Eliminating HIV reservoirs for a cure: the issue is in the tissue

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Purpose of review
Advances in antiretroviral therapy have saved numerous lives, converting a diagnosis with human immunodeficiency virus 1 (HIV-1) from a death sentence into the possibility for a (nearly) normal life in many instances. However, the obligation for lifelong adherence, increased risk of accumulated co-morbidities, and continued lack of uniform availability around the globe underscores the need for an HIV cure. Safe and scalable HIV cure strategies remain elusive, in large part due to the presence of viral reservoirs in which caches of infected cells remain hidden from immune elimination, primarily within tissues. Herein, we summarize some of the most exciting recent advances focused on understanding, quantifying, and ultimately targeting HIV tissue viral reservoirs.

Recent findings
Current studies have underscored the differences between viral reservoirs in tissue compartments as compared to peripheral blood, in particular, the gastrointestinal (GI) tract. Additionally, several novel or modified techniques are showing promise in targeting the latent viral reservoir, including modifications in drug delivery platforms and techniques such as CRISPR.

Summary
Elimination of tissue viral reservoirs is likely the key to generation of an effective HIV cure. Exciting studies have come out recently that reveal crucial insights into topics ranging from the basic biology of reservoir seeding to effective drug targeting. However, there are still many outstanding questions in the field about the relative importance of specific reservoirs, such as the GI tract, that may alter the final strategy pursued.

Keywords
gastrointestinal tract, HIV, lymph node, lymphoid tissue, reservoir, SIV

INTRODUCTION

In-depth characterization of cellular and anatomic reservoirs for human immunodeficiency virus 1 (HIV-1), the tissue compartments in which they reside, salient phenotypic characteristics of viral reservoirs, and environmental signals within defined neighborhoods that are critical for persistence remain poorly understood, yet are likely crucial for understanding how to effectively target and eliminate tissue viral reservoirs. Earlier studies of antiretroviral therapy (ART) in both HIV-1 infected individuals and simian immunodeficiency virus (SIV) infected nonhuman primates (NHPs) demonstrated that viral reservoirs are established rapidly and systemically [1]. Although reduced by ART, these reservoirs constitute a substantial total body burden of potentially infectious virus [2] resulting in plasma viral loads (pVLs) that, while previously undetectable on ART, rapidly rise upon treatment interruption in all but the rarest circumstances. Since natural, ART-free viral remission is highly infrequent and complete viral eradication via hematopoietic stem cell transplant is neither safe nor feasible at scale, the field has

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KEY POINTS

- Tissue reservoirs are a critical source of rebounding HIV-1, which must be eradicated to achieve an HIV cure.
- The tissue distribution of potential therapeutics or treatments and local spatial microenvironment must be taken into account when considering novel cure strategies for HIV.

been working toward targeting tissue viral reservoirs that remain a stubborn barrier to an HIV cure.

TISSUE DISTRIBUTION OF VIRAL RESERVOIRS THROUGHOUT THE BODY

Lymphoid tissues (LT) are a major source of HIV-infected cells at all stages of infection. Our group and others have shown that LT, including the gut, lymph nodes, and spleen, contain $\geq$98% of 'active' ($vRNA^+$) and total ($vDNA^+$) SIV reservoirs before and during ART [2]. Even after ART, HIV-1 and SIV persist in these LT compartments for a variety of reasons (Fig. 1), including: (i) Tissue resident T follicular helper (T_{FH}) cells, which are a preferred cellular reservoir for HIV-1, and follicular dendritic cells (FDC; a noninfected cellular repository of infectious virions) are long-lived viral reservoirs that reside within B cell follicles (BCF) found in secondary LTs [3,4▪▪]; (ii) residual levels of immune activation and inflammatory mediators are heightened in many LTs (in particular the gut-associated lymphoid tissue [GALT]) during ART [5]; (iii) antiretroviral (ARV) drug penetration (particularly protease inhibitors) into tissue sites of viral persistence is very heterogeneous with limited combined exposure to all infected cells, potentially allowing for environments where low level, intermittent viral replication can occur [6,7▪]; and (iv) BCFs represent relative immune sanctuaries that are not highly accessible to HIV-1/SIV-specific cytotoxic $CD8^+$ T cells (CTLs), thereby allowing viral reservoirs that reside in these microenvironments to escape CTL elimination [8]. Although of interest to the field, non-LT viral reservoirs fall outside the scope of this review, primarily because the mechanisms controlling reservoir establishment, persistence, and clearance have aspects that are unique to each tissue site. A prime example is the brain and CNS, in which recent publications have elucidated how HIV-infected microglia and astrocytes are maintained as well as therapeutic strategies specifically designed to cross the blood-brain barrier, a distinct prerequisite for targeting the CNS reservoir (for a review, see [9]). Given the number of recent and exciting publications, separate reviews are required to do justice to these often rapidly developing fields.

In both HIV-1 and SIV infection, seeding of viral reservoirs occurs early and systemically, especially within LT where the majority of $CD4^+$ T cells reside. This is supported by studies in rhesus macaques showing that long-lived reservoirs harboring replication-competent virus are established as early as 3 days after infection [1] and that integrated HIV-1 DNA in acutely infected individuals can be detected in GALT and LNs as early as Fiebig stage I and reach maximal frequencies by Fiebig stage II [10▪]. Moreover, at later stages of infection, the frequency of viral DNA is actually higher in LNs than peripheral blood [10▪], further emphasizing the key role that tissues play in viral reservoir persistence.

Beyond serving as a repository for latently infected cells, LT contribute ongoing viral transcription during ART treatment [11]. The relative contributions of anatomical compartments to total pVL can be challenging to separate, since blood is the primary source of viral dissemination [12▪] and the recurrent interchanges of $CD4^+$ T cells between blood and tissue lead to similar proviral sequences present across both sites. [13▪]. Nevertheless, while viral DNA can be found throughout multiple tissue types during ART suppression, production of viral RNA has been shown by some to be restricted primarily to LT such as within LNs [14] and the gastrointestinal (GI) tract [15], demonstrating the unique contribution of LT to both viral reservoir persistence and production of potentially infectious virus during ART. Moreover, since viral reservoirs are a major barrier to an HIV cure, potential strategies must be able to specifically eliminate all *tissue* viral reservoirs that harbor a replication-competent genome.

FOLLICULAR DENDRITIC CELLS AND T FOLLICULAR HELPER CELLS, TWO DISTINCT BUT IMPORTANT TISSUE RESIDENT VIRAL RESERVOIRS WITHIN B CELL FOLLICLES

Found within BCFs, FDCs comprise a distinctly unique tissue resident viral reservoir. Unlike $CD4^+$ T cells or other infected cell reservoirs (e.g., myeloid lineage populations), FDCs are not permissive for HIV-1 infection. However, they can trap and retain infectious virus in the form of complement (and antibody) opsonized immune complexes, on the extracellular surface or within nondegradative endosomal compartments via CD21 [16] (and FcR) attachment for long periods of time [16–18]. Besides the longevity of this viral reservoir, its exclusive presence within the BCF 'sanctuary' adds additional complexity in directly targeting the FDC reservoir. Indeed, despite recent advances in the development of chimeric antigen receptor (CAR) T cells to

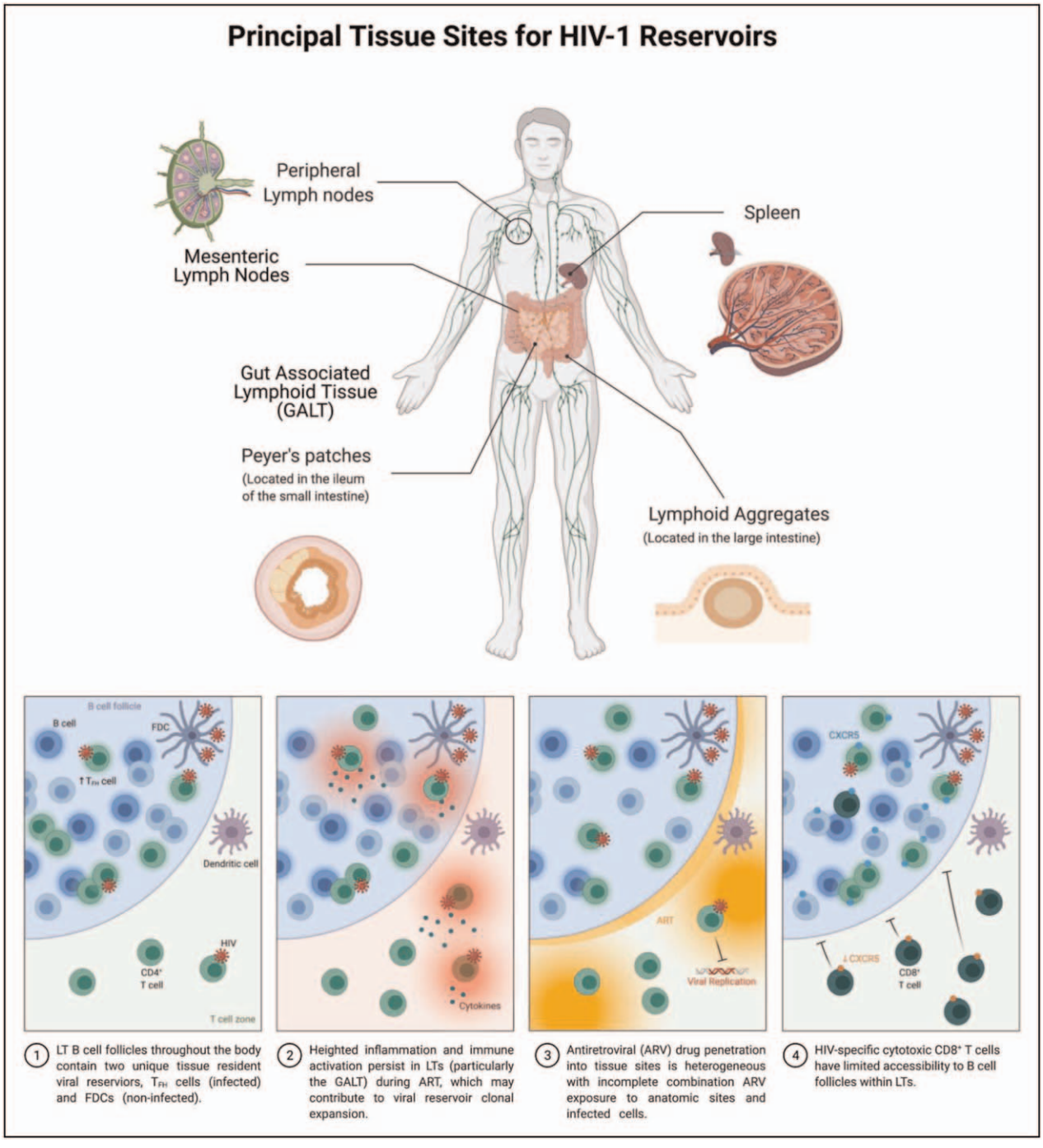

FIGURE 1. Viral Persistence in Lymphoid Tissues. HIV-1 and SIV can persist in LTs throughout the body even after ART due to: (1) persistence of viral reservoirs in BCFs (both T_{FH} cells and FDCs); (2) residual levels of immune activation and inflammation; (3) incomplete ARV penetration into tissue sites potentially leading to low and intermittent levels of viral replication; and (4) limited accessibility of cytotoxic $CD8^+$ T cells into BCFs. Created with BioRender.com. ART, antiretroviral therapy; BCF, B cell follicles; FDC, follicular dendritic cells; LT, lymphoid tissues.

eliminate infected $CD4^+$ T cells (discussed later), they appear unable to eliminate FDCs displaying bound virions [19], likely due to the fact these cells are not infected and thus do not present HIV-1 antigens via MHC class I or II.

Memory $CD4^+$ T cells, including central, transitional, and effector memory (EM), are the major cellular reservoirs for HIV-1 infection [20]. Recent publications have expanded our knowledge of how infected $CD4^+$ T cell subsets vary across multiple

cellular compartments. In blood, the frequencies of $CD45^+CD4^+$ and $CD45^+CD8^+$ T cells correlate directly with the size of the HIV-1 reservoir [21▪] and there is no difference in the frequency of T cells with intact proviruses from HIV-infected individuals on ART across different memory T cell subsets in the peripheral blood mononuclear cell (PBMC) compartment [22▪▪]. However, within LT specifically, T EM and T_{FH} $CD4^+$ T cells harbor the highest frequency of viral DNA and RNA in both acutely SIV-infected and ART-treated rhesus macaques, particularly in the spleen and mesenteric LNs [4▪▪]. The bias toward T_{FH} cells is particularly notable, since $CD8^+$ T cells are unable to effectively target BCF-localized T_{FH} for a variety of reasons, including a deficiency in cytolytic capabilities and the lack of CXCR5 necessary for homing into the follicle [23▪].

Intriguingly, tissue-resident memory $CD4^+$ T cells expressing CD127, the alpha chain of the IL-7 receptor, have diminished levels of multiple factors such as NFκB, NFAT, and Ox40 required for efficient HIV-1 gene transcription, thereby preferentially promoting a quiescent state [24▪]. Although stimulation can reactivate the virus, it remains unclear whether these memory $CD127^+$ $CD4^+$ T cells harbor a significant fraction of the inducible reservoir. Since IL-7 contributes to the persistence of HIV-infected memory $CD4^+$ T cells by promoting homeostatic proliferation [20] and IL-7 administration leads to viral reactivation [25,26,27], the association between high CD127 expression and viral quiescence is perplexing. However, since low expression of CD127 is present on some memory $CD4^+$ T cell lineages that are susceptible to productive infection, such as T_{FH} cells, perhaps this confirms the challenge of designing strategies to induce viral reactivation broadly across all infected T cell subsets. Taken altogether, these studies underscore the importance of understanding how tissue-resident $CD4^+$ T cell subsets within specific immune microenvironments alter the dynamics of HIV-1 infection.

THE GASTROINTESTINAL TRACT: AN IMPORTANT COMPARTMENT FOR HIV-1 PERSISTENCE

We and others have shown that viral infection generates GI tract epithelial barrier damage and permeability, leading to bacterial translocation and enhanced local and systemic inflammation [28–30]. This is likely because the GI tract comprises a substantial majority of the total viral reservoirs present during infection [2]. Therefore, potential treatment modalities should include approaches to measure the efficacy within GI tract viral reservoirs, challenging as that can be. The disproportionate viral reservoir burden seems to be due, in part, to viral infection and/or persistence preferentially within $CD4^+$ T cells that home to GALT, such as $CCR5^+$ $CD4^+$ T cells that additionally express the gut-homing chemokine receptor CCR6 [31] or the mucosal integrin α4β7 [32]. Disappointingly, however, attempts to reduce GI tract viral reservoirs by targeting α4β7 have not borne out [33▪–35▪] despite showing initial promise, even if the antibody is given prophylactically [36▪].

Contraction of $vDNA^+$ reservoirs following ART initiation is tempered within the GI tract compared to LNs [2], skewing the viral reservoir toward greater intestinal localization over the course of treatment. The cause may be that as many as 50% of T cells within the GI tract reside in areas where ARV drug levels are limiting or even undetectable [37▪▪]. Notably, ARVs appear to have different effects within the GI tract as compared to blood and LNs [38▪], which suggests that accessibility may pose a particular challenge when targeting intestinal viral reservoirs. Intriguingly, depletion of $CD4^+$ T cells via an unbiased antibody-mediated approach is markedly less effective in rectal tissues than LNs [39], although, as the authors point out, antibodies should be able to sufficiently penetrate the intestinal mucosa. Indeed, biologics have become staples within the inflammatory bowel disease (IBD) treatment arsenal (e.g., TNFα inhibitors such as adalimumab and infliximab) to moderate inflammation locally within the lamina propria. This difference could be explained by better penetration and distribution of biologics from the blood to sites of GI tract damage and inflammation in the lamina propria (as in IBD), yet more limited access of antibodies into GALT where the majority of intestinal viral reservoirs reside during ART. This highlights how spatial analysis of drug distribution in the context of where viral reservoirs reside within tissue compartments can provide important insight into HIV cure therapeutic efficacy.

Additional factors may specifically complicate GI tract reservoir targeting in ways that aren't yet clear. Indeed, recent findings about the impact of hypoxia in modulating LN viral reservoirs [40▪▪] provokes the question about whether the same is true in the GI tract, at least regionally, given the lower oxygen tension in the lumen of the large vs. small intestine referred to as 'physiologic hypoxia'. Clearly, the extent of therapeutic drug penetration into crucial niches within the GI tract requires further study, as it is critical that any cure option be efficacious in targeting intestinal viral reservoirs. It is encouraging, therefore, that contemporary drug design has focused on ensuring tissue penetration, often including the GI tract, of ARVs and that great

strides have been made in ensuring tissue biodistribution [41–43].

DISTINCT VIRAL STATES OF CELLULAR VIRAL RESERVOIRS

An important consideration of viral reservoirs is their state of activity. HIV-1 infected reservoirs are a heterogenous assortment of infected cells that can be in a variety of viral activation states: (i) deep latency, with no vRNAs expressed, (ii) low transcriptional activation, in which small amounts of vRNAs are produced but not translated or, (iii) dynamic viral activation, in which higher expression of vRNAs are generated and a portion further translated into protein that can result in virion production in viral reservoirs with intact functional genomes (Fig. 2).

Viral rebound following ART discontinuation is often thought to originate from a small number of resting memory $CD4^+$ T cells harboring replication-competent provirus that become activated and produce infectious viral progeny following ART cessation. However, during suppressive ART (in both

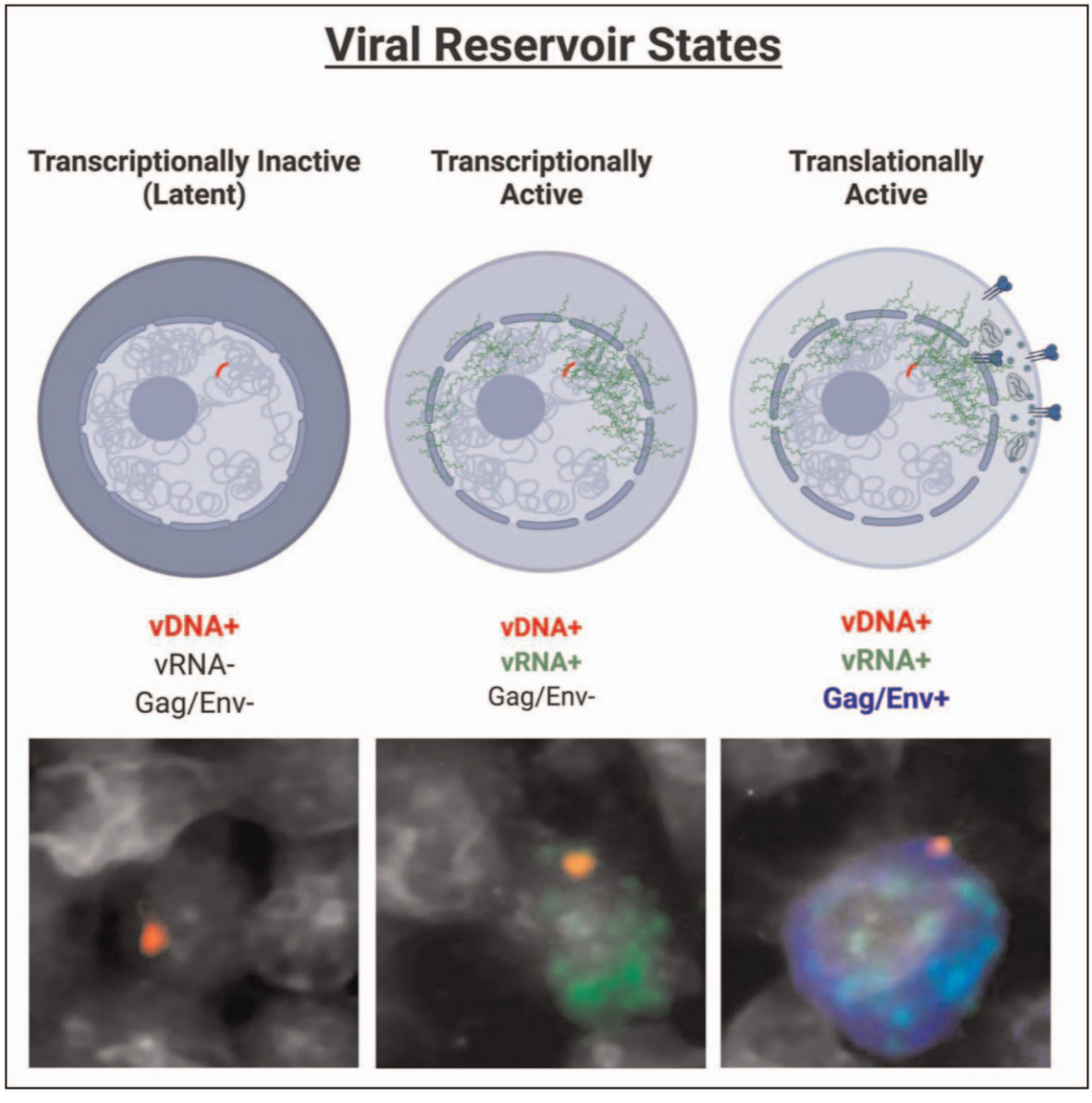

FIGURE 2. Distinct cellular viral activation states. HIV-1 and SIV can persist in LTs even after ART and can be identified using *in situ* spatial analysis with single-cell resolution. The upper panel shows schematic illustrations, and the lower panel fluorescent micrographs of individual latent (vDNA+ only; left), transcriptionally active (vDNA+ and/or vRNA+; middle) or translationally active (vDNA+ and/or vRNA+ HIV/SIV protein+; right) infected cells. Created with BioRender.com. ART, antiretroviral therapy; LT, lymphoid tissues.

HIV-1 infected individuals and SIV infected NHPs), cells that are positive for viral RNA can be detected in both lymphoid and non-LT (e.g., PBMC, GI tract, LNs, and CNS) using both *in situ* and molecular approaches [2,44–46], suggesting that viral particle production taking place at low levels during ART from active viral reservoirs are capable of rapidly reigniting infection following ART discontinuation. Indeed, recent studies have shown that higher levels of HIV RNA expression while on ART are associated with the magnitude and time to HIV-1 rebound after treatment interruption [45,46]. In addition, single genome amplification (SGA) sequencing of the plasma from HIV-1 infected individuals shortly after ART discontinuation identified numerous rebound/founder (R/F) viruses suggesting multifocal origins of recrudescent infection [44]. The rapidity and sequence diversity of viral rebound following ART discontinuation in most individuals implies that numerous viral reservoirs within LTs throughout the body that contain intact proviral genomes and are already actively producing viral RNA prior to discontinuation of ART are a likely source of the recrudescent infection. Recent studies in chronically SIV-infected rhesus macaques provide additional details into the nature of the rebound virus, observing that viral sequences in PBMC and lymph node mononuclear cells (LNMC) during initial rebound closely match viral DNA sequences already present during ART suppression [47▪▪]. This strongly suggests that the source of the viral rebound originates directly from intact proviral DNA in PBMC and LNMC, not from recombinant viruses that only show up 2–4 weeks after treatment interruption.

This presupposes that infected cells producing viral RNA have an intact viral genome capable of producing infectious virus. Because the process of reverse transcription is error prone, with 'skipping' to areas with high homology, subsequent deletion mutations in the genome are common. Indeed, only a small fraction of sequenced integrated HIV-1 viral genomes within a cohort of chronically HIV-1 infected individuals were fully intact, while the vast majority contain deletions, particularly in the 3′ region, as well as hypermutations that rendered them defective. Several assays have recently been developed to accurately assess the 'intactness' of integrated HIV-1 genomes more rapidly and with higher throughput, as whole genome sequencing or quantitative viral outgrowth assay techniques tend to be time consuming, labor intensive, require large quantities of input cells, and expensive. The integrated proviral detection assay (IPDA), is a ddPCR based approach that relies on the detection of 5′ and 3′ probes specifically targeted at two regions of the viral genome based on an in-depth analysis of deleted and hypermutated genomes [48,49]. An alternate PCR-based approach instead relies on 4 different probes [50▪▪,51▪]. In contrast to approaches that target a specific region of the integrated viral genome, an alternate approach relies on a 'tile' based assay of detecting overlapping, sequential PCR amplicons, which allows the mapping of deletions within the integrated viral genome [52▪]. Although all of these new innovations measure the putative intactness of an integrated viral genome, they cannot determine whether a tissue viral genome is latent or being actively transcribed, a key determinant in reservoir maintenance.

TARGETING CELLULAR VIRAL RESERVOIRS: IMMUNE TARGETING AND CELL-BASED THERAPEUTICS

Being able to activate, target, and eliminate viral reservoirs with intact genomes is of utmost importance in developing a functional cure for HIV-1. Several strategies have been proposed for the eradication of latent viral reservoirs, ranging from (i) activating latently infected cells to allow for their removal by cytolysis or clearance through the immune-based mechanisms (the shock and kill approach), (ii) ensuring that the latently infected cells never actively transcribe the virus (block and lock approach), or (iii) removal of the integrated viral genome through gene editing techniques such as CRISPR/CAS. Moreover, since viral reservoirs often exist within immune privileged sites from which $CD8^+$ effector cells are largely excluded, techniques to drive immune effector cells into these sites have been developed, such as the IL-15 superagonist N-803 [53,54]. Alternatively, the generation of CAR-T cells targeted against HIV-1/SIV shows great promise, and it has recently been shown that CAR-T cells can migrate and reside within key tissue reservoir sites such as the GI tract, the central nervous system, and LN, including the relatively inaccessible BCFs [55].

The advent of specific nucleic acid targeting techniques, such as CRISPR based technology may generate a substantial reduction in viral reservoirs by directly targeting the integrated virus. The significant advantage with this approach is that it does not rely on activating the latently infected cells, but rather excises the viral DNA regardless of the cell's viral activation state. This approach has been recently attempted using an NHP SIV model using AAV9 as a carrier, leading to a reduction in viral DNA [56▪▪]. Intriguingly, localization of this vector seemed focused on BCFs within LNs, a notable sanctuary site for tissue resident viral reservoirs (discussed above). Although promising, the

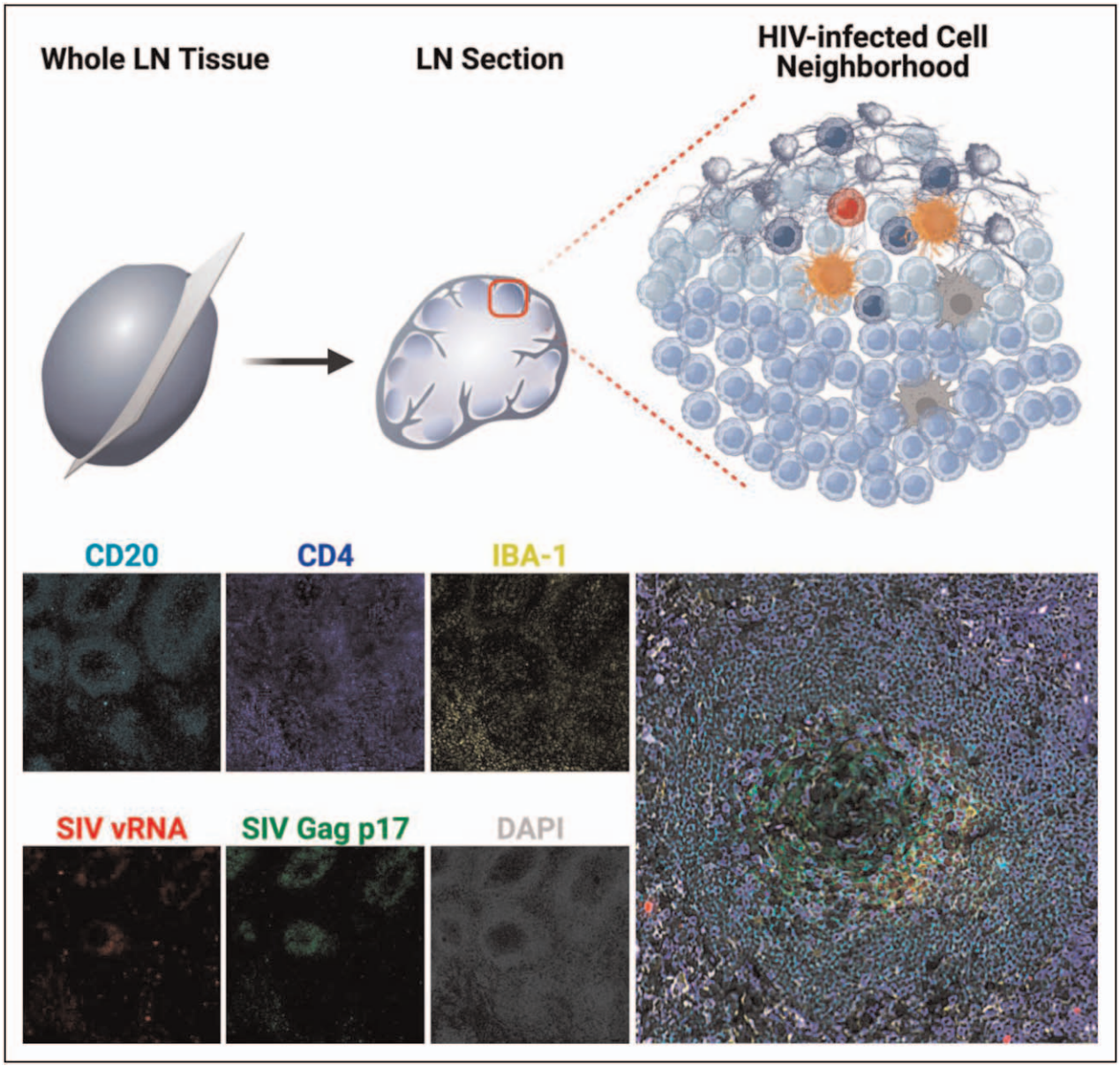

FIGURE 3. Utilizing spatial analysis approaches that comprehensively elucidates the complex and dynamic microenvironments and cellular immune neighborhoods where HIV-1 and SIV reservoirs reside (shown in red) likely hold the key to determining novel therapeutics in the quest for an HIV cure. Created with BioRender.com.

efficiency of such techniques will require substantial improvement, and will likely need to be combined with other approaches to effectively eradicate viral reservoirs.

CONCLUSION

The next break-throughs in the HIV cure field are likely to come from studies understanding the dynamic state of viral reservoirs within the tissue microenvironments in which they reside during ART. Given the increasingly obvious differences that exist between the peripheral blood and tissues, emerging technological advances that enable comprehensive spatial analysis of cellular immune neighborhoods within tissue compartments (such as BCFs) in which viral reservoirs interact and depend for survival will generate enormous rich and nuanced datasets necessary to understand the complex signals that drive HIV-1 latency or activation and viral production (Fig. 3). These rich spatial imaging-based approaches are geared to gain a deeper and more comprehensive understanding of (i) specific viral reservoir phenotypic characteristics, and (ii) key signals and cellular pathways that are unique to cellular immune neighborhoods where

latent and active viral reservoirs reside, with the goal to discover novel and specific virus eradication therapeutics that can either alone, or in combination lead to an HIV cure.

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Conflicts of interest

There are no conflicts of interest.

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