeling

The question of whether or not there is ongoing replication in various tissue sanctuaries in the setting of otherwise suppressive ART remains controversial. For example, there is a paucity of robust phylogenic evidence for evolution of HIV sequences or development of resistant mutations in suppressed individuals over time and ART intensification studies have not demonstrated reductions in low-level, residual plasma HIV RNA levels (98–103). Many of these studies were performed in peripheral blood or limited by the depth of sequence coverage or tissues sampled. Other studies have shown potential indirect evidence of replication such as an increase in unintegrated episomal HIV DNA in blood and cell-associated RNA in tissue (104–106). One topic of interest is the extent to which various ART drugs reach or have activity in various anatomical tissue compartments (107), potentially creating viral sanctuaries that permit low-level replication or, at the very least, allow higher levels of viral transcriptional activity (9, 106, 108). Transcriptionally active cells may also lead to chronic immune activation and inflammation. However, sampling all of the potential sites of persistent HIV for concomitant ART concentrations and viral reservoir persistence

is not practical. It is also difficult to obtain information on the kinetics of drug distribution within tissues outside of peripheral blood. As a result, PET-based imaging of radiolabeled antiretroviral drugs may play an important role in pinpointing areas of poor ART penetration and therefore important sites of persistent HIV burden and potential foci of viral rebound following ART cessation. Imaging studies using fluorine-18-labeled raltegravir (a strand-transfer integrase inhibitor) are ongoing (NCT03174977) and have the potential to locate areas of HIV persistence.

PET Immunoimaging of CD4+ T Cell Dynamics in SIV Infection

CD4+ T cells are the main target of HIV infection. Active disease leads to subsequent and profound reduction in CD4+ lymphocytes throughout the blood and tissues. While counts may improve in many individuals on ART, lasting perturbations to tissues such as the lymph nodes and gut-associated lymphoid tissues are common (8, 109–114). As a result, there has been interest in CD4+ T-cell-specific PET-based imaging techniques to follow CD4+ T-cell dynamics and recovery following various interventions. A recent investigation of the use of an α4β7 mAb in acute SIV infection in macaques demonstrated sustained virological control in mAb-treated monkeys. While these results have yet to be confirmed, the study involved PET-CT imaging using a ^{64}Cu-labeled F(ab′)2 antibody against CD4. The study demonstrated repopulation of CD4+ T cells in a number of tissues, including gut, which was unexpected based on the original study hypothesis that the α4β7 mAb would interfere with CD4+ T-cell trafficking to these areas (67). This investigation is an example of how imaging various cell-specific markers may provide critical information regarding whole-body responses to various immune-based or other therapies for a wide variety of diseases. For example, CD8+ T-cell responses can theoretically be tracked over time in response to interventions such as vaccines or therapies that remove immune checkpoint and reverse T-cell exhaustion (e.g., anti-PD1 therapy).

PET-Based Direct Imaging of SIV Infection

As above, PET-based imaging techniques have the potential to delineate tissue burden and sequelae of HIV infection. PET/CT imaging approaches using a radiolabeled ^{64}Cu-labeled SIV gp120 mAb-specific clone (7D3) have been recently applied to assess SIV envelope protein expression in infected macaques with varying degrees of viremic control and in the setting of early initiation of ART (66). Results from this pivotal study demonstrated that areas of active SIV replication can be visualized and distinguished from non-selective tracer uptake in uninfected animals, with some HIV-related signal detected several weeks following ART initiation. As would be expected, lymphoid-rich areas were localized predominately at sites of persistent SIV protein expression (66). The study also showed that anatomical regions that are often neglected by *in vivo* tissue sampling, such as nasal-associated lymph node tissue, may play an important role in initial HIV seeding and subsequent persistence. A follow-up sub-study of anti-α4β7 treatment in SIV-infected macaques incorporating the radiolabeled SIV gp120 mAb demonstrated a reduction in SIV protein expression in various tissues, including the lung, spleen, and lymph node chains (89). These data suggest that direct SIV or HIV imaging radiotracers have the potential to play a critical role in characterizing HIV persistence and response to curative strategies. As a result, there is currently a high level of interest in direct HIV imaging techniques to humans. However, immunoimaging in SIV infection does have several potential limitations. For example, mAb or antigen binding fragments may have heterogeneous tissue distribution *in vivo*, and humanization or simianization may lead to immunogenicity concerns (115). Finally, the SIV or HIV antigen-specific PET-imaging approaches do not allow for direct discrimination between actively viral producing cells, cells expressing SIV or HIV antigens at the surface, viral particles, or simply viral antigen trapping by non-infected cells.

Human HIV-Specific PET Imaging: Challenges and Promises

Despite the early success of direct SIV specific in the first non-human primate PET/CT imaging studies, there are several challenges in adopting these techniques to human imaging. For example:

1. Non-human primates are typically infected with a clonal SIV strain with known binding affinity to gp120-specific mAb. HIV-infected humans can be extraordinarily diverse with both minority and majority clones capable of harboring resistance mutations to the clinically available HIV-specific mAbs, which have been previously developed as therapeutic broadly neutralizing antibodies (116–122). As a result, there is expected to be a wide range of mAb binding affinities between study participants that will require implementation of mAb resistance testing and careful considerations as to data analysis and interpretation.
2. HIV gp120 expression is expected to be very low among infected tissues in participants on suppressive ART. As a result, there may be insufficient signal-to-noise ratio in order to visualize areas of persistent infection. However, PET imaging may be particularly useful during early infection and for characterizing foci of early tissue HIV recrudescence following cessation of ART; incorporating PET imaging approaches in studies involving analytical ART interruptions is of utmost importance.
3. mAbs do not readily cross the blood–brain barrier. Barring any inflammation and major perturbations of the blood–brain barrier, imaging potential foci of HIV in the central nervous system will be challenging. As a result, the development of small-molecule HIV-specific tracers with improved central nervous system or other immune privileged tissue penetration is urgently needed.
4. Longitudinal human trials are limited by radiation exposure; therefore, multiple imaging time points may be difficult to incorporate into a variety of studies. This may be a particular issue when implementing tracers conjugated with

radioisotopes with longer half-lives *in vivo*, which are likely going to be required given the kinetics of mAb uptake as discussed above. These limitations provide the rationale to incorporate more than one radiotracer in human studies. For example, administering an HIV-specific mAb tracer following PET imaging using a non-viral specific marker of inflammation or immune activation may provide important insights into the relationship between ongoing immune perturbations and HIV persistence.

Fortunately, several strategies exist or are in development to address these challenges using radiolabeled mAbs in PET imaging. For example, smaller affibody proteins or antibody fragments (e.g., minibodies, nanobodies, and single-chain variable regions) (123–125) may have improved tissue penetration and favorable pharmacokinetics for imaging low-level HIV protein expression in various tissues. There is also a high level of interest in the development of dual or multi-targeted molecules for immunoimaging (126) or engineering antibodies to have greater anatomical barrier penetration. One exciting strategy is increasing antibody delivery across the blood–brain barrier by developing bispecific antibodies or designer molecular shuttles that bind to the transferrin receptor (127–130). Animal studies are exciting and can theoretically be applied to HIV-specific mAb or antibody fragments.

The development and implementation of very-high-sensitivity, total-body PET scanners, such as the EXPLORER platform (131–133), are also likely to overcome some of the signal-to-noise limitations of imaging HIV-infected cells in ART-suppressed individuals or those with low overall HIV envelope protein expression. These platforms are just now coming on line for *in vivo* use, and have the potential to revolutionize immunoPET imaging. Approximately 1% of the photons emitted during traditional PET scanning are detected given a limited axial field of view and body length that can be imaged at one time. The field of view in EXPLORER is extended to the entire individual by using a large number of parallel detectors that simultaneously detect photon emission (134). Early data suggest that EXPLORER PET provides a >40-fold gain in effective sensitivity and a >6-fold increase in signal-to-noise ratio compared with standard PET scanners (135). The first-in-human imaging studies have recently been completed (131) and offer an opportunity to significantly advance PET-based imaging of HIV reservoirs. Other emerging technologies include solid-state digital photon counting PET systems, such as those that use solid-state silicon photomultiplier technology (136). These systems have led to improvements in signal-to-noise ratios and enhancing image contrast (137, 138) and may play an important role in improving PET imaging in HIV infection.

Limitations of *in vitro* Modeling of HIV-Specific Immunoimaging Techniques

HIV or SIV envelope-specific PET immunoimaging strategies are likely to be semiquantitative at best. For example, PET/MR or PET/CT imaging techniques reveal relative changes in mAb tracer uptake in various tissue region of interest (e.g., lymph node tissues, gut) before or after initiation of ART or immunotherapy (66, 67). However, questions arise as to what the intensity of the PET signal means in terms of the actual number of infected, HIV or SIV envelope-expressing cells. In other words, can PET imaging be used to directly quantify the burden of HIV *in vivo*? One solution that is often presented is to perform *ex vivo* studies involving PET imaging of three-dimensional clusters of known numbers of infected and uninfected cells (either laboratory infected or derived directly from infected individuals) in order to determine the sensitivity of PET to detect various levels of HIV protein expression. While appealing, these studies are limited by the multitude of variables within living organisms that determine tracer uptake and PET detection. Modern PET scanners are sensitive and able to detect tracer-derived positron emission events above normal background radiation (139). Simply labeling a cell or a group of cells that express HIV envelope will likely lead to a detectable signal. However, regardless of what threshold in the number of infected cells can be detected (e.g., 10, 100, or 1,000 in a sub-centimeter cluster) in isolation, these types of *ex vivo* experiments are unable to account for many biasing factors. For example, radiotracers are often delivered in microdoses, with or without a specified amount of unlabeled antibody. The distribution of these microdoses to various tissues relies on many variables, such as blood flow dynamics, tissue fibrosis, and non-specific tracer uptake, to name just a few. In addition, there is background radiation that is given off by tracers in the macro and microcirculation and from organs involved in tracer metabolism and excretion. Coupled with the need for PET attenuation and tomographic reconstructions in image acquisition and analysis, it will likely be difficult to correlate readout of *ex vivo* PET sensitivity studies with actual uptake in living organisms. In addition, each individual has different metabolic and physiologic dynamics (e.g., liver function, cardiac output, body surface area and mass, renal glomerular filtration rates, local microanatomical variations, etc.). As a result, performing parallel *in vivo* tissue biopsy studies along with PET imaging may be the most useful strategy to provide some quantitative understanding of radiotracer uptake signal and direct cellular measures of HIV burden or cell activation state.

CONCLUSIONS

PET imaging offers several exciting strategies to characterize HIV and HIV-related comorbidities. Despite limitations of traditional of nuclear imaging techniques in identifying HIV-infected cells *in vivo*, proof-of-concept SIV non-human primate studies demonstrate that various immunoimaging approaches have potential to enhance HIV curative and persistence research. Signal-to-noise issues are likely to limit imaging in ART-suppressed individuals when cell-surface HIV protein expression is expected to be low. However, novel approaches such as high-sensitivity, total-body EXPLORER imaging, PET imaging during latent HIV reservoir reactivation or ATI, and development and implementation of non-viral markers of HIV persistence have the capacity to overcome these limitations and provide important tools for the development of novel therapeutic strategies. In addition, technical and data processing advancements may

allow for combination imaging approaches, from tissue-level microscopy to whole-body PET imaging.

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TH, PH, and HV wrote the manuscript and obtained funding.

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REVIEW
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Altered Lipid Tumor Environment and Its Potential Effects on NKT Cell Function in Tumor Immunity

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Natural killer T (NKT) cells are CD1d restricted T cells that mostly recognize lipid antigens. These cells share characteristics with both adaptive and innate immune cells and have multiple immunoregulatory roles. In a manner similar to innate immune cells, they respond quickly to stimuli and secrete large amounts of cytokines, amplifying and modulating the immune response. As T cells, they express T cell receptors (TCRs) and respond in an antigen-specific manner like conventional T cells. There are at least two subtypes of NKT cells, type I and type II, that differ in the nature of their TCR, either semi-invariant (type I) or diverse (type II). The two sub-types generally have opposing functions in tumor immunity, with type I promoting and type II suppressing tumor immunity, and they cross-regulate each other, forming an immunoregulatory axis. The tumor has multiple mechanisms by which it can evade immune-surveillance. One such mechanism involves alteration in tumor lipid repertoire and accumulation of lipids and fatty acids that favor tumor growth and evade anti-tumor immunity. Since NKT cells mostly recognize lipid antigens, an altered tumor lipid metabolic profile will also alter the repertoire of lipid antigens that can potentially affect their immune-modulatory function. In this review, we will explore the effects of alterations in the lipid metabolites on tumor growth, antigen cross-presentation, and overall effect on anti-tumor immunity, especially in the context of NKT cells.

Keywords: lipid metabolism, tumor immunity, natural killer T-cells, antigen presentation, dendritic cells

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INTRODUCTION

Natural killer T cells (NKT cells) are a specialized subset of T-lymphocytes that share characteristics of both the innate and adaptive immune system. By definition, NKT cells are cells that recognize mostly lipid antigens presented by a non-classical class I MHC molecule, CD1d (1). CD1d is a member of the CD1 family, which are involved in presentation of a variety of both endogenous and exogenous lipid antigens to T-lymphocytes (2). NKT cells respond quickly and produce copious amounts of cytokines, further amplifying the immune response, while at the same time acting in an antigen specific manner. They are further categorized into two broad subsets based on their TCR repertoire. Type I NKT cells express a TCRα chain with limited diversity and therefore are referred to as semi-invariant NKT cells or invariant NKT cells (iNKT). The TCRα chain expressed by type I NKT consists of Vα14Jα18 in mice and Vα24Jα18 in humans, which preferentially pairs with Vβ8, Vβ7, Vβ2 in the former, and Vβ11 in the later (3–5). A marine sponge-derived lipid, α-GalCer (α-galactosylceramide) bound to CD1d, is a prototype ligand that binds to and activates virtually all type I NKT cells. In mice, type I NKTs are mostly CD4 single positive and CD4/CD8 double negative cells, whereas in

humans these are CD4 or CD8 single positive as well as double negative cells (6). Type II NKT cells are a distinct CD1d restricted NKT population that does not react to α-GalCer. These cells express a more diverse TCR repertoire. A subset of type II NKT cells that reacts to sulfatide, a self-glycolipid, was the very first subtype to be identified by a specific ligand (7). Although type II NKTs can recognize a variety of lipids presented by CD1d, to date, sulfatide reactive type II NKT cells remain one of the best-described subsets (8). Type II NKT cells appear to be the predominant population in humans (9), but due to the lack of a specific ligand and isolation techniques, they have been difficult to study (10). Although NKT cells recognize lipid antigens, they can recognize hydrophobic peptides in addition to lipids as well, which is beyond the scope of this review and is reviewed elsewhere (11–14). Both Type I and II NKT cells modulate the immune response during tumor development and progression. Although highly contextual, in general, type I NKT cells are shown to have enhanced anti-tumor immune response whereas type II NKT cells generally act in an opposing manner (5, 15–18). However, in some mouse tumor models, type I NKT cells also have been shown to be suppressive of tumor immunity (18–22).

NKT cells recognize a diverse repertoire of both endogenous and exogenous lipids (2, 23). Most information on NKT lipid antigenic repertoire has come from mouse studies. Unlike humans, mice express only CD1d among the CD1 gene family (24). The generic structure of a lipid antigen-loaded to the CD1d molecule consists of a polar headgroup (e.g., a galactose sugar) linked to hydrophobic side chains. The CD1d molecule has two hydrophobic pockets, the A′ and F′ pockets, into which the hydrophobic side chains fit, whereas the polar headgroup sits outside and interacts with the TCR on the NKT cell (13). The length of the hydrophobic side chain as well as structural modifications in both the side chain and the polar headgroup can affect the binding of the lipid antigen presented by CD1d to the TCR on NKT cells. This, in turn, can have a differential effect on their activation status and eventual immune responses (25, 26).

Studies have reported several lipids that bind to CD1d and can potentially be presented to NKT cells. Glycerophospholipids and sphingolipids are the two major lipid groups that bind to CD1d (27). Phosphatidylcholine (PC), phosphatidylethanolamine (PE), Phosphatidylserine (PS), phosphoinositoI, phosphatidylglycerol, and phosphatic acid are the various glycerophospholids that have been shown to bind to CD1d with variable affinities. Several self-lipid antigens stimulate both murine and human NKT cells (28) such as lysophosphatidylethanolamine, and lysophosphatidic acids. Some lipids stimulate type I over type II NKT cells and vice versa. In particular, lysosphingomyelin stimulate only human type I NKT cells. Lysophospahtidylcholine stimulate both type I and type II NKT cells in humans, however, its reactivity with type I NKT is weaker. Additionally, lysophosphatidylcholine also reacts with murine type II NKT cells (29).

There are thousands of lipids within a mammalian cell serving functions ranging from energy storage to structural integrity to signal transduction (30). Any change in the lipid repertoire can disrupt tissue homeostasis leading to cellular transformation, cell proliferation, and migration (31–33). In this review, we will discuss the effect of altered lipid composition on tumor growth, anti-tumor immunity both NKT cell dependent and NKT cell independent. Some of the mechanisms by which lipid changes can modulate NKT cell dependent immune functions, directly or indirectly, that will be discussed here are (1) alteration in the quality of lipid antigen repertoire that can be presented to NKT cells, (2) impaired antigen cross presentation by DCs either by affecting the antigen processing machinery or MHC and CD1d surface expression, (3) modified quality and quantity of lipid reactive NKT cells, and (4) homing of NKT cells to the tumor sites.

ALTERED LIPID METABOLIC STATUS AND EFFECT ON TUMOR GROWTH

Lipids are integral components of the cellular membrane where they participate in lipid raft formation and impact signal transduction (34). Thus, lipids have both structural and functional roles in maintaining cellular homeostasis. Fatty acids (FA) and cholesterol are the building blocks of all lipids in the body and are synthesized *de novo* in specialized tissues from Acetyl CoA. Other than synthesis, FAs are also taken up by the cells from the surroundings such as circulation, nearby tissues, and diet. Short chain saturated FAs are further elongated and desaturated by a specific set of enzymes to generate mono and polyunsaturated fatty acids (31). The human body is unable to synthesize long-chain polyunsaturated fatty acids (PUFAs) called omega 3 (DHA, docosahexaenoic, and EPA, eicosapentaenoic acid) fatty acids and omega 6 (arachidonic acid) at a reasonable rate and therefore, supplementation is required through dietary sources (35, 36). Alteration in lipid repertoire, such as saturated vs. unsaturated lipids, can influence multiple cellular functions. To illustrate, an altered lipid repertoire can impact membrane fluidity, cell-cell interaction, as well as the membrane protein landscape, which in turn can affect the downstream signaling cascade (37, 38). There are several studies that have reported a metabolic reprograming favoring *de novo* synthesis of lipids in cancer (39, 40). Additionally, an association between increased uptake of saturated fatty acids and cancer development has been reported in multiple cancer types (41–44). Also, a diet high in polyunsaturated fatty acids, especially omega 3s, have been shown to be negatively associated with cancer development (45–47). Consistent with that, one recent study reported a significant loss of PUFA especially omega 3 in breast cancer brain metastasis, by downregulation of its specific receptor, Major Facilitator Superfamily Domain Containing 2a (MFSD2a) on tumor endothelium (48).

Tumor cells have high metabolic flux. To sustain growth, they need a rapid and constant supply of FAs and lipids to generate bio-membrane, which is achieved by uptake of FAs from the surrounding tissues as well as upregulation of endogenous lipogenic pathways (49). **Figure 1** outlines the effects of altered lipid metabolism on tumor growth as well as anti-tumor immunity. One pioneering study showed that tumor cells, in addition to uptake from the surrounding tissues, can also synthesize fatty acids *de novo* (39). Additionally, tumors can upregulate metabolic pathways leading to the accumulation of

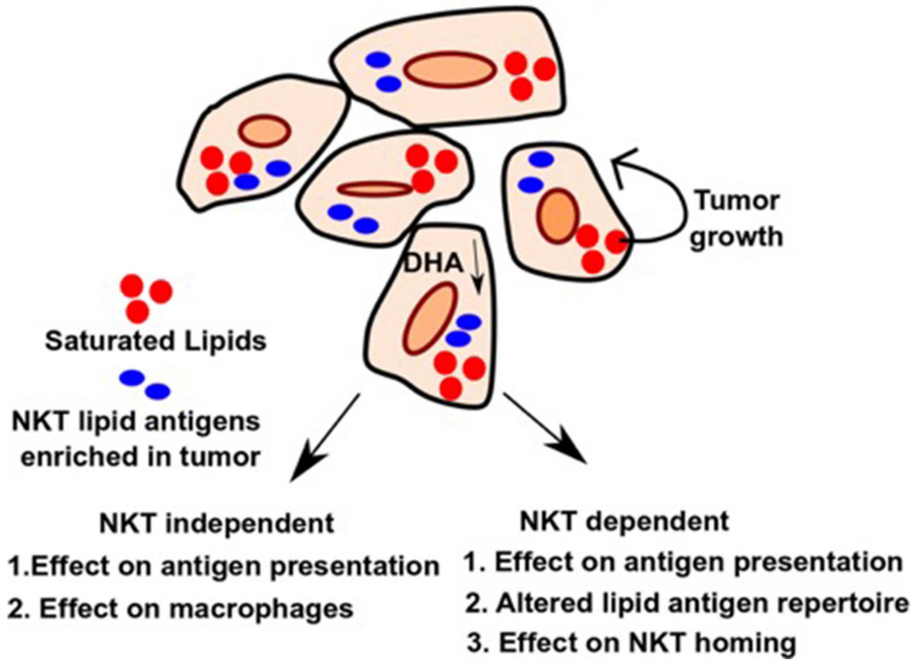

FIGURE 1 | Alteration in lipid metabolism in tumor and potential effects on NKT independent and dependent immune function. Upregulation of *de novo* pathway and loss of tumor suppressive lipids such as DHA leads to differential accumulation of lipids in tumors, which favors tumor growth and provides energy sources and building blocks for bio-membranes. Alteration in lipid pool can affect immune response in an NKT independent or NKT dependent manner as outlined in the figure. It can lead to impaired macrophage function during inflammation and defective antigen presentation. Additionally, altered lipids can also serve directly as antigens for NKT cells and modulate their role in anti-tumor immunity. Homing of NKT cells can also be affected by altered lipids. Therefore, identifying the lipids as well as the pathways that lead to their upregulation and blocking it, can have potential therapeutic benefit in cancer.

specific fatty acids and lipids that promote tumor growth and exclude those that suppress it. Consistent with that, various studies identified upregulation of several key lipid metabolic enzymes (such as ACC, Acetyl Co-A carboxylase, FASN, Fatty acid synthase, and ACLY, ATP-citrate lyase) under tumor conditions, and suppression of these enzymes involved in fatty acid synthesis has been shown to be preventive against tumor growth and metastasis (50–52). Additionally, sterol regulatory element-binding protein (SREBP), a master regulator of lipid biogenesis (53), is aberrantly upregulated in multiple cancer types and leads to upregulation of its target genes, promoting cancer growth (54). Furthermore, genetic or pharmacological inhibition of SREBP in pre-clinical studies, shows anti-tumorigenic effect by altering tumor specific lipid metabolism (55, 56).

EFFECTS OF ALTERED CELLULAR LIPIDS ON NKT CELL INDEPENDENT IMMUNE RESPONSES

Lipid mediators are at the crux of both initiating an inflammatory response as well as resolving it (57–59). Therefore, metabolic deficiencies, pathogenic conditions, tumors, and dietary habits can cause an imbalance in the lipid metabolism that can skew the balance toward the accumulation of certain lipids over others, leading to aberrant immune activation.

Effect of Altered Lipid Metabolism on Antigen Presentation

A high-fat diet that predominantly contains saturated fatty acids (SFAs) positively correlates with cancer development and progression (60–62). Although, both SFAs and PUFAs can have immunomodulatory effects under various pathological conditions (63), their effect on the immune system in the context of cancer development and progression is not well-understood. Many cancers accumulate SFAs by upregulating the *de novo* fatty acid synthesis pathway. These SFAs are preferentially taken up from the surrounding milieu. Additionally, tumors exclude PUFAs from their lipid pool. Alterations in the fatty acid pool of a cell can lead to gene expression changes as well as structural changes in the bio-membrane. Not much is known about the effect of altered lipid metabolism on lipid antigen presentation, recognition, and consequent activation of cytotoxic T cells (CTLs) and NKT cells, especially in cancer.

Dendritic cells (DCs) are the professional antigen presenting cells in the body. Efficient antigen presentation by DCs results

in enhanced activation and the cytotoxic response of $CD8^+$ T cells. Several studies have shown that a high-fat diet, enriched in SFAs, can significantly impair the ability of DC's to activate naïve T cells. In addition to SFAs, PUFAs can also diminish the immunogenic function of DCs (64). APCs, when treated with high levels of palmitic acid (PA), express significantly reduced levels of class I MHC on their cell surface (**Figure 2A**). Additionally, this also leads to an impaired conjugation rate of APCs and lymphocytes (65) (**Figure 2C**). This effect is primarily due to altered membrane dynamics, and defects in membranes generated by high PA. Furthermore, co-treatment of oleic acid (a monosaturated fatty acid) with PA, sequesters PA into lipid droplets and negates its effect on cytoskeletal organization. This has important effects on antigen presentation and can thereby rescue the antigen presentation ability of APCs even when PA is present.

Other than treatment with exogenous fatty acids, endogenous fatty acids also affect DCs, both qualitatively and quantitatively. One study reported a significant reduction in the number of DCs as a result of blocking cell intrinsic fatty acid synthesis (66). However, their antigen presentation ability was not compromised. The study further reported a diminished maturation, yet an upregulated expression of TLRs on DCs upon inhibition of FA synthesis. Additionally, blocking FA synthesis led to increased production of inflammatory cytokines as well as enhanced antigen capture by the DCs. Taken together, these data suggest that an immune response elicited by DC-mediated antigen presentation, irrespective of peptide or lipid antigen, is highly contextual under physiological conditions and is dependent on the nature and levels of fatty acids.

Tumor cells can alter the DCs causing them to become dysfunctional and inefficient in antigen presentation (67). DCs can take up lipids from the tumor microenvironment, which can significantly affect their antigen presentation ability and hence immunogenicity (68). During growth tumors accumulate high levels of triglycerides (TAGs). DCs from a tumor-bearing mouse become significantly enriched for TAGs when compared to DCs from a naïve mouse. Further, this accumulation of lipids in the DCs from tumor-bearing mice is mainly by upregulation of scavenger receptor A in DCs. Additionally, high lipid content in DCs from tumor-bearing mice negatively affects the antigen processing machinery (69, 70). Also, the DCs in peripheral blood in persons with cancer show a lipid excess, and their numbers as well as their antigen presentation ability is significantly compromised (71). One hypothesis why DC vaccines or DC-based cancer therapies may not work is due to the accumulation of lipids when these cells are either in circulation or in the tumor microenvironment and a subsequent loss of antigenicity. If that turns out to be the case, then use of autologous monocytes to produce autologous DCs *ex vivo*, pulsing or transducing them with antigen, and maturing the DCs *in vitro* could produce tumor-targeted DC vaccines that evade this suppressive mechanism in the tumor microenvironment. Such a strategy is already being applied to avoid other immunosuppressive effects of tumors on DC maturation (72, 73). Interestingly, the defects of DC function induced by high lipid content seems to be reversed by reducing the lipid levels, thereby restoring their antigen presentation function and enhanced efficacy of DC-based cancer vaccines (69). Recently, one study reported defective antigen cross-presentation by tumor-associated DCs due to the accumulation of lipid bodies in the DCs containing oxidatively truncated lipids. The defect in the cross-presentation was due to impaired trafficking of MHC class I molecules to the cell surface (74). Another recent study reported an impaired antigen presentation of peripheral blood DCs in late-stage lung cancer patients due to high levels of TAGs (71). Together, these data suggest that an altered lipid environment in the tumor environment can directly affect DC function, both at the tumor site and peripherally.

Effects of Altered Lipids on Macrophages

Macrophages are diverse cell population found in every tissue (75). Tissue-specific environmental cues define their characteristics (76, 77). During inflammatory conditions, macrophages play distinct roles in an orchestrated manner, where initiation state is marked by the M1 phase, whereas, the M2 phase defines the beginning of the resolution, re-epithelialization and return to the homeostatic stage (78). Both M1 and M2 phenotypes of macrophages are dependent on specialized lipid mediators. A lipid class switch from pro-inflammatory AA (arachidonic acid) derived lipid mediators to an anti-inflammatory, DHA (docosahexaenoic acid) and EPA derived lipid mediators is important to push the macrophages to the resolution state, thereby inhibiting inflammation and re-establish homeostasis (58). **Figure 3** outlines the effect of different lipids on macrophage function in inflammation. Tumor-associated macrophages (TAMs) play roles in promoting tumor growth. One study recently reported that debris generated by chemotherapy in tumors can stimulate TAMs to secrete pro-inflammatory cytokines thereby facilitating tumor growth. This effect was reversed by resolvins, which are a class of pro-resolving lipid mediators generated by DHA, thereby stimulating debris clearance by macrophages and suppression of tumor promoting inflammation (79).

EFFECTS OF ALTERED LIPIDS ON NKT CELL FUNCTIONS

Alteration in cellular lipids can directly influence NKT cell function via affecting antigen cross presentation by DCs, altered lipid antigen repertoire leading to different CD1d:lipid complexes that are presented to NKT cells, and modulating expression of CD1d. Here, we will outline the effect of altered lipid repertoire in metabolic defects and cancer on NKT cell function as well as CD1d expression on DCs. Since antigen cross presentation can also influence NKT independent immune responses, we will cover that in a separate section.

Effects of Metabolic Disorders on NKT Cell Development

Lipids are essential for development of NKT cells (80). Mice deficient in a lysosomal enzyme β-galactosidase (β-Gal) or lysosomal lipid transfer enzyme Niemann Pick C (NPC) 2 have

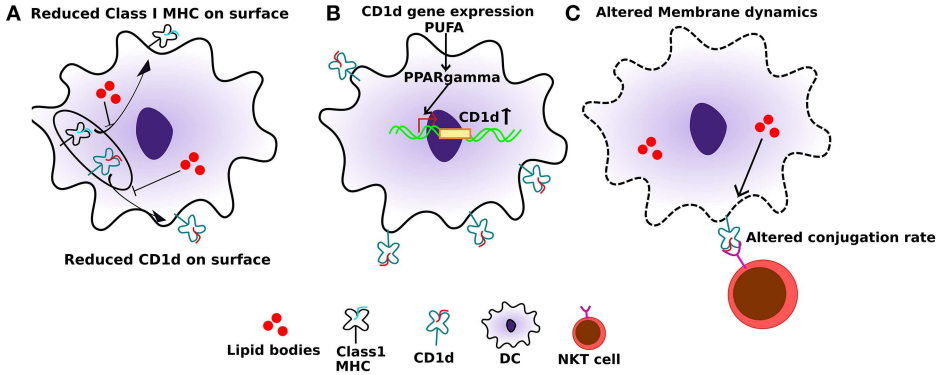

FIGURE 2 | Effect of altered lipids on antigen presentation. **(A)** Accumulation of lipid bodies, mostly saturated lipids, negatively affects the localization of both class I MHC as well as CD1d. **(B)** Also, polyunsaturated fatty acids (PUFAs), especially DHA, can induce PPARγ levels in dendritic cells which in turn induce the expression of CD1d. **(C)** Lipid excess can also affect membrane dynamics, which in turn can interfere with CD1d:lipid antigen conjugation rate with TCRs on NKT cells, leading to sub-optimal NKT function.

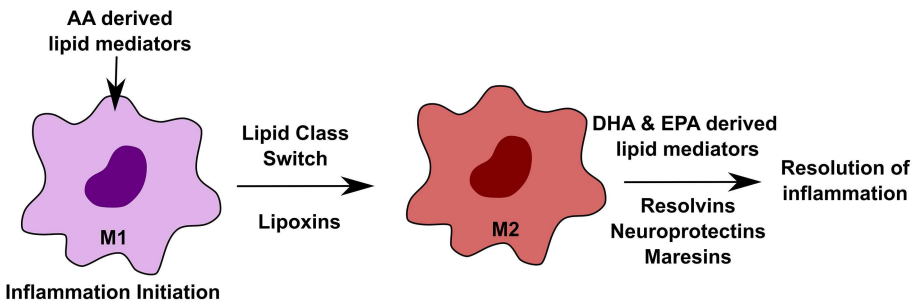

FIGURE 3 | Effect of altered lipids on macrophages. Macrophages play important roles during inflammation in a highly orchestrated manner. During initiation of inflammation, arachidonic acid (AA)-derived lipid mediators, such as prostaglandins and leukotrienes, are required. M1 to M2 transition is mediated by lipoxins, which are also derived from AA. The resolution state of inflammation requires docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) derived lipid mediators such as resolvins, neuroprotectins, and maresins.

reduced numbers of lipid-reactive type I NKT cells (81). This is largely due to defective CD1d antigen presentation and impaired thymic selection of type I NKT cells. Even though the number of NKT cells is reduced, there are still residual NKT cells with differential TCR Vβ usage and CD4 expression in both β-Gal$^{-/-}$ and NPC2$^{-/-}$ mice. This effect is due to the accumulation of different lipids leading to altered CD1d: lipid antigen complex formation. This in turn gives rise to NKT cells with different functional subsets where a significant decrease in Vβ8.2/Vβ7 ratio in β-Gal$^{-/-}$ but not in NPC2$^{-/-}$ was observed, in contrast to an increased ratio of CD4^{-}/CD4^{+} in NPC2$^{-/-}$ but not in β-Gal$^{-/-}$ mice was observed. This suggests a direct effect of the type of lipid antigen presented on both quality and quantity of NKT cells. Several other mouse models of the lysosomal storage disease (Tay-Sachs, GM1 gangliosidosis, Fabry, NCP1) also show a reduced number of type I NKT cells, not due to defective CD1d presentation or lack of APCs, but due to impaired loading of lipid antigen on to the CD1d molecule (82). In addition to the decreased number, some lysosomal mouse models also show a defective function of type I NKT cells (82). Interestingly, in human patients with lysosomal storage disease, harboring NPC1 mutations, there does not appear to be any change in the number of type I NKT cells. Additionally, APCs from the patients can present lipid antigens to type I NKT cells efficiently (83). Although the quantity remains unchanged, the effect on the quality of type I NKT cells in response to altered lipids in lysosomal storage disease (84) is not known in humans.

Effect of Altered Lipids on CD1d Antigen Presentation

DCs are professional APC that carry antigens from local tissues to the draining lymph nodes and are necessary to prime T cells including NKT cells. For the NKT cell priming, the expression level of CD1d is critical. One study reported increased expression of CD1d on human keratinocytes undergoing terminal differentiation upon increased cellular ceramide synthesis as well as exogenous ceramide application (85). Under physiological conditions, one study showed that peroxisome proliferator-activated receptor γ (PPARγ) upregulates CD1d in monocyte-derived DCs at the transcriptional level (86) (**Figure 2B**). Moreover, PPARγ mediated upregulation of CD1d is via activation of the retinoic acid pathway. PPARγ also

enhances internalization activity and effective lipid antigen presentation to iNKT cells, leading to their activation and expansion, when α-GalCer is present (87). Interestingly, DHA-derived lipid mediators act as potential PPARγ agonists (88). Also, DHA has been reported to generate a tumor suppressive effect via PPARγ (89, 90). Consistent with that DHA can specifically upregulate PPARγ expression and levels of its target genes in DCs, and this upregulation is reversed by blocking PPARγ activity (91). However, DHA and lipid mediators derived from it are missing from the tumor environment (48). Several studies report an anti-tumor effect of DHA. DHA dietary supplementation, as well as its use as an adjuvant, has been shown to improve disease outcome in cancer patients (92). Additionally, PPARγ functions as a tumor suppressor and its expression is lost in many cancers (93). We can hypothesize that accumulation of tumor specific lipids in the tumor microenvironment can affect the expression of CD1d on both tumor cells and DCs, thereby suppressing their immunogenicity and facilitating eventual immune evasion. Immunogenic cell death as a result of intratumoral treatment of tumors with anti-cancer agents can lead to release of tumor-specific antigens, which then can activate T-cell mediated immunity and confer long term immunologic memory against tumor (94). The use of EPA/DHA alone or in combination with various chemotherapeutic agents has shown anti-tumor effects, mostly via apoptosis (92). We propose that co-treatment of tumors with EPA/DHA and intratumoral anti-cancer agents may provide a novel effective immunotherapy by mediating presentation of tumor antigens to T-cells and induction of long term anti-cancer immunity.

Effects of Altered Lipids on NKT Cell Function in Inflammation and Cancer

Non-alcoholic fatty liver (NAFLD) is considered as a pre-malignant stage in the liver. One study in an obese mouse model for NAFLD reported a reduction in the number of hepatic NKT cells, as a result of activation-induced death of NKT cells by activated Kupffer cells due to lipid excess (95). Additionally, lipid excess in high fat diet (HFD)-induced obese mice activates type I NKT cells and skews the balance toward a pro-inflammatory cytokine environment. Further, lipid excess also causes obesity-induced insulin resistance and hepatic steatosis in an NKT dependent manner and can be reversed by deficiency of either type I NKT cells or CD1d (96) Another study reported a role of type II NKT cells in HFD induced obesity in mice (97). The study reported minimal weight gain, reduced inflammation, hepatic steatosis and insulin resistance in $CD1d^{-/-}$ mice compared to $Ja18^{-/-}$ mice. In addition to that, a direct role of CD1d mediated presentation of endogenous lipid antigens to activate NKT cells in mice fed with HFD was shown (98). Moreover, deletion of CD1d in adipocytes led to decreased weight gain and higher insulin sensitivity in mice. In a contrasting study, type I NKT cells were reported to suppress diet induced obesity and development of type II diabetes. The study further showed an increased infiltration of pro-inflammatory macrophages and decreased type I NKT in adipocytes during development of obesity. Moreover, an adoptive transfer of iNKT into $Jα18^{-/-}$ obese mice or α-GalCer treatment of WT mice abrogated obesity induced disorders (99). Yet another study, reported no difference in weight gain, insulin sensitivity, inflammation and liver steatosis between $CD1d^{-/-}$ vs. WT mice when fed with HFD (100). In context of hepatocellular carcinoma (HCC) as a result of NAFLD, one study reported no significant change in the NKT cell number as a consequence of increased lipid content in the liver in a transgenic mouse model (101). Another study identified a subset of NKT cells reactive to lysoPC lipid species in myeloma patients (102). In Gaucher disease (GD), another pathology caused by a lipid metabolic defect, it was shown that accumulation of β-glucocyceramide (β-GL1-22) and glucosylsphingosine (LGL1) led to induction of a different subset of type II NKT cell in both mice and humans (103). This specific subset of type II NKT cells leads to aberrant activation of humoral immunity and increased risk of B-cell malignancy.

Ceramides are released when cancer cells are exposed to chemotherapeutics or ionizing radiation leading to apoptotic death of tumor cells (104, 105). As ceramide is a major species of lipid that can be presented by CD1d to be recognized by NKT cells, the activation of NKT cells by ceramides released from treated tumors likely modulates the anti-tumor immune response. Interestingly, in the 4T1 pre-clinical tumor model, radiotherapy in mice deficient in type I NKT cells significantly enhanced tumor regression compared to WT mice with intact type I NKT cells (106). Additionally, administration of α-GalCer, NKT cell agonist that induces strong anti-tumor immunity, did not enhance the response to radiotherapy in WT mice, suggesting a potential immunosuppressive role of type I NKT cells that were exposed to tumor-derived lipids.

Gangliosides are yet another sialic acid-containing diverse group of glycosphingolipids that bind to and activate a subset of NKT cells (107, 108). Any alteration in lipid repertoire can also lead to altered ganglioside milieu. In regard to that, gangliosides disialoganglioside 2 (GD2) and disialoganglioside 3(GD3) have been reported to be overexpressed in cancer and shown to regulate tumor growth and metastasis (109). Mice immunized with melanoma cells expressing GD3 were found to have GD3 reactive NKT cells that were shown to be CD1d restricted (110). Additionally, coimmunization of GD3 loaded APCs along with GM3 loaded APCs suppressed the type I NKT cell function (108). GM3 also suppressed IL-4 production but not IFN-γ by type I NKT cells in response to α-GalCer. Also, GM3 is expressed in several malignancies and targeting it by specific antibody has anti-tumorigenic activity (111–113). In an ovarian cancer model, GD3 was shown to be enriched in tumor microenvironment and inhibit NKT cell activation. Also, GD3 abrogated a α-GalCer mediated NKT cell activation *in vivo* and *in vitro* by competing for the binding to CD1d (114). Furthermore, increased VEGF levels in tumor enhances GD3 levels in ovarian cancer (115). CD1d expressing APCs treated with GD3 significantly suppress NKT cell activation, suggesting a direct role of GD3 as a lipid antigen enriched in tumor in suppressing anti-tumor immunity in an ovarian cancer model through presentation by CD1d to NKT cells. Additionally, both GD3 and GM3 were recently reported to be present in TLR9 stimulated DCs (116) and synthetic versions of β-linked GM3

and GD3 were able to activate type I NKT in mice, both *in vivo* and *in vitro* in a CD1d dependent manner. Taken together, an altered lipid environment in the inflammatory conditions and tumor microenvironment can potentially affect NKT cell function and fine tune the immune response. Understanding the biology behind this can open up several therapeutic avenues such as therapeutically targeting synthesis of tumor promoting (e.g., GD3) lipids and/or using tumor inhibitory lipids (e.g., DHA) as adjuvants to enhance anti-tumor immunity.

EFFECT OF LIPIDS ON HOMING OF NKT CELLS

Localization of an immune cell to the site of injury is critical for resolution of inflammation and tissue homeostasis. In cancer, there are very limited studies that report localizing of NKT cells to the tumor site. CCR2 (expressed by NKT cells) and CCL2 (expressed by a subset of MYCN non-amplified neuroblastoma cells) mediated homing of NKT cells to neuroblastoma was shown in subset of neuroblastoma patients. Also, the survival of patients with NKT cell infiltration was significantly longer than that of patients without infiltration (117). In a follow-up study, it was demonstrated that MYCN repressed the expression of CCL2, thereby preventing homing of NKT cells to the tumor site in both mouse models and human patients (118). Interestingly, MYCN inhibition resulted in reduced tumor growth and improved survival in a transgenic mouse model. At the same time, there was an accumulation of lipid droplets in neuroblastoma cells which were treated with MYCN-inhibitors, suggesting a potential role for lipid metabolites involved in tumor regression (119). Not much is known about the nature of the lipids and mechanisms by which they may affect the recruitment of NKT cells to tumor site, which remain open questions.

One of the early studies reported a role of leukocyte function associated antigen-1 (LFA-1) on accumulation of NKT cells in the liver and LFA-1 deficient mice were shown to have significantly fewer NKT cells. (120). Also, LFA-1-intercellular adhesion molecule 1 (ICAM1) interaction was shown to be critical for tissue resident NKT cells in mice, such that blocking of either LFA-1 or ICAM1 led to a rapid release of NKT cells in circulation, in a parabiotic mouse study. Furthermore, this LFA-1-ICAM1 mediated tissue homing of NKT cells was shown to be dependent on the transcription factor promyelocytic leukemia zinc finger (PLZF) (121). Yet another study revealed the role of a chemokine receptor CXCR6 expressed on the NKT cell surface, and its specific receptor, CXCL16 (a transmembrane chemokine which is expressed on liver, lung and spleen cells), in homing of CXCR6 expressing NKT cells to the liver (122). This pathway is also lipid-dependent because the gut microbiome's metabolism of lipid bile acids affects the induction of CXCL16 and thus NKT cell homing to the liver and ability to control liver cancer (123).

CONCLUSIONS

To date, most immune therapy treatment regimens in cancer focus on peptide-antigen-recognizing conventional T cells. However, lipid-reactive NKT cells have emerged as one of the major immune-modulators in tumor immunity, in pre-clinical mouse models. Although contextual, it is generally acceptable that type I NKT cells exert anti-tumorigenic effect whereas type II NKT cells have an opposite effect. Notwithstanding that both type I and II NKT cells constitute a small percentage of lymphocytes as compared to the conventional T cells, both NKT cell types mediate substantial immunomodulatory effects. Therefore, a deeper understanding of their differential regulation under normal and tumor conditions could unravel novel therapeutic nodes that can prove beneficial for anti-tumor immune therapy. Deregulated lipid metabolism is reported in several cancers. Unlike functional studies of DNA and proteins, knowledge of both the structural and functional roles of lipids in the process of cellular transformation and tumor growth has lagged behind. Changes in lipids can have a global effect on immune response and can influence anti-tumor immunity in both NKT-dependent and NKT-independent manners. Functional studies focused on understanding these aspects of tumor immunity can provide some unique and clinically useful therapeutic interventions.

AUTHOR CONTRIBUTIONS

ST, MT, and JB wrote and edited the manuscript.

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HTLV-1 as a Model for Virus and Host Coordinated Immunoediting

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Immunoediting is a process that occurs in cancer, whereby the immune system acts to initially repress, and subsequently promote the outgrowth of tumor cells through the stages of elimination, equilibrium, and escape. Here we present a model for a virus that causes cancer where immunoediting is coordinated through synergistic viral- and host-mediated events. We argue that the initial viral replication process of the Human T cell leukemia virus type I (HTLV-1), which causes adult T cell leukemia/lymphoma (ATL) in ∼5% of individuals after decades of latency, harmonizes with the host immune system to create a population of cells destined for malignancy. Furthermore, we explore the possibility for HIV to fit into this model of immunoediting, and propose a non-malignant escape phase for HIV-infected cells that persist beyond equilibrium.

Keywords: immunoediting, HTLV-1, HIV, viral reservoir, ATL (adult T-cell leukemia)

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INTRODUCTION

Cancer immunoediting describes the dynamic reciprocity in which the immune system both protects against cancer while inadvertently sculpting a population of cells that may become malignant, progressing through three distinct phases: elimination, equilibrium, and escape [reviewed in (1, 2)]. During elimination, the coordination of innate and adaptive immunosurveillance enables the detection and destruction of early potential tumor cells, while some cells evade the immune response. Cells that survive elimination persist through what can be decades of equilibrium in a dormant state with continued selection pressure that promotes the survival of cells that have developed immunoevasive phenotypes. These cells proceed into the escape phase with acquired somatic mutations and genetic instability that drive their ability to proliferate indefinitely while maintaining invisibility from immunosurveillance, ultimately causing malignancy in the individual (1, 2).

As we discuss what is known about immunoediting in cancer to elucidate the capacity for immunoediting to occur in HIV infection, as discussed in detail in our sister publication in this issue, we explore the intersection of cancer caused by a virus to highlight differences between host- vs. viral-mediated immunoediting, to reveal whether we can untangle the two concepts. Human T cell leukemia virus type 1 (HTLV-1) was the first retrovirus identified to infect humans and was discovered as the etiological agent of adult T cell leukemia/lymphoma (ATL) (3, 4). The prevalence of ATL is as high as 20% among carriers who were born with or contracted HTLV-1 around birth, and infected children have a 25% lifetime risk of developing ATL (5). ATL is extremely aggressive with poor survival outcomes, and even with intensive chemotherapy or allogeneic hematopoietic stem cell transplantation (HSCT), relapse remains high (6). While cancers caused by viruses may undergo what is classically defined as immunoediting, there exists another layer encoded within the viral genome itself to specifically modify how that cell is able to survive or how it can interact with

the immune system. During the decades of latency prior to T cell transformation the processes of mutation, clonal selection and expansion, and selection by the immune system allows for HTLV-1 infected cells to complete the immunoediting process in carriers who develop ATL. This is due to the myriad of epigenetic and genetic changes that accumulate over time, initiated early by viral-mediated events that fated this specific cell to eventual transformation.

EVENTS DURING EARLY HTLV-1 INFECTION SET THE STAGE FOR THE ELIMINATION PHASE OF IMMUNOEDITING

There exist many parallels between HTLV-1 infection and the course of HIV infection, especially in the case of people living with HIV who are treated with suppressive antiretroviral therapy (ART), including their preferential infection of activated memory $CD4^+$ T cells (7) followed by integration into transcriptionally active regions within the host genome (8, 9). Unique integration sites arise through the infectious spread of both viruses, with decades of mitotic division resulting in the clonal expansion of infected cells (10–12). In contrast, unlike the exhaustive viral replication of HIV that leads to an average of 10^5 virions per mL of plasma (13–16), the productive replication of HTLV-1 spreads through the virological synapse (17) in the absence of detectable cell-free virions in peripheral blood (10).

Early in viral replication, the HTLV-1 Tax protein acts as a transcriptional transactivator of the viral long terminal repeat (LTR) (18, 19) analogous to HIV Tat (20). Tax acts through binding host cAMP response element binding protein (CREB) to recruit histone acetyl transferases to the Tax-responsive element 1 (TRE-1) to promote viral transcription (21, 22). Tax can also transcriptionally activate the expression of or alter function of cellular proteins with roles in T cell activation, proliferation, and survival (23–34). Particularly, Tax can inactivate the transactivation function of cellular p53 by inhibiting its N-terminal activation domain (35), abrogating the p53-induced G_1 cell cycle arrest required to allow appropriate repair in response to DNA damage (36). Tax alters the expression of many host proteins associated with cell cycle, accelerating progression through G_1 and disabling checkpoints at cell cycle transitions, meanwhile stimulating antiapoptotic signals, and affecting telomerase expression (37). Maintaining the cell in a metabolically active state confers a fitness advantage for viral replication but with grim consequences to the host cell, potentially enabling chromosomal instability, and the accumulation of host genomic mutations (37).

Infectious spread of HTLV-1 establishes thousands of infected-cell clones and then peaks within 3 months of infection before plateauing (38) to a level that is dependent upon the quality of the individual's mounting immunity (39). The proviral genome encodes its own mechanism to impede the activity of Tax protein. The 3′LTR drives an antisense transcript, expressing HTLV-1 basic leucine zipper factor (HBZ) (40) which can outcompete Tax in binding CREB, blocking interaction with TRE-1 and downregulating viral transcription (40, 41). Tax and HBZ rival in many host cell signaling pathways to alter viral replication and change expression profiles within the cell to coordinate proliferation and survival of HTLV-1 infected cells (42). Where Tax activates pathways including NF-kB, AP-1, NFAT, and Wnt signaling, HBZ acts to repress them (30, 32, 43–46). By hindering ongoing viral replication, cells expressing HBZ are driven into a latent state (40).

Although the control of latency is not as well-defined as in HIV, there are specific cellular attributes that repress HTLV-1 expression. DNA methylation accumulates along the provirus after seroconversion and throughout chronic infection, which is not correlated with the methylation patterns of host genes surrounding viral integration sites (47–49). Early methylation is observed in regions that encode Gag, Pol, and Env, with the 5′LTR becoming heavily methylated over time; sometimes associated with hypoacetylated histones, silencing viral sense transcription (47–49). The 3′LTR remains unmethylated, permitting continued expression of HBZ to drive clonal proliferation (47, 48). These distinct patterns of epigenetic modification are established through the 11-zinc finger protein CCCTC-binding factor (CTCF)—a host insulator element that restricts the spread of epigenetic modifications to define boundaries between transcriptionally active and inactive regions of the genome (50, 51). The binding of CTCF to proviral DNA at the defined epigenetic border modifies viral transcription and splicing (50), contributing to the regulation of latency as similarly observed in EBV (52) and KSHV (53).

Virus-coordinated events early in replication alter the population of $CD4^+$ T cells infected with HTLV-1. Whether Tax expression is a prerequisite for malignancy remains debated in the field, yet we theorize that initial Tax activity is a major driver of immunoediting. Tax-induced changes to the cell that promote viral protein expression and the presentation of neoantigens provoke immunosurveillance and progression into the elimination phase of immunoediting. Initial Tax activity changes the cell and may predestine it to become malignant (31, 34), should it survive the robust HTLV-1 specific immune response and acquisition of appropriate somatic mutations through decades of latency.

ELIMINATION: HOW THE HOST IMMUNE RESPONSE SCULPTS THE PERSISTENCE OF HTLV-1 INFECTED CLONES

The interplay between early viral protein expression and the establishment of HTLV-1 latency synchronize with host immunosurveillance into the elimination phase of immunoediting, where a strong immune response remains unable to eradicate the virus and does not intrinsically prevent ATL (54). $CD8^+$ Cytotoxic T lymphocyte (CTL) responses are detected against viral Gag, Pol, and Env, although Tax remains the immunodominant target (55–61). Studies in rats

Abbreviations: HTLV-1, human T cell leukemia virus type I; ATL, adult T cell leukemia/lymphoma; CTL, cytotoxic T lymphocyte; HIV, human immunodeficiency virus; ART, antiretroviral therapy.

demonstrate that siRNAs against Tax sufficiently downregulate Tax expression, repressing Tax-specific CTL killing of HTLV-1 infected cells (62). In asymptomatic individuals, whilst Tax expression remains low or undetectable immediately *ex vivo*, short-term culture of $CD4^+$ T cells is sufficient to reactivate viral expression from latency which is rapidly controlled with the addition of autologous $CD8^+$ T cells (61). There exists sequence heterogeneity across HTLV-1 isolates, although not as extensive as the diversity observed in HIV that drives the emergence of CTL escape mutants (63, 64). Natural variation in the *tax* gene, however, can lead to peptide presentation that cannot be recognized by consensus-sequence Tax-specific CTLs (63). These variants render severe functional impairment of Tax activity, and therefore a survival advantage that enables the maintenance of a population of cells with reduced sense transcriptional activity that continue to evade immune recognition (59, 63).

Chronically active HTLV-1 specific CTLs are present in otherwise asymptomatic carriers of HTLV-1 without associated disease (58), and the proliferation rates of memory $CD8^+$ T cells are 3-fold higher than in uninfected controls (65). The frequency of HTLV-1 specific CTLs does not correlate with proviral loads, while transcriptomic analysis of $CD8^+$ T cells reveals that individuals with low proviral loads highly express gene clusters associated with improved effector function, and with CTL-mediated lysis (66). Additionally, Tax-expressing $CD4^+$ T cells increase the expression of molecules, i.e., ICAM-1, Fas, and TRAIL-R1/2, improving the susceptibility of these cells to CTL-mediated lysis (60). These data support the notion that bursts of antigenic stimulus throughout latency drive persistent immunosurveillance and depletion of infected cells expressing antigen, suggesting an equilibrium is established between replicating virus and the immune response (58, 59, 66, 67).

The infected individual's human leukocyte antigen (HLA) alleles restrict the repertoire of antigen presented to CTLs (60, 68). *HLA-A*02* binds various Tax epitopes, with a particularly strong affinity of Tax_{11-19} for the peptide binding groove of A*02, which confers a lower proviral load and selective pressure against Tax-expressing cells in asymptomatic carriers (54, 58, 69–71). HBZ also binds to the protective alleles *A*02* and *CW*0801,* leading to lower proviral loads in asymptomatic carriers (54). However, the frequency of HBZ-specific CTLs is significantly lower than Tax-specific CTLs, and the binding affinity of HBZ to HLA molecules is notably weaker than Tax peptides (54, 60). The low immunogenicity of HBZ in asymptomatic carriers is mirrored in subsequent malignancy—ATL cells constitutively express HBZ, yet HBZ-specific CTLs fail to lyse transformed ATL cells (72). However, more recent work has demonstrated that vaccines harboring specific HBZ epitopes, i.e., $HBZ_{157-176}$, can elicit anti-HBZ CTLs in model ATL mice (73), warranting further research to improve the immunogenicity of low-affinity HLA-associated HBZ peptides to enhance ATL immunotherapy (74). That HBZ does not stimulate strong CTL responses may be surprising, given its imperative action in maintaining the survival and proliferation of infected cells throughout all phases of immunoediting. This could be due to the additional function elicited by *HBZ* in its RNA form, inducing distinct antiapoptotic activity, therefore precluding the expression of antigen while promoting cell survival (75).

The capacity of HBZ to downregulate Tax-induced viral transcription occurs, in part, to evade the stronger immunodominant Tax-specific CTLs. The dynamic coordination between early virus- and immune-mediated events permit the sufficient control of active viral replication while creating longevity in a population of infected cells refractory to immune recognition, inherently sculpting the persistence of certain clonal populations of infected $CD4^+$ T cells that do not express Tax. This elimination phase of immunoediting synchronized by the virus and the host immune response orchestrates the immortalization of infected cells without transforming them, securing their persistence for years.

HIV also evades immune recognition, albeit through different mechanisms including Nef-directed downregulation of CD4 and MHC class I receptors (76, 77). In our sister article, we describe in depth, models of immunoediting during HIV infection and the progression to AIDS, as well as through suppressive ART. Entry into latency is established soon after HIV transmission, either by infected activated $CD4^+$ T cells reverting back to a resting state (78–81) or through the direct infection of resting $CD4^+$ T cells (82–85). Individuals living with HIV who have access to ART can achieve undetectable viral loads (13–16). This medical intervention (which is not required to inhibit the infectious spread of HTLV-1) allows for the recovery of $CD4^+$ T cells (13–16). Consequently, this recovery enables clonally infected populations that exist before the initiation of ART (86) or that are seeded as viral replication wanes, to expand and contract (87) through years of equilibrium congruent with this model of immunoediting.

EQUILIBRIUM: VIRAL AND HOST FACTORS THAT CONTRIBUTE TO THE CONTINUED SELECTION AND SURVIVAL OF HTLV-1 INFECTED CLONES

HTLV-1 infected cells that have survived the elimination phase, and exist in latency with an immune-evading phenotype, will endure into the equilibrium phase of immunoediting. During equilibrium, sustained polyclonal expansion of infected cells will favor clonal populations that continue to accumulate somatic changes that facilitate cell survival (**Figure 1**).

HTLV-1 integrates preferentially into transcriptionally active regions of the host genome (88, 89), with a modest preference for integration near host transcriptional start sites (90), with a small percentage of ATL cases (<6%) containing proviruses near genes associated with hematological malignancies (91). Asymptomatic carriers harbor between 10^4 and 10^5 clones with unique integration sites (92, 93) capable of indefinite proliferation (51) and a preference for the expansion of clones containing proviral integration within the long arm of acrocentric chromosomes 13, 14, 15, and 21 (91). The hypothesized survival of these particular integration sites is that these chromosomes are physically associated with the nucleolus of non-dividing cells, and this nucleolar periphery remains transcriptional quiescent—such that these cells evade HTLV-1 specific CTL killing (51, 91).

Throughout decades of equilibrium, clonal populations are selected for, expand and contract, and integration site sequencing

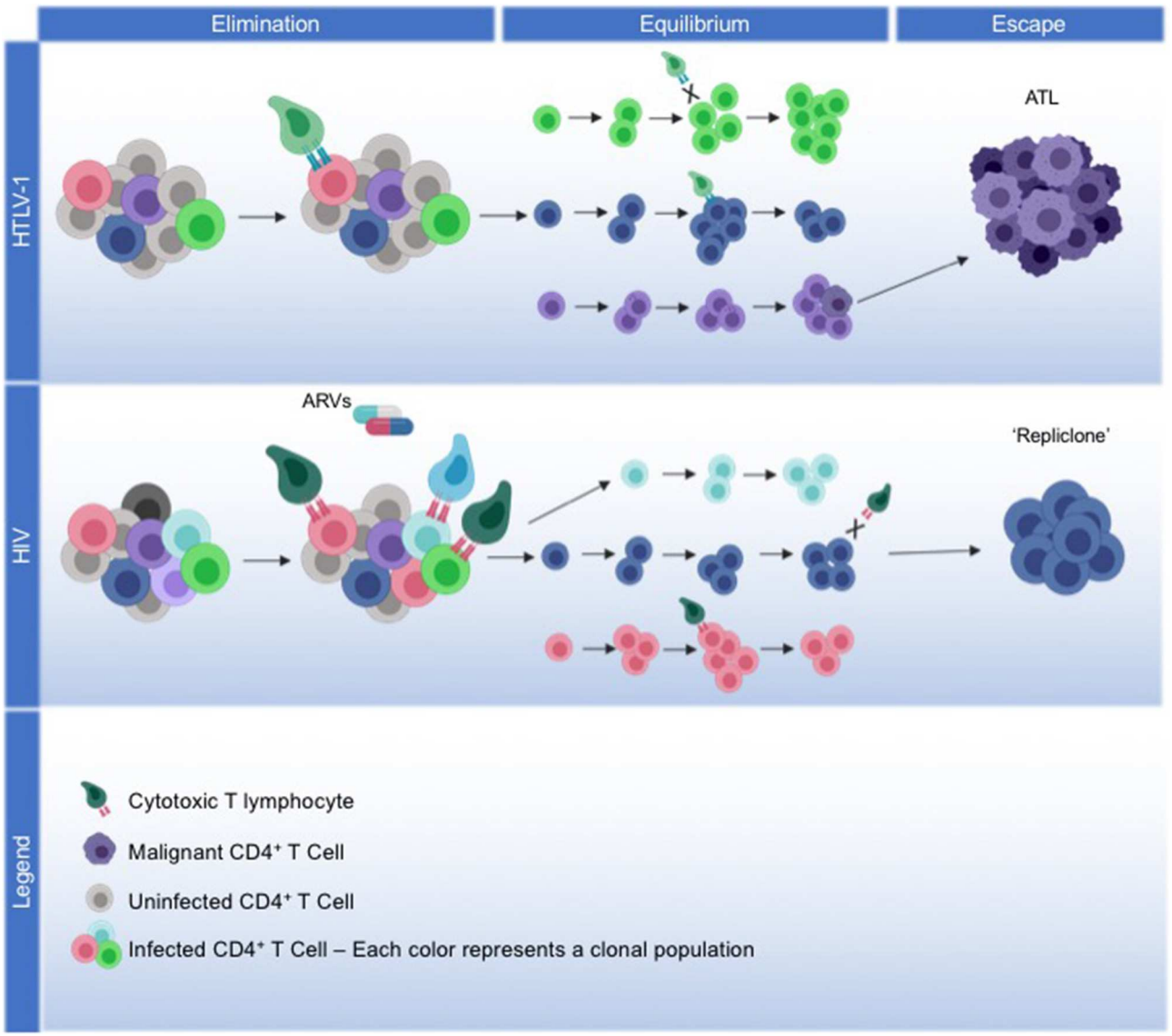

FIGURE 1 | Model for viral and host coordinated immunoediting. **HTLV-1**: We propose a model of immunoediting that leads to ATL, mediated through HTLV-1 replication, and the host immune response. HTLV-1 infection spreads through the virological synapse without cell-free virions, maintaining low proviral loads. The viral protein Tax drives viral replication and alters the expression or activity of many host proteins involved in survival and proliferation, facilitating the immortalization of infected cells. During the elimination phase, the development of a robust Tax-specific CTL response will kill off cells that continue to express Tax, and infection plateaus. HBZ expression from the antisense transcript will repress viral transcription to protect cells from CTL killing, meanwhile driving them into latency. Immortalized cells that can evade the immune response are selected into equilibrium, where continued HBZ expression maintains the survival and proliferation of latently infected cells. Over decades of equilibrium, clonal populations with identical proviral integration sites continue to proliferate, accumulating somatic mutations and epigenetic modifications that may lead to the eventual transformation into ATL. Through the escape phase, a malignant cell emerges from a monoclonal population, with HBZ expression and the acquired somatic mutations enabling ATL cells to continue to proliferate and evade cancer immunosurveillance. **HIV**: We propose that HIV, in part, fits into this immunoediting model, albeit with differing mechanism. During the elimination phase, robust viral replication of HIV initially establishes high proviral load, with strong HIV-specific CTL responses enabling infection to plateau. With the addition of antiretrovirals (ARVs), infection is driven into latency and as viral replication ceases, latently-infected cells not recognized by CTLs will persist into the equilibrium phase. Throughout decades of latency, cells with the same proviral integration sites will clonally expand, with clonal populations waxing and waning over time. Less is known about what drives the proliferation of certain HIV infected clonal populations. Recently, the concept of a "repliclone" has been established, representing the expansion of a monoclonal population with replication competent provirus defined by a single integration site. Although these cells are not malignant, they persist into the escape phase given a yet unknown selection advantage for survival and proliferation.

reveals that 90% of cancer cells from patients with ATL are expanded from a single predominant malignant clone (91, 94). What drives the selection of this monoclonal population of malignant cells in the background of polyclonal populations that fail to transform? The prevalence of each clonal population represented by the proportion of observed integration sites to

PVL suggests that a malignant clone emerges from an initially low-abundant clonal population (91), rather than a largely expanded clonal population (11, 93). While high-abundance clones manage to progressively increase over time, low-abundance clones tend to decay, likely due to their modest levels of Tax expression during latency (51, 93). Low-abundance clones that progress to ATL display the highest level of integration within acrocentric chromosomes 13 and 15 (91), potentially contributing to sporadic Tax expression during cell division- or stress-induced dispersal of nucleoli, temporarily releasing viral transcriptional repression (51). There are documented plus-strand transcriptional bursts during chronic infection that enable intermittent expression of Tax protein (95) that can trigger antiapoptotic machinery (96), thought to contribute to survival of infected cells and lasting Tax-specific CTL responses (51) and an additional push toward transformation into ATL cells.

Each clone has a particular susceptibility to experience malignant transformation, initially predisposed through Tax-induced changes to the cell (51), as described above. While Tax is not commonly expressed in latency or transformed ATL cells, HBZ is constitutively expressed throughout chronic infection and in ATL, and displays oncogenic properties (51, 97). HBZ promotes proliferation by targeting retinoblastoma tumor suppressor protein (98) and further inhibits apoptosis by repressing the transcriptional activity of p53 (99, 100), and by suppressing the pro-apoptotic genes *Bim* and *Fas ligand* by downregulating their transcriptional activator, FoxO3a (101). Even in the absence of Tax expression, HBZ can maintain the immortalized status of the cell and continues to drive clonal expansion.

Proliferation of these clones over decades also allows for the accumulation of somatic mutations due to random errors during DNA replication, i.e., somatic mutations (102). Independent of Tax- or HBZ-induced inhibition of p53 function, which occurs in the presence of wild type p53 protein, 30–40% of ATL patients have acquired mutations in the p53 gene (23, 103–105). In an incredible integrated molecular analysis of ATL cells from 426 individuals, Kataoka et al. investigated the whole-genome exome, transcriptome, and methylome of ATL cells (97). They identified significant mutations in 50 genes, with over 30% of mutations observed in both the phospholipase C γ1 (PLCγ1) gene and a member of the PKC family of proteins (PRKCB), additionally correlated to mutations in the cytoplasmic scaffolding gene *CARD11*, with RNA sequencing confirming transcripts with acquired mutations (97). Other hotspot mutations were observed in genes within the same pathway, and although there were no functional analyses on the acquired mutations in this study, literature in other cancers indicate that together the observed changes in amino acid sequence are gain-of-function mutations in this set of genes, and can act to increase TCR signaling and antigen-receptor induced NF-kB activation of T cells (97). Interestingly, 56% of ATL cells exhibited deleterious mutations that would predispose them to evade immunosurveillance, including within the major histocompatibility complex (MHC) class I, immune checkpoints, and death signaling pathways. The MHC class I gene was also discovered to be extensively hypermethylated, with 90% of ATL cases harboring mutations and/or methylation patterns within this gene that would render a loss of expression of MHC class I (97). Other mutated pathways discovered through this study are common in other human malignancy, including DNA repair mechanisms, epigenetic regulation, and telomere preservation. Overall, the accumulation of these mutations over time demonstrate the ability of cells to continue to proliferate while evading immune response.

In addition to the integration site and accumulation of somatic changes, another mechanism for persistence is the accumulation of clonal populations containing defective provirus. Defective proviral genomes may lack the 5′LTR and flanking genomic regions that encode immunogenic gene products, particularly Tax, contributing to immune evasion (106, 107). Defective proviral genomes that explicitly express HBZ can proliferate and avoid immunosurveillance, while driving cells toward malignancy. HBZ is sufficient to stimulate T cell lymphoma in mice in the absence of any other viral proteins (108) even after a period of latency (109), and the concept that HBZ alone could induce ATL has been observed *in vivo* (107). While HBZ is ubiquitously expressed in ATL (97), defective proviruses are observed in up to 56% of ATL cells (107, 110, 111). Tamiya et al. identified the existence of what they termed a type II defective provirus as one that contains a large recombination between *env* and the 5′LTR, partially deleting the LTR and most of the genome, whilst others have identified the complete deletion of the 5′LTR (112, 113)—in both cases, only an active 3′LTR remains, and individuals with type II defective proviruses have the most aggressive forms of ATL (107). Cells that harbor defects in the 5′LTR that preclude Tax expression would completely evade Tax-specific CTLs. The discovery of these type II defective proviruses was made over a decade before the identification of HBZ; Tamiya et al. could only speculate at this point that a viral gene other than *tax* could potentially drive transformation, and they were correct. It is now widely accepted that an intact 3′LTR and *HBZ* gene are essential for oncogenesis (51, 113, 114).

Further supporting Tax immortalization but HBZ oncogenesis is the closely related retrovirus, HTLV-2, which is not associated with malignancy (115, 116). The Tax protein of HTLV-2 has demonstrated behavior in driving T cell immortalization by promoting survival and abnormal proliferation through similar mechanisms observed by HTLV-1 Tax (117). HTLV-2 also encodes an antisense protein, APH-2, which can repress HTLV-2 Tax-mediated transcription (118, 119), yet does not exhibit the oncogenic properties of HBZ, and does not promote malignancy (120). These studies suggest the oncogenic behavior of HBZ is distinct from Tax-driven immortalization of $CD4^+$ T cells.

HTLV-1 infected clonal populations are selected for through decades of equilibrium from the initial immortalization of these cells by Tax, with maintained survival achieved from the ubiquitous expression of HBZ. In addition, certain common integration sites, proviral defects, and the acquisition of genetic and epigenetic changes that drive proliferation whilst protecting against immune responses promote further survival of these clones. While HIV latently-infected cells with identical integration sites are also demonstrated to undergo clonal proliferation (9, 12, 121, 122), it remains

unknown what drives this expansion. Similar to HTLV-1, HIV preferentially integrates within introns of actively transcribing genes. And while HIV does not express a protein analogous to HBZ to promote cell proliferation, HIV has demonstrated integration patterns within genes responsible for controlling cell division and growth, perhaps contributing to their clonal expansion (12). Despite different mechanisms, both retroviruses promote the clonal proliferation of their latently infected cells. HTLV-1 infected $CD4^+$ T cells expand in a polyclonal manner, with a dominant clone selected for malignancy in a fraction of individuals (**Figure 1**). Although HIV does not transform cells, polyclonal populations wax and wane throughout latency (87), raising the possibility that particular populations could be selected for through the escape phase of immunoediting.

ESCAPE: HOW A SINGLE HTLV-1 CLONE BECOMES CANCER

Now that HTLV-1 infected cells have survived through decades of equilibrium, a select monoclonal population that has accumulated the appropriate set of mutations, and which remains resistant to immune defenses, will enter the escape phase of immunoediting, and become the malignant population of ATL cells in 5 to 20% of individuals living with HTLV-1. ATL is classified into four clinical subtypes, acute, lymphoma, chronic, and smoldering; each with varying clinical manifestations, pathogenesis, and treatment strategy (123). Cells that have undergone transformation into ATL display hallmarks of malignancy, the majority of which are driven by HBZ activity—some identified hallmarks have enabled the development of new targeted therapies for ATL.

Now that an HTLV-1 infected clonal population has become malignant, it must continue to counteract cancer-specific immunosurveillance, not only HTLV-1-specific immune responses. A variety of cancers can manipulate immune checkpoint expression to evade immunosurveillance, leading to the development of immune checkpoint blockade as a successful therapeutic strategy for certain cancers (124). The co-inhibitory receptor T cell immunoglobulin and ITIM domain (TIGIT) is a well-characterized inhibitory checkpoint. When upregulated on $CD4^+Foxp3^+$ T cells, TIGIT induces the expression of IL-10, contributing to dysfunctional $CD8^+$ T cells within tumor microenvironments (125). Classically, $CD4^+Foxp3^+$ T cells are defined as T regulatory (Treg) cells, and up to 70% of transformed ATL cells express Foxp3 (126–130). These findings initially associated ATL with Tregs, even suggesting that ATL originates from the Treg subset infected by HTLV-1 (131). More recent findings have demonstrated that HBZ can modify the immunophenotype of conventional $CD4^+$ T cells to exploit the desired properties of Treg cells while impairing their suppressive function (109). HBZ can enhance *Foxp3* transcription and hijack its function to stimulate proliferation of ATL cells (44, 109, 132). TIGIT is also highly expressed on ATL cells (132–134). HBZ directly increases the expression of TIGIT to protect ATL cells from immunosurveillance (132), meanwhile abrogating the inhibitory effect TIGIT generally has on T cell proliferation, allowing the cells to continue to proliferate (133). HBZ can also induce the expression of the immunosuppressive cytokine IL-10, increasing its secretion from ATL cells, further supporting the role HBZ plays in the evasion of anti-viral and anti-cancer host defenses (132).

Expression of the host CC chemokine receptor CCR4 is observed in 90% of tumor cells isolated from individuals living with ATL (135–137). Gain-of-function mutations in the *CCR4* gene enhance the chemotactic properties of ATL cells, thought to drive infiltration into organs by impairing CCR4 internalization, and improve cellular metabolism and survival by prolonging PI3K/AKT signaling (97, 138). HBZ can induce the expression of CCR4 through its major transcription factor (GATA3), driving migration and proliferation of ATL cells (139). These findings led to the development of mogamulizumab, a defucosylated monoclonal antibody against CCR4, which was recently approved for treatment of relapsed ATL (137, 140, 141). The defucosylated portion of the Fc region of mogamulizumab enhances antibody-dependent cellular cytotoxicity (ADCC) against $CCR4^+$ ATL cells given an increased affinity to bind the Fc receptor on effectors cells (137, 142, 143). In a small clinical study to investigate the dynamics of this successful monotherapy, mogamulizumab was demonstrated to reduce proviral load, with a particularly rapid reduction of the abundance of the $CCR4^+$ malignant clone (144). Researchers suggest that individuals with high levels of $CCR4^+$ HTLV-1^+ cells could benefit from this therapy to prevent the development of ATL. This type of immunotherapy, however, must proceed with caution. CCR4 is expressed on Tregs to drive their migration to and mitigate inflammatory responses in tissue (145), and while a reduction in Tregs may boost immunity in the tumor microenvironment, it may simultaneously create autoimmunity in tissue sanctuaries (146). Although mogamulizumab treatment is demonstrated to maintain ATL remission and decreased HTLV-1 proviral loads, the associated reduction in normally functioning Tregs can cause severe adverse events, as observed in individuals with ATL treated with mogamulizumab; one individual developed fatal Stevens-Johnson syndrome due to reduced Treg populations (146).

Overall, immunoediting coordinated by the virus and by the host immune response has inherently compelled particular HLTV-1 infected cells down a pathway toward cancer. This transpires in at least 5% of individuals, and within those individuals, only occurs from 1 in 10^5 unique clones. ATL is rare, yet very aggressive. It takes 40–50 years for infected cells to transform and depends on the initial immortalization of cells by Tax, years of proliferation driven by HBZ, and the accumulation of the appropriate set of host cell genetic and epigenetic changes, all while evading HTLV-1- and cancer-specific immunosurveillance. In this circumstance, immunoediting has enabled the immune system to protect against viral infection whilst promoting the persistence of cells fated to become malignant, and the historical evolution of HTLV-1 with human cellular machinery has created a symbiotic relationship between the virus and its host cell, at eventual the cost of human lives.

WHAT DOES HIV LOOK LIKE IN THE ESCAPE PHASE UNDER THIS MODEL?

There have been no reported cases to date of HIV causing malignancy in $CD4^+$ T cells, which is likely due to the fact HIV does not express a protein homologous to HTLV-1 HBZ, the driver of ATL. Although HIV and HTLV-2 both encode an antisense element [ASP (147) and APH-2 (118), respectively], neither display oncogenic properties (120, 148). What does the escape phase of immunoediting look like for HIV to fit into this model, even without malignancy? We propose that the final "escape" of clonally expanding cells that remain refractory to immunosurveillance are those recently termed "repliclones," an emerging concept in which $CD4^+$ T cells harbor full-length replication-competent proviral sequences with identical integration sites, sometimes contributing to clinically-detectable viremia in individuals on long-term suppressive therapy. Although these integration sites represent rare events in the background of the multitude of defective proviral genomes, the clones themselves are large, with an estimated expansion between 50 to 300 million $CD4^+$ T cells (Halvas EK; Conference on Retroviruses and Opportunistic Infections 2019; Seattle, Washington) (**Figure 1**). Currently, there is no evidence that they are selected for, and research is warranted to define mechanisms that drive their capacity to expand and potentially contract, and to elucidate their ability to produce clinical viremia in the presence of circulating HIV-specific $CD8^+$ T cells.

There are multiple suggested and demonstrated mechanisms that drive the clonal expansion of HIV latently infected cells, encompassing progression through normal T cell function undergoing homeostatic proliferation while enduring bursts of expansion in response to antigen (149). In a case study, Simonetti et al. reported on an individual that developed low-level viremia after 12 years on ART. Single genome sequencing of plasma viral RNA revealed a portion of this population was genetically diverse and viremia was attributable to drug resistance. The other portion of viral RNA was identical, and lacked drug resistance mutations. Changing ART regiment suppressed the diverse sequences with resistance mutations, but did not affect the identical sequences. These sequences were mapped to an integration site in an ambiguous region of the human genome, thus labeled AMBI-1, with an estimated expansion to 9 million cells over time (150). The authors suggest that this clone could persist and expand over years whilst producing virus particles because this individual never achieved full T cell recovery, potentially impairing immune responses. Additionally, this individual developed squamous cell carcinoma, and AMBI-1 RNA sequenced from plasma waned post-cancer treatment, but reemerged with its relapse. Autopsy of the metastatic lesions revealed that infiltrating $CD4^+$ T cells in tumor tissue were enriched for AMBI-1 clones (150). This suggests proliferation of this clone could have been driven through response to tumor antigen (12), and is consistent with a model where response to antigens or homeostatic proliferation are the major drivers of clonal expansion. In some cases, the proviral integration site itself may also contribute to enhanced clonal expansion and persistence of cells. This is supported by studies which have reported over-representation of expanded clones with integrations into genes associated with proliferation, i.e., *MKL2* and *BACH2* (12). The study of factors responsible for driving clonal expansion in HIV remains an active and important area of study.

Although the repliclone does not become malignant as observed in HTLV-1, it follows the concept that a clonal population can be selected for, refractory to immune response, and can survive for years. Given these expanded clones are difficult to study, evidence for them is limited, with more reports emerging at conferences. It appears that the escape of a repliclone has many mechanisms at play and will likely differ across individuals. Whatever the mechanism of expansion, it remains clear that these repliclones do persist, and perhaps have accumulated survival phenotypes throughout decades of elimination, antiretroviral therapy, equilibrium, and escape. As discussed in our partner manuscript, we have recently described that replication competent HIV-infected cells from individuals on suppressive ART are resistant to HIV-specific CTL killing, even when stimulated to reactivate latent infection (151). The inherent survival of these cells that have undergone robust T cell activation and are otherwise hardwired to achieve successful viral replication and antigen presentation with susceptibility to CTL killing, suggests these cells have acquired a unique feature or set of features through the various phases of immunoediting that facilitate proliferation and survival and the acquisition of an immune-evading phenotype. Research to uncover these mechanisms of persistence and immune evasion is extremely relevant for the field, and may elucidate treatment strategies to curb populations that otherwise continue to expand and greatly contribute to the perseverance of the stable, replication competent latent reservoir.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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CAR Talk: How Cancer-Specific CAR T Cells Can Instruct How to Build CAR T Cells to Cure HIV

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Re-directing T cells via chimeric antigen receptors (CARs) was first tested in HIV-infected individuals with limited success, but these pioneering studies laid the groundwork for the clinically successful CD19 CARs that were recently FDA approved. Now there is great interest in revisiting the concept of using CAR-expressing T cells as part of a strategy to cure HIV. Many lessons have been learned on how to best engineer T cells to cure cancer, but not all of these lessons apply when developing CARs to treat and cure HIV. This mini review will focus on how early CAR T cell studies in HIV paved the way for cancer CAR T cell therapy and how progress in cancer CAR therapy has and will continue to be instructive for the development of HIV CAR T cell therapy. Additionally, the unique challenges that must be overcome to develop a successful HIV CAR T cell therapy will be highlighted.

Keywords: T cell, lentiviral (LV) vector, immune escape and surveillance, clinical trials, immune privilege

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HOW INITIAL HIV STUDIES PAVED THE WAY FOR SUCCESSFUL CD19-DIRECTED CAR THERAPY

From a T cell perspective, controlling HIV replication and cancer growth share many of the same challenges: antigen escape, antigen persistence resulting in T cell exhaustion, and active mechanisms employed by both HIV and tumors to avoid T cell recognition and elimination. Thus, the use of CARs to redirect T cells toward both HIV and cancer as a means to bolster T cell control of these maladies was an attractive concept, which led to the preclinical studies using both HIV and cancer models. In the 1990s when antiretroviral therapy (ART) was in its infancy and not yet able to provide durable control of HIV replication, the rationale to treat HIV infection with CAR T cell therapy advanced more rapidly, and in this setting, the first CAR T cell trials were performed. These studies tested the ability of T cells expressing a major histocompatibility complex (MHC)-unrestricted chimeric receptor consisting of CD4, as the natural ligand of the HIV Envelope (Env) glycoprotein, and the CD3 zeta (ζ) chain (1) to suppress viral replication in HIV–infected individuals (2–4). While clinical success was not achieved with these early efforts in the just-emerging CAR T cell field, these efforts were not a "failure," but in fact, successfully laid fundamental groundwork that enabled success using CAR T cells to treat CD19-expressing tumors. Several key observations and discoveries foundational to the overall field of CAR T cell therapy were made during the clinical investigation of CD4-ζ CAR T cells. For one, the field gained an appreciation that a combination of CAR-modified CD4 and CD8 T cells, rather than purified CD8 T cells alone, resulted in a marked improvement in CAR T cell persistence (3). This was ultimately confirmed by demonstration of >10 years of durable CD4-ζ CAR T cell detection in treated subjects (5). Additionally, these early studies demonstrated that rapid and

reproducible CAR T cell manufacturing could be achieved both from uninfected and viremic HIV-infected subjects following 10-day culture incorporating T cell co-stimulation with anti-CD3 and anti-CD28 immuno-magnetic beads. This manufacturing process resulted in improved functional properties of CD4-ζ CAR T cells as well as stable and durable *in vivo* persistence (3–5). Moreover, evidence in randomized trials suggested modest anti-viral activity in HIV-infected subjects through demonstration of trends in reduction of blood- and gut-associated HIV reservoirs, and a reduction in transient viral rebound in plasma (or "blips") in aviremic subjects (2, 4). Finally, these studies demonstrated a lack of immunogenicity of the fully human CD4-ζ construct and an absence of depletion of MHC class II expressing cells, suggesting that CD4-MHC class II interaction was not sufficient to trigger CAR activity. Of note, these early trials with CD4-ζ CAR T cells were performed with the first generation CAR constructs using gamma-retroviral vectors and including only the CD3-ζ cytoplasmic domain without the benefit of co-stimulatory molecules, such as CD28 or 4-1BB, included in successful modern CAR T cell trials. Additionally, these early HIV-specific CAR T cells were not protected from HIV infection, a risk that is further exacerbate by using CD4 as a retargeting domain. Recently, a CD4-based CAR that was re-engineered (see details below) to incorporate lessons learned from successful cancer targeting CARs (6), was shown to confer greater antiviral activity than widely-investigated broadly neutralizing antibody (BNAb) based CARs. This CAR coupled with agents to protect the CAR from HIV infection (7–10) has recently entered the clinic (NCT03617198) to determine whether these changes augment HIV CAR T cell activity and provide some durable control of HIV replication and/or reduce the latent reservoir. The evolution of CAR design is summarized in **Table 1.**

CANCER AND HIV: SHARED CHALLENGES AND OPPORTUNITIES

Persistent Antigen and Exhaustion

Persistence of antigen at high levels drives exhaustion of T cells, which limits the functional properties of T cells and is characterized by high expression of immune checkpoint (IC) molecules, such as programmed death-1 (PD-1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), ultimately hindering clearance of tumors and chronic infections (13–16). An advantage of CAR T cell therapy is that new, fully functional T cells can be redirected toward HIV or tumor antigens. Once re-infused, however, these CAR T cells are susceptible to becoming exhausted if they are unable to clear the targeted antigen in a timely manner. Thus, the reversal or prevention of T cell exhaustion may represent a mechanism whereby dysregulated immunity is prevented, allowing CAR T cells to have a longer therapeutic window to control either HIV replication or tumor cell growth.

Antibodies targeting ICs (e.g., PD-1, PD-L1 or programmed death-ligand 1, and CTLA-4) have shown clinical responses in multiple tumor types, including melanoma, renal cell carcinoma, non-small cell lung cancer (17), and bladder cancer (18). So far, there are six U.S. FDA-approved immune checkpoint inhibitors (ipilimumab, nivolumab, pembrolizumab, avelumab, atezolizumab, and durvalumab) and their objective response rates have ranged from 27% in melanoma patients, to 30% in non-small cell lung cancer patients, and 63% in Kaposi sarcoma patients (19). However, there have been significant immune-related toxicities, including onset of type 1 diabetes, colitis, and dermatological issues (20) that may represent an acceptable risk/benefit to advanced cancer patients, but may be unacceptable to HIV-infected individuals whose viral load is well-controlled by ART. Several clinical trials are currently underway to explore the effect of anti-PD-1 based therapies in HIV-infected individuals who also have tumors known to be responsive to PD-1 blockade (NCT03367754, NCT02408861) (19) and one trial is treating non-tumor bearing HIV-infected individuals (NCT03787095). It will be interesting to see if and, if so to what extent, anti- PD-1 therapies can re-invigorate the HIV-1 specific immune response and whether side effects of this anti-PD-1 therapy in this otherwise healthy population confer an overall benefit/risk sufficient to permit wider exploration in HIV Cure studies.

Furthermore, some studies show that PD-1 also contributes to the establishment and maintenance of HIV latency, so checkpoint blockade may be a promising approach to reverse latency (21). In order for the remaining hidden pool of virus to become recognized by HIV–specific T cells, it must be reactivated first and this could be accomplished by various latency reversing agents (LRAs) (e.g., histone deacetylase inhibitors (HDACis) and protein kinase C class drugs) (22). IC blockades could also function to reverse HIV latency through limiting inhibitory signals sent from IC molecules into cells harboring latent HIV. CTLA-4 blockade results in significant increases in plasma viremia and T cell activation (23). Thus, the combination of IC blockade coupled with HIV CAR T cell therapy may be an effective "shock and kill" (24) strategy.

If systemic checkpoint inhibitor approaches prove too toxic for routine use in HIV-infected individuals, specific targeting of checkpoint genes within HIV-specific CAR T cells via clustered regularly interspaced short palindromic repeats (CRISPR) or small hairpin RNA (shRNA) technologies may prove an effective and safe way to make HIV-specific CAR T cells exhaustion resistant because only the HIV CAR T cells will have their checkpoint genes disabled (25, 26). Here, cancer-based therapies are paving the way for HIV-specific therapies. A clinical trial using a CRISPR-based approach to disable PD-1 is currently underway (NCT03399448) to determine if this improves the anti-tumor efficacy of engineered New York esophageal squamous cell carcinoma 1 (NY-ESO-1), a cancer-testis antigen expressed in a wide range of tumor types -targeted T cells. If successful, this trial could establish sufficient safety and feasibility to warrant coupling HIV CARs with PD-1 CRISPRs. Other immune checkpoint inhibitors, such as those targeting T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), lymphocyte-activation gene 3 (LAG-3), and T-cell immunoreceptor with Ig and ITIM domains (TIGIT), may also help enhance anti-HIV CAR T cell therapy by overcoming T cell exhaustion, possibly with a more acceptable safety profile (27–30).

TABLE 1 | Evolution of CARs used in HIV and cancer cell and gene therapy.

Component	First generation HIV CARs (11)	CD19 CARs that led to first FDA approval (12)	Current HIV CARs being tested in NCT03617198 (6)	Functional impact
Viral vector	γ Retrovirus (MMLV-based)	Lentivirus (HIV-based)	Lentivirus (HIV-based)	Safety, sustained expression
Promoter	PGK	EF1α	EF1α	Higher expression (MFI), sustained expression
Hinge	None	CD8α	CD8α	Flexibility
Transmembrane	CD4	CD8α	CD8α	Helps prevent infection, dimerization to promote activation
Signaling motifs	CD3ζ	CD3ζ, 4-1BB	CD3ζ, 4-1BB	Improved *in vivo* expansion, survival, and persistence
Extracellular domain	CD4 EC domains	scFv domains	CD4 EC domains	No immunogenicity or off target recognition. HIV's ability to escape will likely be limited

Antigen Escape

Antigen escape and efforts to limit T cell recognition of targeted cells are major hurdles for effective T cell-based HIV and cancer control (13). Most common mechanisms of antigen escape in cancers are (1) the immune selection of cancer cells, which lack or mutate immunogenic tumor antigens or lose expression of the antigens targeted by CAR T cells, (2) the acquisition of defects or deficiencies in antigen presentation [e.g., loss of major histocompatibility (MHC) expression], or (3) deficits of antigen processing machinery (31–33). Multiple compelling studies suggest that aberrant signal transducer and activator of transcription 3 (STAT3) signaling plays a key role in facilitating tumor escape from immune detection by impairing antigen presentation and reducing production of immunostimulatory molecules (34). Thus, STAT3 inhibition in concert with other immunostimulatory agents, such as toll-like receptor (TLR) 3, TLR7, and TLR8 agonists like stimulator of interferon genes (STING) or retinoic acid inducible gene (RIG)-I, could provide promising combination immunotherapeutic strategies. Additionally, a variety of CD19 mutations and alternative splicing have been observed with development of acquired resistance of acute lymphocytic leukemia (ALL) to CD19 targeted CAR T cells (35). In this regard, CARs targeting distinct motifs on the tumor surface may be an effective strategy to prevent resistance through tumor escape. For example, Ruella et al. demonstrated that the combination of CD123-targeted and CD19-targeted CAR T cells prevented relapses caused by antigen loss in preclinical models (36). Another study used bispecific CARs that targeted both CD19 and CD20 in order to minimize antigen escape from CD19-negative leukemia. Those bispecific CAR T cells were able to eradicate heterogeneous populations of leukemic cells in NSG mice (37).

In the case of HIV, the virus has evolved features to escape from immune monitoring with quick selection for cytotoxic T lymphocytes (CTL) escape mutations prior to antiretroviral therapy (ART) due to an error prone reverse transcriptase (10). Additionally, the HIV-1 negative regulatory factor (Nef) protein modulates expression of MHC class I, CD28, and other proteins involved in immune recognition to evade CTLs (38–40). As a result, recent efforts have focused on introducing a potent engineered immune response designed to overcome HIV's escape mechanisms instead of solely relying on the endogenous immune response to control HIV replication in the absence of ART (41–43). One advantage of CARs to target HIV is that HIV Env expression on the cell surface is not affected by Nef; thus, CAR T cells may recognize HIV-infected cells better than natural HIV-specific T cells. HIV can rapidly escape from a single BNAb (44–46), and will likely escape from a CAR that uses a BNAb as its targeting domain, though those targeting the CD4 binding site seem to be more resistant to escape (47). However, like in cancer, bi- or multi-specific HIV CARs have been constructed and have demonstrated superior efficacy against several HIV-1 primary isolates *in vitro*, warranting further *in vivo* investigation (8, 10, 48, 49). Moreover, it is not clear whether use of BNAb is advantageous as a means to redirect T cells to HIV as BNAb binding relative to non-BNAb binding promotes Env internalization (50). Thus, in both HIV and cancer, loss of target recognition by CAR T cells via antigen escape is an issue, but through simultaneous targeting of multiple antigens or the targeting of biologically important functions such as HIV binding to CD4, this issue seems to be solvable.

Immune-Privileged Sites

Immune privileged sites are anatomical regions (CNS, testes, and eyes) in which the immune response is purposely attenuated, usually to protect sensitive tissue from immune-related, off-target damage. These immune sanctuaries are often used by HIV and some tumors to hide from the immune attack. To overcome these issues, recent preclinical studies have shown the antitumor efficacy and safety of intracranial administration of EGFRvIII, HER2, and IL13Rα2 redirected CAR T/NK cells. Brown et al. described a patient who received multiple infusions of IL13Rα2-CAR T cells over 220 days via infusions to the resected tumor cavity and the ventricular system (51, 52).

Immune privilege coupled with HIV latency is an even more daunting problem for T cell-based therapies targeting HIV. Recent data have highlighted the fact that the >99% of all HIV-infected CD4+ T cells are found outside the vasculature within secondary lymphoid organs (SLOs), gut, brain, lung, and other tissues (53). Immunologic clearance of these infected cells

is thought to largely involve cytotoxic CD8+ T cells, specifically CD8+ T cells with a fully differentiated "CTL" phenotype (CCR7-CD62L-CD27-CD45RA+) (54–59). CTLs, however, do not bear the markers (CCR7 and CD62L) necessary to enter lymphoid tissue (60–63). Betts and colleagues recently demonstrated that peripheral blood CTLs are rarely found in HIV-infected lymph nodes, and instead lymph nodes are populated by HIV-specific CD8+ T cells with very limited cytotoxic function (64, 65). In addition, it has been demonstrated that intestinal mucosal tissue is similarly populated with CD8+ T cells that have limited cytotoxic function (66). HIV-infected CD4+ T follicular helper cells (T_{FH} cells) in B cell follicles of lymphoid tissue are a major compartment for persistent virus replication during combination ART (cART) (67–69). Even though virus-specific CTLs have been detected in lymph nodes, they are largely absent from the B cell follicles because they lack expression of CXC-chemokine receptor 5 (CXCR5), which is responsible for the trafficking of cells into the B cell zone along a CXC-chemokine ligand 13 (CXCL13) concentration gradient (70, 71). Therefore, the lack of CXCR5 expression on virus-specific CTLs is one mechanism that promotes the persistence of infected CD4+ T_{FH} cells within an immune-privileged site (72). On the other hand, increasing evidence suggests the existence of tissue-resident macrophages as HIV-1 reservoirs (73, 74). Allers et al. found that macrophages were significantly enriched in the gut of untreated HIV patients (75). This also corresponds with a decrease in blood monocytes and increased expression of gut homing receptors (e.g., chemokine receptor CCR9 and integrin α4β7) on those monocytes, suggesting that blood monocytes may be a major source of macrophages that infiltrate gut mucosa. It has been reported that α4β7 is able to bind HIV-1 Env protein gp120 and is 3-fold larger than CD4 receptor, allowing it to capture HIV efficiently (76). Lastly, it is unclear whether engineered T cells will be able to transverse the blood brain barrier in HIV-infected individuals in order to target the HIV reservoir hiding in the CNS (77).

Taken together, there are at least three major issues facing HIV CAR T cells: (1) Will the latent reservoir of HIV-infected cells express sufficient levels of the target antigen (e.g., HIV Env) to drive CAR T cell recognition after a latency reversal agent is used? (2) Will the HIV CAR T cell be able to traffic to the site where the HIV-infected cell is hiding? and (3) if it is expressing antigen and the HIV CAR T cell is able to recognize the infected cell, will the CAR T cell have the necessary machinery (perforin and granzyme) that may be lost as part of the T cell exhaustion program to kill the HIV-infected cell and eliminate the latent reservoir?

CANCER AND HIV: UNIQUE CHALLENGES AND OPPORTUNITIES

Cancer CAR T Cells Are Infused When Antigen Level Is High; HIV CAR T Cells Are Infused When Antigen Level Is Low

Unless employed to prevent tumor relapse or treat minimal residual disease, cancer-specific CAR T cells are generally infused when there is abundant target antigen available. CAR T cells that quickly recognize their target have an engraftment advantage (78, 79). Moreover, CAR T cell recognition and killing of target cells can result in massive expansion of CAR T cells. In one celebrated case, a single CAR T cell whose vector integrated into and disrupted the function of the Tet methylcytosine dioxygenase 2 (Tet2) gene preferentially expanded to >90% of all of the CAR T cells within the body and this clone was able to maintain durable control of the targeted leukemia (80), indicating that CAR T cells have massive expansion potential. Thus, for individuals with established tumors, it may be possible to infuse a small number of well-engineered T cells and let the body serve as the bioreactor to generate enough T cells to eradicate the targeted tumor. However, CAR T cells that enter a body without significant target antigen may massively contract with a small subset becoming memory T cells, similar to what happens in a natural T cell response once antigen is cleared. Initial studies (NCT03617198) propose to infuse HIV CAR T cells in individuals whose ART has fully suppressed viral replication. It is unclear how well these adoptively transferred T cells will engraft in the absence of high levels of target antigen; however, it is reassuring that first generation CAR T cells targeting CD4-ζ demonstrated brisk expansion and prolonged persistence following infusion into aviremic patients effectively managed with ART therapy (2). For approaches that attempt to block viral rebound once ART is removed, there needs to be a sufficient quantity of T cells present that are widely distributed throughout the body to recognize the vast majority of cells expressing HIV Env as soon as they emerge. Thus, strategies such as infusion of very high numbers of T cells or vaccination approaches that maintain high levels of HIV-specific CAR T cells in the presence of minimal antigen may be required for HIV-specific CAR T cells to be used as part an HIV cure strategy (68).

HIV Can Be Specifically Targeted, but HIV Can Target the CAR T Cells

The search for a CAR target that uniquely recognizes a tumor has proven very challenging. Currently, targets fall into two categories: (1) those with acceptable on-target/off-tumor toxicity, i.e., loss of "expendable" tissue such as B cells in the case of CART-19 therapies or (2) targets highly expressed on tumors and weakly expressed on a limited set of healthy cells, which may allow the CAR T cells to preferentially kill tumor with minimal effects on healthy cells. On-target/off-tumor recognition of CAR T cells has been observed in a variety of organ systems, including gastrointestinal, hematologic, and pulmonary (81). A fatal example of on target/off tumor CAR T cell recognition was observed with the cancer-associated antigen HER-2/neu. Rapid respiratory failure, multi-organ dysfunction, and subsequent death was attributed to reactivity against pulmonary tissue expression of HER-2/neu (82). Fortunately, for HIV CAR T cell therapy, HIV is non-self and thus highly specific agents can be developed that are unlikely to cross-react with human tissue. However, while HIV can be uniquely targeted, there are some challenges: (1) only the HIV Env protein is expressed on the cell surface after latency reversal, making it the only target suitable for CAR T cell therapy, and thus limiting some combinatorial

approaches that may improve the efficacy and/or safety; (2) extensive sequence diversity within Env making it challenging to find antibody-based targeting agents that can bind all strains of HIV. Consequently, the natural HIV ligand, CD4, is attractive for use in a CAR construct, because HIV escape from binding to CD4, would likely result in a virus with greatly reduced fitness; (3) HIV Env expression levels are not fixed as in most cancer targets. Rather, the number of HIV Env targets on the cell surface increases over time as HIV replicates within the cell. However, the best chance for HIV CAR T cells to control HIV replication is to recognize and kill HIV-infected targets as soon as possible after infection when there is minimal HIV Env on the cell surface in order to limit the spread of the virus. Thus, CAR constructs that can redirect T cells to recognize minute levels of HIV Env on the cell surface will likely be very successful to limit HIV spread. This race between the CAR T cell to recognize HIV and HIV's effort to infect new cells has no clear parallel to cancer CAR T cells. It will therefore be interesting to see how this difference impacts the ability of HIV CAR T cells to control HIV replication in HIV-infected individuals.

Additionally, whereas tumors create hostile environments for T cells to function (83), HIV actively infects and kills T cells. While CD4 is a necessary binding receptor for most HIV strains, CD8 T cells can temporally express CD4 after T cell activation permits making both CD4 and CD8 HIV-specific CAR T cells susceptible to infection (6, 84, 85). For these reasons, HIV-specific CAR T cells will need to be protected from HIV infection. A variety of strategies exist including chemokine co-receptor disruption and fusion inhibitors that provide robust protection of T cells from infection (41). The only challenge in these strategies is the additional engineering that is required during the T cell manufacturing process.

The Bar by Which Therapies Are Deemed Successful Differs Considerably Between HIV and Cancer Cell and Gene Therapy

Current cancer treatments such as chemotherapy, surgery, and/or radiation, have significant side effects and in most cases low rates of cure in advanced disease settings. CAR T therapies are currently being explored in patients with advanced/refractory malignancies and are FDA approved in chemotherapy refractory leukemia and lymphoma. Clinical success and FDA approval for Sipuleucel-T (Provenge), a dendritic cell-based therapeutic vaccine, was based on ∼4 month increase in survival time for prostate cancer patients. In contrast, ART is nearly universally successful in compliant individuals with access to healthcare, and those individuals whose virus remains undetected due to ART have lifespans approaching those of non-HIV infected individuals (86). Thus, both commercial and clinical success for cancer therapies is measured by increasing mean survival time whereas for HIV, only a cure, whether functional or sterilizing (87, 88), is considered a success. Given that only two people have been cured of HIV infection (89, 90), having a lifetime cure as the only measure of success is quite a high bar. This is why analytical treatment interruptions (ATIs) are crucial to advance the HIV CAR T cell field. Here, individuals involved in an IRB approved clinical trial voluntary stop taking ART after receiving an experimental agent and the time to viral rebound is measured. Most individuals rebound within 2–4 weeks; therefore, individuals who are part of an interventional study that is able to limit the virus from replicating significantly longer provide evidence that the experimental therapy is having some effect. As the field matures and many approaches are studied, one can then analyze ATI data to propose combination trials to determine whether further delays in viral rebound occur. This

TABLE 2 | Synergy between HIV and cancer cell and gene therapy.

Advance	Initial impact	Impact on other disease
Bone marrow transplant	Lifesaving approach to restore patient bone marrow after severe cancer therapy that can induce graph v. tumor effects (96)	Part of the regimen of the individuals cured of HIV (89, 90)
Retroviral vectors	The first time a genetically modified cell was infused into humans was when neomycin was expressed by a retroviral vector in cancer infiltrating T cells (97)	The clinical development of retroviral vectors in cancer paved the way for the first CAR T cell trial in HIV (3, 4)
CD3/28 bead culture system for T cell Stimulation	Development of a GMP compliant, robust method to expand HIV-infected CD4 T cells in the absence of ART due to CCR5 downregulation (98–101)	Used widely to manufacture T cells for cancer CAR therapy including in the first indication that led to FDA approval (12, 102–104) using SOPs initially developed for HIV
CAR T cell	Fusion of CD4 with the CD3 zeta chain created the first CAR construct tested in humans and demonstrated the long term persistence of CAR T cells (5)	Manufacturing advances and safety data obtained from HIV CAR T cell studies paved the way for development of the first FDA approval of any gene therapy- and the first CAR T product (12, 102, 105, 106)
Lentiviral vectors	A lentiviral vector that expressed anti-sense HIV Env in transduced T cells represented the first time lentiviral vectors were used in humans (107)	Lentiviral vectors have preferred integration pattern (108), improved expression (6), and are the preferred vector for cancer CAR T cell therapy
Genome editing	Infusion of CCR5 ZFN treated T cells into HIV-infected individuals represented the first time genome edited T cells were employed (109)	NYESO-1-specific T cells with disrupted TCR and PD-1 alleles were recently infused into cancer patients (NCT03399448)
TCR enhanced affinity	T cells expressing an affinity enhanced TCR specific for MAGE-A3 resulted in two treatment related deaths due to unexpected off-target toxicity (110, 111)	A clinical trial using similar technology to redirect T cells to HIV was stopped because the TCRs used did not undergo an improved screen for off target recognition (NCT00991224)

combinatorial, iterative approach is likely the best chance we have to develop an effective and safe HIV Cure regimen. To date, carefully monitored ATIs have not resulted in ART escape or increased the viral reservoir (91–93), suggesting that there are no long term adverse outcomes for individuals participating in clinical trials that have ATIs (94).

OUTLOOK: RECENT LESSONS FROM CANCER WILL INFORM THE NEXT GENERATION OF HIV SPECIFIC–CARS

The development of cancer and HIV CAR T cell therapy has a long, intertwined, and symbiotic relationship (95), and this relationship is highlighted in **Table 2**. Exactly how did success with cancer CAR T cell therapy inform the design and implementation of HIV CAR T cell therapy? The initial CD4-ζ CAR was housed in a murine gammaretroviral vector, contained the CD4 transmembrane domain, lacked costimulatory domains, and was driven by the phosphoglycerate kinase (PGK) promoter (1, 112). In a side-by-side, step-by-step study, Leibman et al. compared this first generation HIV CAR with the vector design of CARs that achieved FDA approval for CD19-expressing tumors (6). Surprisingly, the choice of vector delivery made a huge difference in CAR expression and this translated into greater control of HIV replication. Substituting the EF-1a promoter resulted in both more stable and higher CD4 CAR expression. Replacing the CD4 transmembrane domain with the CD8 hinge region resulted in slightly less expression, but rendered the HIV CAR T cells less susceptible to infection and improved the overall efficacy of these T cells. Lastly, endowing the CD4 CAR with 4-1BB costimulation promoted both the survival and expansion *in vivo* as previously observed in tumor models (6, 113).

In a convergence of fields, much attention is now focused on where a CAR vector integrates. Pioneering studies by the Bushman lab demonstrated that HIV (and HIV-based vectors) prefers to integrate in coding regions, whereas murine gammaretroviruses target promoter regions (108, 114). More recently, the site of HIV integration has been shown to play a role in whether T cells will become part of the latent reservoir (115), suggesting that the site of integration can impact a T cell's long term persistence and ability to homeostatically expand. Using approaches to study how HIV integrates, Fraietta et al. uncovered how a CD19 CAR vector fortuitously integrated into the TET2 locus, and this integration resulted in a central memory-like T cell phenotype with an incredible ability to expand and function (116). As genome engineering becomes more effective, safer and less expensive (117), one can imagine that it will be possible to specifically insert a CAR vector into a precise spot in the genome to provide a functional advantage or survival benefit to either HIV or cancer CAR T cells.

As mentioned in the beginning, the field of T cell manufacturing was in its infancy when the first HIV CAR T cell therapy trials were performed. The field has matured considerably, but there is much more to learn in order to improve how T cells are produced for use in adoptive T cell applications. Cancer CAR T therapy has seen a strong correlation in how well T cells expand *ex vivo* with their *in vivo* function and persistence (118). Additionally, it has been demonstrated that changes in T cell manufacturing such as expanding T cells in the absence of human serum (119) improves the *in vivo* efficacy of CAR T cells. Here, developers of cancer CAR and HIV CAR can support each other as many of the developments in T cell manufacturing are likely to benefit both fields. One possible difference is that for HIV CAR T therapy large quantities of HIV CAR T cells may be required to have enough effectors on hand to prevent viral rebound after ART removal since there is minimal antigen present to induce *in vivo* CAR T cell expansion. In contrast, for cancer CAR T cell therapy, infusion of less CAR T cells may be safer, less expensive and just as effective so the manufacturing for these two therapies are reasonably similar now but they may diverge considerably once we learn more about what is required to obtain therapeutic responses. Lastly, HIV-infected individuals are currently excluded from receiving CAR T therapy in part because the commercial manufacturers have not developed a process by which HIV-infected T cells can be GMP manufactured. Perhaps one of the last gifts HIV CAR therapy can give to cancer CAR therapy is to share the best practices by which HIV CAR T cells are manufactured using T cells from HIV-infected individuals so that HIV-infected individuals can benefit from this life saving, cancer CAR T cell technology.

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REVIEW
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Crossroads of Cancer and HIV-1: Pathways to a Cure for HIV

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Recently, a second individual (the "London patient") with HIV-1 infection and concomitant leukemia was cured of both diseases by a conditioning myeloablative regimen followed by transplantation of stem cells bearing the homozygous CCR5 Δ32 mutation. The substantial risks and cost associated with this procedure render it unfeasible on a large scale. This strategy also indicates that a common pathway toward a cure for both HIV and cancer may exist. Successful approaches to curing both diseases should ideally possess three components, i.e., (1) direct targeting of pathological cells (neoplastic cells in cancer and the HIV-infected reservoir cells), (2) subsequent impediment to reconstitution of the pool of pathological cells and (3) sustained, immunologic control of the disease (both diseases are characterized by detrimental immune hyper-activation that hinders successful establishment of immunity). In this review, we explore medications that are either investigational or FDA-approved anticancer treatments that may be employed to achieve the goal of curing HIV-1. These include: thioredoxin reductase inhibitors (phases 1–3), immune checkpoint inhibitors (phases 1, 3), Jak inhibitors (FDA approved for arthritis and multiple cancer indications, summarized in Table 1). Of note, some of these medications such as arsenic trioxide and Jak inhibitors may also reversibly down regulate CCR5 expression on $CD4^+$ T-cells, thus escaping the ethical issues of inducing or transferring mutations in CCR5 that are presently the subject of interest as it relates to HIV-1 cure strategies.

Keywords: HIV, immunomodulator, inflammation, eradication, latent reservoir HIV infected CD4 T cells, apoptosis of HIV infected CD4 T cells

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INTRODUCTION

Human Immunodeficiency Virus (HIV-1) is currently well-managed and achieves plasma viral suppression with existing combination antiretroviral therapy (ART). Despite durable virologic suppression, a major barrier to eradication of HIV-1 remains the effective elimination of cells harboring integrated HIV in a latent or low-level replication state, including pharmacological sanctuaries such as the central nervous system (CNS) (1–6). Further, many reports now demonstrate that chronic immune activation and exhaustion transpire in people living with HIV (PLWH) even with well-controlled viremia. These markers include elevated levels of inflammatory and immunomodulatory cytokines including IL-1α/β, TNF-α, IL-6, D-dimer, C reactive protein (CRP), IL-7, IL-15, sCD14, and sCD163. These correlate with increased morbidity and mortality in PLWH (2, 6–16). For example, IL-7/15 drive homeostatic proliferation and IL-15 reactivates

HIV-1 from latent stores, thereby expanding the viral reservoir (2, 10, 11). Elevated inflammation is a driver of immune exhaustion (elevated PD-1), which is also associated with disease progression for PLWH (7, 17, 18).

Existing antiretroviral agents do not completely eliminate ongoing inflammation and immune activation for all virologically suppressed individuals, nor do these agents target the latent or persistent viral reservoir (19–21). These limitations have led to the burgeoning exploration of well-defined anti-cancer agents that target key cellular and immunomodulatory pathways.

The interface between cancer and HIV-1 treatment is not new; the first FDA-approved antiviral agent was azidothymidine, an adenosine nucleoside analog originally explored as an anticancer agent to block DNA synthesis given its lack of the 3′ hydroxyl group (22–24). The wealth of FDA-approved agents with well-described mechanisms of action and clinical profiles provide a robust foundation that can be readily leveraged for HIV-1 treatment. This review focuses on key anti-cancer agents that either are FDA-approved or have already begun clinical investigation in PLWH.

HEMATOPOIETIC STEM CELL TRANSPLANTATION AND CELLULAR THERAPY

The concept of disease eradication followed by reconstitution of depleted cell lines using a living, unrelated donor is over 60 years old. The first allogeneic hematopoietic stem cell transplantation (HSCT) was performed in 1957 by E. Donnall Thomas on six individuals with various malignancies (25). Only two patients engrafted after conditioning chemotherapy with radiation, and all died within 100 days. Allogeneic transplants require the bone marrow graft to be derived from a healthy donor, in contrast to an autograft (self) transplantation. The procedure usually requires an initial round of high dose chemotherapy along with total or selective body irradiation to reduce the tumor burden and weaken native immunity prior to transplantation. Thereafter, donor cells are infused, followed by the administration of immunosuppressive agents initially to prevent rejection and later to minimize graft-vs.-host disease (GVHD). After the introduction of histocompatibility matching in the 1980s, disease-free survival dramatically improved as graft rejection and GVHD decreased (26). In the modern era of HSCT, the majority of patients undergoing this procedure have refractory leukemia or multiple myelomas well as other malignancies such as lymphomas (non-Hodgkin's and Hodgkin's), gliomas and neuroblastomas. HSCT has also been an effective therapeutic strategy for non-malignant diseases including autoimmune disorders and sickle cell disease (27, 28). According to the WHO, over 50,000 HSCT are performed worldwide each year for malignant indications, with more than 90 % resulting in a cure (29).

In the early years of the HIV epidemic, HSCT was explored as an option to treat cancer or reconstitute the immune system for people living with HIV (PLWH). For untreated, advanced HIV infection, most patients experienced little clinical benefit and ultimately succumbed to AIDS. Interestingly, several of the allogeneic HSCT recipients had, at necropsy, undetectable levels of HIV from various tissues as late as 10 months after transplant (30). Interestingly, syngeneic transplants recipients remained viremic throughout the post-transplant period indicating the potential value of a graft-vs.-virus effect. As ART became available, the interest in developing HSCT as a strategy to specifically treat HIV disease waned. Outcomes of autologous HSCT for PLWH with lymphoma continued to improve and approached those of HIV negative individuals (31). However, the effectiveness of moderately intensive chemotherapy or HSCT on the HIV reservoir had been minimal due to reinfusion of infected $CD4^+$ T cells contaminating autografts, new infection of donor-derived $CD4^+$ T cells, and chemotherapy-resistant infected cells.

In 2007, Timothy Brown (the "Berlin patient") underwent HSCT for relapsed acute myelogenous leukemia (AML). Gero Hütter, the treating physician, identified a donor lacking the CCR5 coreceptor (homozygous for the Δ32 deletion) on $CD4^+$ T cells, which is critical for R5-tropic HIV viral entry. ART was discontinued, and subsequently HIV in blood and various tissues were undetectable (32). After more than a decade since transplantation, he remains free from both HIV and AML becoming the first patient ever cured of HIV by this strategy (33). Two additional patients in Boston (who were themselves heterozygous for the Δ32 deletion) underwent HSCT using a reduced-intensity conditioning regimen and $CCR5^+$ wild-type donors (34). Unfortunately, both patients experienced viral rebound 12 and 32 weeks after ART cessation despite maintaining undetectable levels while receiving ART until full chimerism was achieved. More recent evidence of HIV remission following HSCT has been documented from a patient in London who is now over 18 months undetectable (35). Numerous protocols are underway to examine this approach in various international settings (30). The Berlin patient prompted new research aimed at knocking–down CCR5 on $CD4^+$ T cells using CRISPR/Cas9, zinc-finger nuclease and transcription activator-like effector nuclease genome editing systems (36). There are some concerns around the use of lentiviral transduction resulting in insertional oncogenesis and the potential effect of losing the CCR5 co-receptor for immune function and mortality (37). Furthermore, without fully myeloablative chemotherapy or high efficiency of graft transduction, it is unclear how to best to achieve complete chimerism in the host, and identification of donor matched Δ32 for all PLWH is not possible, given this mutation is a rare mutation in the human population at large (38). Additionally, there are ethical considerations for altering the human genome; modifying CCR5 signal transduction may have implications for specific pathogens and long-term immunity that are incompletely understood.

Abbreviations: HIV, Human Immunodeficiency Virus; CRP, C Reactive Protein; PLWH, People Living with HIV; ART, antiretroviral therapy; HSCT, hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; TrxR, Thioredoxin reductase; ATO, Arsenic trioxide; PML, promyelocytic leukemia protein; Jak-STAT, Janus activating kinase signal transducer and activator of transcription; PKC, protein kinase C; TLR, toll-like receptor.

Despite encouraging evidence, this procedure incurs a significant risk of complications, challenging pharmacological interactions and substantial financial costs. Survival at 1 year is around 60% with the underlying malignancy often described as the cause of death. The major adverse events include infection, liver injury due to veno-oclusive disease, and GVHD. At a median total healthcare cost at 100 days of $289,283 for the myeloablative HSCT and $253,467 for reduced-intensity HSCT (39), allogeneic HSCT is not a viable treatment option for the nearly 37 million PLWH globally. This reason alone has weakened support of HSCT as a viable strategy for HIV cure (40–43). More recently, the use of virally transduced chimeric antigen presenting autologous T-cells (CAR-T) has broadened the potential utility of immune directed anticancer therapy with approval of several agents in this class by the US FDA for the treatment of refractory leukemia and lymphoma (44). While the success of HSCT has been limited to hematologic malignancies, CAR-T has the potential to positively impact the treatment of solid malignancies in the near future. Despite the advancement of this potential intervention for cancer, implementation of CAR-T cells to HIV-positive individuals presents with significant logistic limitations since it requires transplantation, limiting its application to the nearly 37 million PLWH worldwide.

CHECKPOINT INHIBITORS

There are a number of approved immunotherapeutic agents directed at CTLA-4 (ipilimumab), programmed cell death 1 protein or PD-1 (nivolumab, pembrolizumab, cemiplimab), and PD-L1 (atezolizumab and durvalumab) (45). See **Table 1** for summary of indication and route of administration for this class of agents. Each of these monoclonal antibodies present critical pharmacokinetic challenges. For example, they are not orally bioavailable and there is poor tissue delivery of these agents at adequate concentrations to confer efficacy.

Immunologic dysfunction associated with HIV infection and persistence, including T cell exhaustion, is related to overexpression of checkpoint molecules including CTLA-4, PD-1, LAG-3, and TIM-3. This overexpression is a major contributor of the viral reservoir in ART-suppressed, HIV-positive patients and non-human primates (46–48). Recently, a report from Fromentin et al. demonstrated that PD-1 blockade potentiates HIV latency reversal *ex vivo* in $CD4^+$ T cells from ART-suppressed individuals (49), further underscoring the role of PD-1 in HIV-1 latency, reversal, and overall reactivation.

Clinical trials are already underway (NCT02408861, NCT03354936) or have been completed to test checkpoint blockade. In a previous case report, ipilimumab was given to a HIV positive patient with melanoma. This patient experienced an increase in $CD4^+$ T cell quantity, T cell activation and cell-associated unspliced HIV RNA with a subsequent decline in plasma HIV RNA (50). Moreover, a HIV-positive patient with lung cancer was given nivolumab with a subsequent reactivation of latently-infected T cells (51). Significant adverse effects have been reported when using these agents in cancer; as these molecules are involved in antigen self-tolerance, disruption can lead to autoimmune or inflammatory side-effects, reactivation of underlying autoimmune conditions, or new autoimmune conditions such as type 1 diabetes mellitus (52). Several case reports have described colitis, skin toxicities, endocrinopathies, pneumonitis, and hepatitis (53, 54). Finally the substantial cost of these agents necessitates a careful consideration of which patients and populations would be ideal candidates for this class of drug (55). Together, these significant safety limitations coupled with cost of treatment, likely preclude their development for the indication of HIV-1 cure.

TABLE 1 | Summary of anti-cancer agents that have been explored for the indication of HIV.

Agent	Target	Route of administration
Nivolumab, pembrolizumab, cemiplimab), and PD-L1 (atezolizumab and durvalumab	PD-1	Monoclonal antibody; infusion
Auranofin	Thioredoxin reductase	Oral
Arsenic trioxide	Thioredoxin reductase	Intravenous
Ruxolitinib	Jak 1/2	Oral
Baricitinib	Jak 1/2	Oral

THIOREDOXIN REDUCTASE INHIBITORS

Thioredoxin reductase (TrxR) is a key suppressor of oxidative stress and regulates cell death and differentiation. It is a selenoprotein which reduces the oxidized from of thioredoxin (Trx), turning this protein into its active reducing form, thus maintaining the functional levels of one of the main cellular antioxidants (56). The presence of a selenocysteine in the active center of TrxR renders it sensitive to inhibition by a number of metal and metalloid ions, which directly bind the selenium ion of selenocysteine thus blocking the active center of the protein (57).

Auranofin is the only gold salt which is orally available and FDA-approved, see **Table 1** for summary of indication and route of administration (58, 59), although it is rarely prescribed in the modern era due to toxicities, and development of other more specific, safe and well tolerated agents. Auranofin was developed for RA treatment in the 1970s, but, at that time, the mechanisms behind its effects on the immune system were largely unknown (58). It was known, however, that the compound inhibited lymphocyte proliferation (60), and, in this light, its anticancer potential soon became apparent (61). A recent human clinical trial with five HIV-positive individuals was conducted (NCT02961829) (62). The findings demonstrate that no severe adverse events were reported for the duration of the study, apart from a decline in total CD4 T cells at week 8 and week 12. A sample size of five individuals per group, statistical analysis to confidently perform appropriate statistical tests to determine significance of findings cannot be performed; nonetheless, the trial demonstrates that auranofin may be safely tolerated in HIV-positive individuals; further studies are needed to better understand the impact of this agent on the viral reservoir.

To date, auranofin has been largely replaced by modern-era anti-cancer agents that demonstrate a significant improvement in safety and specificity profiles. Nonetheless, the ability of this agent to block activation based events that drive immune activation add to a better understanding of links between inflammatory events and HIV persistence.

ARSENIC TRIOXIDE (ATO)

Early reports demonstrated that ATO potently suppressed lymphocytic proliferation in acute promyelocytic leukemia (APL) (63), however the fact that it blocks T cell proliferation provides serious concern for application toward PLWH, given CD4 T cell loss is a major hallmark of disease pathology in this population. A case-report study demonstrated that oral arsenic trioxide–based maintenance regimens conferred complete remission of APL in a 10-year follow up study, underscoring that agent can be tolerated in this cohort to achieve remission (64). APL requires a 15:17 chromosome translocation and chimerization of the retinoic acid-RAR-α and the promyelocytic leukemia protein (PML). PML is a primary constituent of the nuclear bodies, a molecular "hub" attracting chromatin-modifying enzymes and transcription factors regulating cell death and proliferation and, interestingly, HIV-1 transcription (65). A combination of the RAR-α ligand all-trans retinoic acid and ATO, found to be a PML ligand (65), has become an effective, FDA-approved treatment for APL, inducing stable remission of the disease (66). See **Table 1** for summary of indication and route of administration. Despite this approval, arsenic-based compounds are considered to be toxic, although the benefits to patients with cancer may outweigh the risks. It remains to be seen whether this risk-benefit ratio will be similar for PLWH. More recently in the past decade, the inhibitory effects of ATO on TrxR were discovered (67). ATO was thus tested, alone or in combination with other drugs in a wide variety of cancers without the 15:17 chromosome translocation including solid malignancies such as melanoma and small cell lung cancer (68, 69) and was approved as a treatment for hepatocellular carcinoma in China (70).

Currently, ATO could provide insight into mechanisms for cure-based work for several reasons: First, ATO has been reported to demonstrate efficacy in the traditional "shock and kill" strategy, with a mechanism that is related to TxR inhibition (65, 71), which implies a relationship between TxR inhibition and viral reactivation. Additionally, ATO reduces the susceptibility of subsequent HIV infection down-regulating CCR5 expression on $CD4^+$ T-cells without the need of a bone marrow transplantation. A recent report also stated that ATO (72) conferred a delay in viral rebound for two SIV-infected macaques (out of four total animals in the study), with doses similar to those administered to humans with APL. A larger sample size to determine the impact of these agents in macaques is warranted, although concern for overall T cell loss with an anti-leukemic agent in PLWH will require a thorough evaluation of the risk/benefit ratio. CCR5 down-regulation is the likely result of the pro-differentiating effects of ATO in lymphocytes: similarly to auranofin, ATO induces CD27 down-regulation, thus limiting their potential to become activated (73). A Phase 1 human study (20 total participants randomized to control or treatment groups) to examine this agent is currently recruiting in China, which may provide critical insight into the safety and efficacy of this agent in PLWH (https://clinicaltrials.gov/ct2/show/NCT03980665). Together these mechanisms provide insight into control of the viral reservoir, although direct clinical application of ATO to HIV-positive individuals is uncertain at present. Weighing the risk/benefit ratio for an agent that may block pro-HIV events, vs. its clinical safety profile must be carefully considered with agents, especially those that are not prescribed currently due to their safety profiles.

JAK INHIBITORS

The Janus activating kinase signal transducer and activator of transcription (Jak-STAT) pathway is activated within 2 h of HIV-1 envelope gp120 binding to CD4, in both primary T-cells and macrophages, in a co-receptor independent manner (74). Downstream activation results in Jak activation, subsequent STAT phosphorylation, and extracellular production of pro-inflammatory and cytokines that are key drivers of HIV persistence, disease progression, reservoir magnitude, and decreased CD4 T cell counts (7, 75–78).

Jak 1/2 inhibitors including ruxolitinib (Jakafi; Jakafi.com), and baricitinib (olumiant; olumiant.com) are FDA-approved for myelofibrosis or polycytemia vera (ruxolitinib), and rheumatoid arthritis (baricitinib), respectively. Baricitinib is FDA-approved for long-term use, including in children, rendering its safety profile favorable for consideration in PLWH. See **Table 1** for summary of indication and oral administration for jak inhibitors. Jak 1/2 blockade represents an attractive cellular target because Jak 3 but not Jak 1/2 blockade induces natural killer cell depletion and systemic side effects that can promote immunosuppression (79–82). Ruxolitinib has demonstrated potent inhibition of reservoir establishment, maintenance and expansion in primary T cells and macrophages *in vitro* and *ex vivo* (7), and demonstrated reduction in immune activation markers associated with HIV-1 persistence including CCR5, HLA-DR, CD38, CD25, Ki67, and PD-1. Further, Jak inhibitors significantly reduce Bcl-2 expression in non-dividing $p24^+$ primary T cells *ex vivo*, thereby offering the potential to reduce the lifespan of reservoir cells by down-regulating a key marker that controls lifespan of cells (7, 14, 78, 83, 84). These data provided the foundation for a recently completed multi-site Phase 2a AIDS Clinical Trial Group (ACTG)-funded study (https://www.clinicaltrials.gov/NCT02475655. It was recently reported that ruxolitinib was safe and well-tolerated in a highly-selected cohort of PLWH on suppressive ART (85). The ruxolitinib arm demonstrated a trend in reduction of IL-6, and a statistically significant decrease in sCD14 (85), coupled with an increase in circulating T cells through undefined mechanisms. Data are forthcoming about the impact of this agent on viral reservoirs, and key markers of viral persistence.

Additional work has begun to explore another Jak 1/2 inhibitor, baricitinib for HIV (86). Baricitinib is an FDA approved once-daily dosed, orally bioavailable inhibitor that is renally cleared, approved for long-term use in children (primary indication rheumatoid arthritis, and under investigation for

various inflammatory or malignant indications). A recently published study demonstrated that baricitinib reverses HIV associated neurocognitive disorders in a severe combined immunodeficiency (SCID) mouse model and reservoir seeding *in vitro* (86). Importantly, baricitinib was shown to significantly reduce activated phagocytic cells from the periphery that recruit to the CNS during HIV infection, highlighting the link between blockade in the periphery and potential application to CNS infection with HIV. Together, these data provide a rationale for future studies of Jak inhibitors in PLWH who have residual inflammation or immune dysfunction despite long-term suppressive ART.

PREVIOUS WORK PAVES A ROAD TOWARD A BRIGHT FUTURE

Many early studies exploiting anti-cancer agents were based on the "shock and kill" concept (87, 88). These agents, notably panobinostat (primary indication: multiple myeloma) and vorinostat (primary indication: cutaneous T cell lymphoma) failed due to toxicity and lack of efficacy (89). Other modalities targeting key pathways reactivating the latent virus, including protein kinase C (These markers include elevated levels) agonists and toll-like receptor TLR agonists, are being explored, however to date no agent has demonstrated durable reduction in the latent reservoir (90). These agents also may promote systemic immune activation since they are reactivation agents, which could have fuel HIV persistence, representing a major limitation associated with these approaches. Nonetheless, these studies provide a better understanding of potential application of anti-cancer agents and the effect of the shock and kill approach on the HIV reservoir (i.e., reactivation of the latent virus followed by elimination of the infected cell).

COMMON STRATEGIES FOR CURING HIV AND CANCER

Curing cancer has a major mechanistic hallmark of stopping proliferation of malignant cells and/or inducing cell death, while maintaining immune function and reducing toxicity of uninfected cells. HIV-1 eradication strategies are now beginning to adopt this paradigm to target and eliminate only HIV-infected cells. This archetype represents the beginning of a new horizon, where better understanding of the complex and delicate interplay between cellular signaling, inflammation, autocrine and paracrine events, and the impact of these events both locally and across organ compartments. The field of HIV has been able to move forward with much greater speed and knowledge due to the wealth of information collected from anti-cancer approaches spanning diverse mechanisms of action. The data collected to date have provided insight into some approaches that are not viable, while simultaneously providing insight into why they may have failed, providing a potential pathway to re-evaluate these mechanisms with different agents. Other approaches have provided promising preliminary data in humans and will require further rigorous evaluation in PLWH. Careful consideration for agents that are safe, specific, and potent that can be translated for large-scale use in PLWH, including children, must be considered. Further, the bioavailability of the agent, its pharmacokinetic profile, and ability to be administered without drug-drug interactions to PLWH who are receiving ART are critical components to repurpose oncology chemotherapeutic agent for use in HIV infection. The data generated to date will facilitate better understanding of the potential impact of these agents on the viral reservoir and end-organ disease, and provide great potential to identify a candidate agent that can lead to a functional HIV cure.

CONCLUSIONS

Targeting and eliminating HIV-infected cells without conferring toxicity to uninfected cells systemically remains a critical key to HIV eradication. The information gained from oncology and its rapidly advancing target library will undoubtedly continue to guide eradication strategies for HIV-1. Data learned from previous work provides hope that eradication of HIV-1 is possible, when guided by the lighthouse of cellular-factor targeted agents and anti-cancer therapies.

AUTHOR CONTRIBUTIONS

CG was the primary author and also edited all other contributing portions into the doucment. AS provided sections for thioreductase inhibitors, auronafin, and historical perspectives. TO provided sections for anti-cancer agents and crosstalk to background and clinical application in cancer. VM was the senior author and wrote historical sections, edited document, and guided CG toward completion and structure of the manuscript.

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Moving Immunoprevention Beyond Virally Mediated Malignancies: Do We Need to Link It to Early Detection?

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Vaccines can successfully prevent viral infections and have emerged as an effective strategy for preventing some virally mediated malignancies. They also represent our major hope for cost-effective reduction of the cancer burden. The concept that the immune system mediates surveillance and editing roles against tumors is now well-established in murine models. However, harnessing the immune system to prevent human cancers that do not have a known viral etiology has not yet been realized. Most human cancers originate in a premalignant phase that is more common than the cancer itself. Many of the genetic changes that underlie carcinogenesis originate at this stage when the malignant phenotype is not manifest. Studies evaluating host response in human premalignancy have documented that these lesions are immunogenic, setting the stage for immune-based approaches for targeted prevention of human cancer. However, recent studies suggest that the hierarchy of T cell exhaustion and immune-suppressive factors have already begun to emerge in many preneoplastic states. These considerations underscore the need to link immune prevention to earlier detection of such lesions and to personalize such approaches based on the status of the pre-existing immune response.

Keywords: preneoplasia, early detection, cancer prevention, T cell exhaustion, cancer vaccine, cancer interception

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WHY PREVENTION?—LESSONS FROM VIRALLY MEDIATED MALIGNANCIES

Despite major advances in therapies for several cancers, most patients with advanced cancer eventually succumb to the underlying malignancy. Many cancers carry considerable genomic complexity at diagnosis and acquire mechanisms of resistance to current therapies, including chemotherapy, targeted therapy, and immune therapies. Even the most successful cancer immune therapies, such as immune checkpoint inhibitors and adoptive transfer of engineered T cells, only benefit a subset of patients and are not amenable to easy application for the prevention of cancer, particularly in the developing world. In addition to the need to reduce human suffering and mortality from cancer, the increasing and unsustainable costs of cancer care also create an economic argument to reduce the cancer burden, even in rich nations (1). One such approach to prevention is vaccination, which has been highly effective against some pathogens. In the setting of virally mediated disease, evidence is emerging that preventive vaccines for reducing viral infections are also effective for preventing virally mediated cancers (2). The risk of chronic liver disease and hepatocellular carcinoma (HCC) following hepatitis B virus (HBV) infection is higher in children who acquire the infection before the age of 5 years (3). HBV infant vaccination programs have

shown remarkable efficacy in the reduction of HCC incidence compared to non-vaccinated controls (3, 4). Vaccines against human papillomavirus (HPV) represent another success story in terms of protection from virus-induced malignancy (2, 5, 6). Two currently approved HPV vaccines provide protection not only against chronic infection with HPV types 16 and 18 but also against cervical intraepithelial neoplasia (CIN), adenocarcinoma *in situ*, and cervical cancer. Vaccines targeting E6 and E7 antigens from HPV 16 and 18 have also shown remarkable efficacy in mediating the regression of CIN lesions (2). For example, women with grade 3 vulvar lesions vaccinated with long peptides derived from these antigens experienced high rates of complete regression of these lesions (7). In the Papilloma Trial against Cancer in young Adults (PATRICIA trial), HPV vaccination led to complete protection from CIN as well as adenocarcinoma *in situ* lesions (5, 6). In view of its effects on precursor lesions, it is projected that HPV vaccination will lead to a major reduction in cervical cancer mortality in the next 20–30 years. One important lesson from this experience is that vaccines incorporating antigens that do not lead to regression of established cancers are still highly effective in preventing early lesions.

IMMUNE SURVEILLANCE AND EDITING: INSIGHTS FROM MOUSE MODELS

Although it has been over 50 years since the initial evidence for immunity against carcinogen-induced tumors in mice was published, the concept that the immune system could mediate surveillance against tumors has now overcome initial skepticism (8). Several strains of immune-deficient mice have been shown to be deficient in immune surveillance in one form or another in models that include both carcinogen-induced and spontaneous cancers. Schreiber and colleagues proposed the term cancer immune editing, which incorporates three distinct phases: elimination, equilibrium, and escape (8). An important aspect of the equilibrium phase, as different from prior concepts of dormancy, is that the tumor is not really static but is likely engaged in ongoing interactions with the immune system leading to evolution (or editing) until there is escape from immune destruction (9). A deeper understanding of the equilibrium phase is particularly critical for translation to secondary cancer prevention in the clinic, as it resembles the premalignant or clinically silent phase preceding cancer.

HOST RESPONSE TO PRENEOPLASTIC LESIONS IN HUMANS

Most studies of cancer immunity in humans have focused on patients with clinical cancer, which represents the escape phase. In this setting, the presence of immune infiltration within tumors has emerged as a strong predictor of outcome, in some cases more dominant than the clinical staging systems currently in place (10). Indeed, the presence of pre-existing tumor immunity forms the basis for the clinical success of immune checkpoint therapies (11). However, genomic studies have shown that many of the oncogenic mutations are acquired long before the clinical malignancy is manifest (12). Studies on such human precancer lesions are limited, as these lesions (e.g., colon polyps) are typically resected at the time of initial diagnosis. However, even in these settings, it has been shown that there are changes in adjacent "normal" mucosa that predict the risk of recurrence (13), thereby making a case for targeting these abnormal cells to reduce recurrence. The presence of immune infiltration has now been demonstrated in diverse preneoplastic states including intraductal papillary mucinous neoplasms (IPMNs) that precede pancreatic cancer (14, 15), oral leukoplakia as a precursor to oropharyngeal cancer (16), non-invasive bladder cancer (17), bronchial lesions preceding lung cancer (18–20), and ductal carcinoma *in situ* (DCIS) of the breast (21–24). One of the earliest examples of specific immune responses to human preneoplasia in the tumor microenvironment was in the setting of monoclonal gammopathy of undetermined significance (MGUS), which serves as a precursor to myeloma (MM) (25). In contrast to other cancers, tumor cells in MGUS cannot be surgically resected at initial diagnosis, and therefore it provides an important and unique model for studies on early response to preneoplastic lesions in humans (26). Notably, although MGUS lesions carry many of the genetic changes found in MM cells, only a small proportion go on to develop clinical malignancy (26, 27). Prior studies have shown that the immune system does recognize these lesions, and this leads to alterations in both innate and adaptive immune cells in the bone marrow (25, 28–31). Importantly, pre-existing T cell immunity was a strong predictor of reduced risk of progression to clinical myeloma in a large prospective clinical trial, with protective effects manifest across all major genetic subtypes of MGUS (32, 33). As is the case with precursor states to more common solid tumors, MGUS lesions are quite common and can be detected even with less sensitive methods in up to 3% of individuals over 50 years of age (26). It is important to note that while MGUS is not surgically resectable as in some other preneoplastic lesions, several aspects of the biology and genetics of these lesions resemble the more common solid tumor counterparts. For example, genome sequencing studies have shown that precursor and pre-invasive lesions in solid tumors carry many of the genomic alterations found in their clinically malignant counterparts, and this is true in the setting of MGUS as well (27, 34).

Chronic immune responses can lead to T cell dysfunction or exhaustion (35). As the premalignant phase of cancer is immunogenic and lasts much longer than the malignant phase itself (typically several years), an important question arises—how does the host maintain such a chronic immune response? In mouse models of chronic viral infections such as lymphocytic choriomeningitis virus (LCMV), the maintenance of chronic immune responses and the prevention of the attrition of exhausted T cells depend on the presence of a subpopulation of stem-like T cells (36–38). Loss of this subset leads to attrition of the immune cells and loss of immunity in these models (36). Similar biology may also be operative in the setting of premalignancy. Utilizing complementary single-cell technologies, T cells infiltrating MGUS lesions were found to be less differentiated than those seen in MM (39). These cells

were also enriched for TCF1/7+ memory T cells as well as those with tissue-resident phenotypes (39). Therefore, the hierarchy of T cell exhaustion seems to be established early in the setting of cancer development. Another insight from these studies is that changes in innate immunity as well as in the myeloid compartment also occur early (15, 30, 39). Early emergence of suppressive myeloid populations may be an important obstacle to immune-based prevention targeting these lesions (15, 40). An important challenge in terms of studying the biology of host response to human preneoplasia relates to the limitations of existing models in terms of permitting the growth of human preneoplastic cells *in vivo*. Recent advances with humanized models do permit the growth of human premalignant MGUS cells *in vivo* (41) and may provide a useful tool for probing these questions.

ANTIGENIC TARGETS FOR CANCER PREVENTION

Ideally, an antigenic target for a preventive vaccine would be highly tumor-specific, essential for tumor biology, expressed by the entire clone (or clonogenic progenitors), and capable of eliciting an immune response of sufficient potency to mediate protection. Advances in cancer genetics have shown that the genomic complexity of cancer is established early, even during the premalignant stages and that the tumor in each patient has a distinct set of genomic alterations and oncogenic mutations that yield neoantigenic targets (42). While this suggests the need to consider personalized approaches such as those targeting mutation-associated neoantigens (MANA) to prevent cancer (discussed later), strategies that target non-mutated tumor-associated antigens shared between tumors present fewer logistical challenges and are more amenable to clinical testing. One such antigen is MUC1, which is immunogenic in several human preneoplastic states and has therefore emerged as an attractive target for such preventive approaches (43). For example, intraductal papillary mucinous neoplasms (IPMNs) as precursors to pancreatic cancer express a hypo-glycosylated form of MUC1 and develop IgG antibodies against this antigen (15). Heavy smokers with preneoplastic lung lesions were shown to develop IgG antibodies against cyclin-B1 (44). HER2 is overexpressed on tumor cells in ductal carcinoma *in situ* and leads to the induction of immune responses in this setting (22, 45). Progression to invasive breast cancer is associated with a decline in these responses, setting the stage for targeting this antigen in the context of preventive vaccines (22, 45). The efficacy of vaccines against these antigens has also been demonstrated in murine models of breast cancer (46). An antigen screen for immune-reactivity in MGUS suggested that shared antigenic targets of host response in MGUS may differ from the malignant counterpart, myeloma (28). Specifically, the top antigenic targets in MGUS were genes such as SOX2 that are known to play a role in the biology of embryonal stem cells and are enriched on clonogenic progenitors (28, 47). In murine models, vaccines targeting early-stage antigens were more effective than vaccines targeting antigens expressed later in the course of the cancer (48). The presence of a T cell response against SOX2 emerged as an independent predictor of reduced risk of malignancy in MGUS in a large prospective study (32). Recent studies have also shown that OCT4, another embryonal stem cell-associated gene, can be immunogenic in humans (49). T cell responses against these antigens have also been observed in the setting of tumor regressions in the setting of checkpoint blockade, chimeric-antigen-receptor (CAR)-T cells, and chemotherapy of highly curable germ cell tumors (49–51). Further studies are needed to better understand whether immune targeting of stemness pathways in preneoplastic lesions can be clinically exploited for immune prevention (52, 53).

MUTATION-ASSOCIATED NEOANTIGENS AS TARGETS FOR PREVENTION

As much of the antitumor-response in preneoplastic lesions seems to be specific to an individual lesion (25), mutation-associated neoantigens (MANA) may be an important target for T cell response-targeting for cancer prevention. The importance of the T cell response against MANA has been demonstrated in mouse models and can impact the evolution of tumors during the equilibrium phase (54, 55). Serial analyses of human cancer have also provided evidence of immune-mediated regulation of cancer evolution, including that involving neoantigens (55, 56). However, whether T cells against neoantigens are essential for effective cancer prevention in the clinic remains to be established. Several studies have tried to vaccinate cancer patients against neoantigens in order to elicit MANA-specific T cells *in vivo* (42). While these studies have shown the feasibility of eliciting such responses, they seem to be of low frequency compared to immune responses following viral infections, and whether they mediate clinically meaningful anti-tumor effects remains to be established. It should be noted that as the genomic makeup or tissue of origin of cancers cannot currently be predicted before they develop, most of the efforts toward cancer prevention are only feasible as secondary cancer prevention, such as in patients with preneoplastic states. Primary cancer prevention is, however, potentially attractive in the case of hereditary cancer syndromes with defined patterns of organ-specific cancer, such as patients with Lynch syndrome.

INSIGHTS FROM VACCINES IN CHRONIC VIRAL INFECTIONS

If preventive vaccines in cancer can realistically only be tested in the setting of pre-existing preneoplasia at present, then some of the lessons learned from mouse models and human studies of chronic viral infections such as human immune deficiency virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) are worth considering. In chronic viral infections, the T cells target non-self-epitopes, similar to their response against neoantigens. Vaccines generally lead to poor T cell expansion in the case of chronic infection with the clone 13 strain of lymphocytic

choriomeningitis virus, which leads to chronic viral infection (57). In the case of simian-immunodeficiency-infected primates, prior reduction of viral load with anti-retroviral therapy was required in order to elicit strong T cell responses to gag antigens (58). Peptide and viral vaccines against hepatitis B and C have led to only mild increases in T cells against target antigens in infected patients, although the ability to elicit T cells in uninfected individuals was much greater (59–61). Chronic exposure to the virus also leads to a loss or a reduction in the loss or deletion of T cells with the highest affinity to the antigen. For example, in chronic gamma-herpesvirus infection, high-affinity clones mediate early robust expansion but undergo attrition, while intermediate/low-affinity clones are maintained longer (62). Similar observations have been made in human HIV infection (63). These considerations raise the possibility that T cell responses, even to neoantigens, may not be as impressive as currently hoped if applied late in the course of preneoplasia.

LESSONS FROM THERAPEUTIC VACCINATION IN CANCER

The discovery of the T cell response to tumor-associated antigens, beginning with the MAGE family (64), not only provided the foundation for the field of cancer immunology but also led to studies of therapeutic vaccination. Several strategies have been utilized for inducing immunity to tumor-associated antigens. These include injection of peptides with adjuvants, DNA vaccines, viral vectors, dendritic cell vaccines, and prime-boost approaches (65). Prime-boost approaches have also commonly been utilized in the case of chronic viral infections. With increasing appreciation of the importance of MANA, several of these strategies are currently being applied to try to elicit immunity to neoantigens in the clinic (42). However, many of the initial studies focused on patients with clinical malignancy but often lacking measurable disease, and the clinical efficacy of such approaches remains to be established (42). The vaccination field was greatly aided by the discovery of dendritic cells (DCs) as critical antigen-presenting cells and led to several studies targeting mature DCs (66–68). However, while monocyte-derived DCs led to T cell responses in several patients, these studies led to tumor regressions in only a small proportion of patients, although some of these responses have been long-lasting (67, 69). Only one of the DC vaccines, Sipuleucel-T, has to date led to improved survival in the setting of cancer (70). It is important to note that the initial studies did not target the immune-suppressive pathways, including immune checkpoints and regulatory T cells. More recent studies have successfully targeted human DCs *in situ*, which is more amenable to larger-scale clinical trials (71). However, these studies were also conducted without addressing immune-suppressive factors in the tumor bed. Vaccine-based studies exploiting the biology of human DC subsets, and in particular those with enhanced potential for cross-presentation, have not yet been carried out, although evidence for the feasibility of targeting these subsets is emerging (72, 73). Strategies that target DCs directly *in situ* may also be preferable to those that target DCs *ex vivo* because the former may allow targeting of naturally occurring DCs in greater numbers compared to those limited by the effect of *in vitro* culture (71). In this regard, specific targeting of DC subsets *in situ* remains an unmet need. It is possible that combinatorial targeting of defined DC subsets may be essential for robust immunity (73, 74). An important desired goal of vaccines is to elicit T cells that mediate long-term protection (75). It has been suggested, for example, that vaccines that elicit tissue-resident memory T cells may be needed to mediate protective immunity (76). Studies with yellow fever vaccine, one of the most effective vaccines in humans, have provided important insights into the properties of long-term protective immunity, involving the induction of a broad immune response and the generation of long-lasting memory T cells (77, 78). It remains to be demonstrated whether T cells with similar properties can be elicited in the context of vaccination against tumor antigens.

DIVERSITY OF PRENEOPLASTIC LESIONS—DO WE NEED TO LINK IMMUNE PREVENTION WITH EARLY DETECTION?

It is now well-appreciated that preneoplastic lesions can exhibit significant diversity. At the clinical level, this includes features such as size, dysplasia, and genomic changes in preneoplastic cells that confer an increased risk of malignant transformation. However, these lesions may also differ considerably in terms of the nature of the host immune response. As discussed earlier, many of the oncogenic mutations found in cancer cells originate in the precursor phase. The initial studies describing the presence of expanded hematopoietic clones carrying genomic mutations have now been extended to clones of cells with somatic mutations within normal tissues in otherwise healthy individuals (79, 80). The long natural history of these lesions, typically spanning several years, implies (although it is not proven) that the immune system has already undergone chronic exposure to these antigens. The application of single-cell technologies to study the immunology of these lesions has illustrated the diversity of human preneoplastic states, wherein the immune response evolves over time (**Figure 1**) (18, 19, 39). As discussed earlier, the persistence of exhausted T cells in models of chronic viral infection depends on a subset of T cells that exhibit more stem-like features (36). Recent studies in MGUS patients have shown that similar hierarchies of T cell exhaustion that are responsible for maintaining chronic T cell responses are established early during carcinogenesis (39). Advanced lesions also carry greater dysfunction of innate cells including NK cells, innate lymphoid cells, and altered polarization of myeloid cells (30, 39). Changes in the myeloid compartment may therefore be an important driver of the malignant phenotype and the loss of immune control (15, 39, 40). Strategies that target innate immunity may therefore also be explored for cancer prevention (81). The concept that precursor lesions are not immunologically silent suggests that strategies that overcome immune checkpoints may also be effective in these patients. While current strategies for

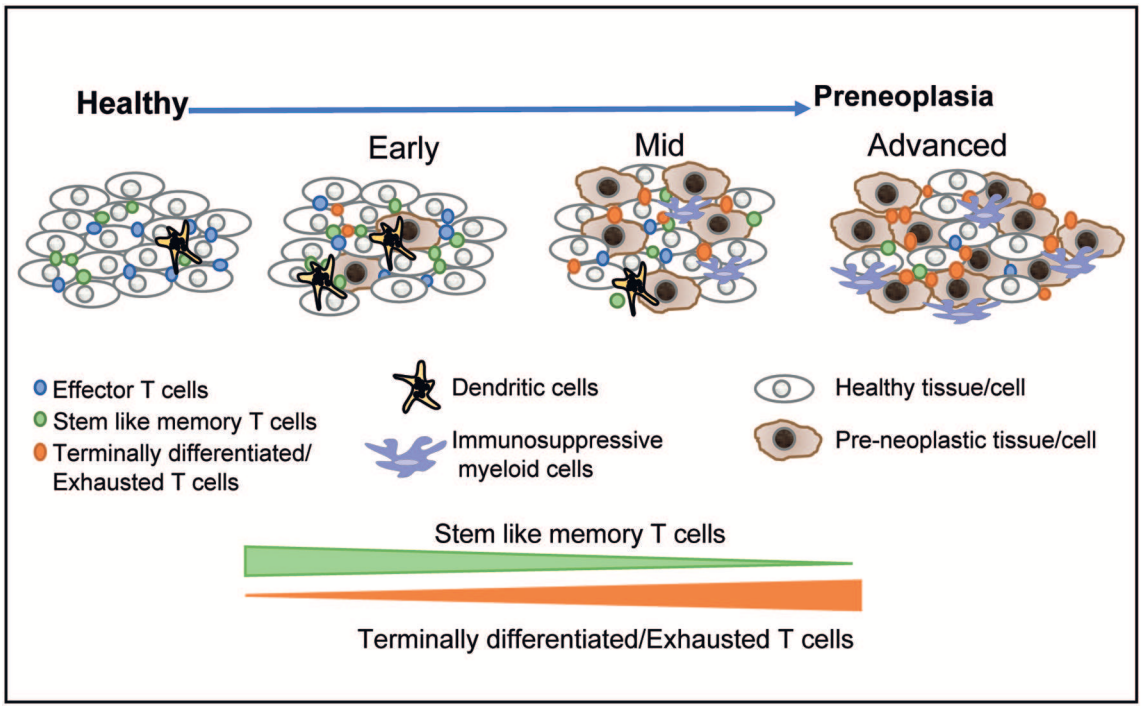

FIGURE 1 | Immunological diversity and evolution of precursor states. The application of single-cell technologies to studying precursor states has shown that the earliest lesions are associated with changes in the immune microenvironment. The hierarchy of T cell exhaustion is established early and is associated with a relative decline in stem-like and resident memory T cells over time. More advanced lesions are associated with infiltration by more immune-suppressive myeloid populations. These data suggest that immune prevention through vaccination may be most effective for earlier lesions, with more advanced lesions requiring combination strategies.

checkpoint blockade do carry the risk of adverse autoimmune events that may be unacceptable for this patient population (82), advances in preventing such complications (83) may make it more feasible to pursue checkpoint blockade to target high-risk precursor lesions.

The concept that the immunologic evolution of the tumor microenvironment begins early also has important implications for the timing of immune prevention. It may be desirable to target lesions that still have high levels of stem-like and tissue-resident T cells and low levels of immune-suppressive myeloid cells in order to achieve a durable response to immune-mediated prevention. This, in turn, may require that strategies that pursue immune prevention are directly linked to early detection before the adverse aspects of the preneoplastic immune microenvironment are fully established. Alternatively, combination approaches (such as are being pursued in the context of therapeutic manipulation of immunity in established cancers) may be required for immunologically altering the natural history of more advanced preneoplastic lesions. Traditionally, the rationale for early detection in cancer has been limited to enhancing the potential for the surgical resection of the lesion, presumably with curative intent (84). Here we suggest that even in a setting wherein surgical resection is not feasible (e.g., hematologic premalignancies) or is clinically not indicated, early detection may be essential for achieving a window of opportunity for effective immune prevention.

CLINICAL STUDIES OF IMMUNOPREVENTION

In contrast to the large body of literature evaluating therapeutic vaccination in cancer, data about preventive vaccination, particularly for non-viral vaccines, are limited. One of the antigens evaluated in more advanced studies is the tumor-associated antigen MUC-1. The safety and immunogenicity of a MUC-1 peptide vaccine have been demonstrated in initial clinical studies (85). While colon polyps are typically resected at diagnosis, the rationale for vaccination in this setting is based on reducing the recurrence of polyps. In the initial studies, the immunogenicity of the vaccine was impaired in patients with elevated myeloid suppressor cells, suggesting that vaccination in the earlier stages of preneoplasia should be considered, as discussed earlier (85). Nonetheless, MUC-1 vaccination is currently being tested in the context of a phase III trial. Instillation of Bacillus Calmette Guerin (BCG) has been shown to mediate the regression of *in situ* bladder cancer lesions but is ineffective in the setting of more advanced muscle-invasive lesions (17). Vaccination in the neoadjuvant setting has been trialed to evaluate the induction and anti-tumor effects of vaccination for preneoplasia. Vaccination of women with DCIS of the breast with dendritic cell vaccines presenting Her2-derived peptides led to the induction of immunity and provided some early evidence of antitumor effects, with a reduction in DCIS

lesion seen in some patients at surgery (45). Preneoplastic lesions that cannot be resected (as is the case with MGUS, a precursor to myeloma) represent an attractive model for establishing the principles of immunomodulation for the prevention of human cancer. In a recent study, patients with smoldering myeloma (an intermediate preneoplastic stage between MGUS and myeloma) were randomly assigned to either observation alone as the standard of care or administration of single-agent lenalidomide, an immunomodulatory drug (86). Lenalidomide led to a significant prolongation of progression-free survival compared to observation, with a nearly 70% reduction in the risk of clinical malignancy (86). These data provide an example of successful immune-modulation-based interception of human cancer (87), in this case utilizing an oral therapy that would otherwise be inadequate as a single agent in the setting of established cancer. These findings may not only change clinical practice for the subset of patients at highest risk of clinical progression; they also set the standard for future studies testing immune-based prevention in MM.

CHALLENGES AND BARRIERS TO PREVENTIVE VACCINES AND OTHER APPROACHES

In spite of an improved understanding of the immunology of precursor states, there are several potential challenges to preventive vaccination of cancer, even when targeting preneoplastic lesions (88). At present, it is not feasible to accurately predict which specific antigen (or combination thereof) will serve as a rejection antigen or effectively prevent cancer in an individual patient. While peptide-based strategies have been employed to target both shared antigens and neoantigens, several variables, such as the choice of peptides and their immunogenicity, clearance, and expense may impact the clinical efficacy and application of peptide-based vaccines. Targeting a limited set of antigens also carries the potential for antigen-loss variants as a mechanism for immune escape. Antigen-loss has been shown to be a potential mechanism of tumor immune escape in murine models (54, 89). However, the degree to which this occurs in the setting of preventive vaccination in the clinic remains to be established. One potential strategy may be to target "trunk" mutations or genes essential for a malignant phenotype, but this has, to date, proven challenging in the clinic, and several of the trunk mutations may not be immunogenic (42). Other barriers that limit therapeutic cancer vaccines may also apply to preventive vaccination, particularly if the latter is approached in the setting of more advanced precursor lesions. These include intra-tumoral heterogeneity, stem-like features of tumor cells or even putative cancer stem cells, and other immune-suppressive features in the tumor microenvironment (90). If true, this would imply that preventive vaccination would also need to use combination approaches as is currently being explored in the setting of established cancer. As discussed previously, these considerations further reinforce the need to link immune prevention to early detection, and perhaps even before clinically meaningful preneoplastic lesions are manifest. In addition to vaccines, other strategies such as T-cell redirection (e.g., bispecific antibodies) and other immunomodulatory antibodies are being considered for immune-based interception. Recent success with lenalidomide in the prevention of myeloma, as discussed earlier, may encourage such studies. However, given the cost and potential toxicity, it would be important to limit such approaches to patients at highest risk and with careful attention to long-term effects.

SUMMARY

In the preceding sections, we have discussed the emerging evidence in support of immunological approaches to preventing cancer. In contrast to therapeutic vaccination, these are still very early days for clinical or even translational studies testing these hypotheses. However, advances in cancer genetics and recent successes in cancer immunotherapy have begun to set the blueprint for strategies to harness tumor immunity to prevent cancer. It is now being appreciated that clonal expansions of cells carrying potentially oncogenic mutations are common in healthy tissues (80). As the biological and immunological principles underlying these strategies are being established, careful clinical investigation will be required to move the field forward. One of the challenges that makes cancer a formidable foe is its ability to adapt and evolve, as is also the case with pathogens. Therefore, the immune system, with its capacity to adapt, evolve, and persist, may be our best defense against cancer, as is already evident from its success in preventing pathogens (91). Planned investments in defining the landscape of precursor states to human cancer should go a long way toward helping us achieve these goals (12, 92).

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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